PROCEEDINGS OF

National Seminar on Marine Resources

Organized by :

Department of Zoology, The M.D.T. Hindu College, Tirunelveli

In Collaboration With

Seaweed Research and Utilisation Association

CAS in Marine Biology, Annamalai University, Parangipettai - 608 502.

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Proceedings of
National Seminar on Marine Resources
NSMR'16

Editor

Dr. V. Saravanan

Associate Editor

S. Elango Subramanian

Organised by

Department of Zoology, The M.D.T. Hindu College

Tirunelveli. Tamilnadu - 627 010.

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ISBN : 978-81-931973-9 -4

Citation : V. Saravanan and S. Elango Subramanian (2016) Proceedings of National Seminar on Marine Resources (NSMR'16) at The M.D.T. Hindu College, Tirunelveli 3rd and 4th March 2016. 250pp.

Compilation of Articles:

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Published by:

Department of Zoology

The M.D.T. Hindu College

Pettai, Tirunelveli – 627 010.

Email : mdthinducollge@gmail.com

www.mdthinducollege.org

Printed by:

Galaxy Printograph

Tirunelveli – Town

Email : galaxyprintograph@gmail.com

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PANANGAD P.O., KOCHI 682 506, KERALA, INDIA
☎ 0484- 2701308, 2700598; **Fax**: 0484-2701308; ***e-mail***: k.padmakumar@gmail.com; pvc@kufos.ac.in ***website***: www.kufos.ac.in

Prof. K. Padmakumar, Ph.D.
Pro Vice-Chancellor

29 February 2016

The Ocean is considered as Earth's most valuable natural resources which provide various kinds of food, medicines, minerals and oil. The marine environment also plays a crucial role in absorbing approximately 25 percent of the CO_2 added to the atmosphere from human activities each year and regulates Earth's climate. More than three billion people depend on marine and coastal biodiversity for their livelihoods. Food from marine environment forms world's largest primary source of protein, with more than 2.6 billion people depending on it. But today, 40% of the world oceans are heavily affected by human activities, including pollution, depleted fisheries, and loss of coastal habitats and ultimately valuable marine biodiversity. Hence it is high time to create awareness among the students, researchers, administrators and general public on the importance of marine resources and sustainable utilization of it. I wish the National Seminar on Marine Resources organized by the M.D.T Hindu College, Tirunelveli during 3 – 4 March 2016 a grand success.

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……..…….

Dr. A. John De Britto

Registrar

FOREWORD

It's with deep satisfaction that I write this foreword to the proceeding of the "National Seminar on Marine resources" (NSMR'16). Our vast beautiful oceans and seas have always been an endless source of inspiration and recreation while sustaining the livelihoods of millions. They provide us with numerous resources and services of which oxygen, food, energy, transport and tourism are just a few examples. For toolong, we have taken it all for granted and little attention has been paid to our unsustainable use of these resources. Today the degradation caused by inefficient growth model we have been locked into is threatening many marine ecosystems, though this may sometimes be masked by the immensity of sea itself.

Marine Strategy Framework Directive(MSFD) of EU aims to ensure that our seas and oceans are healthy that is in 'Good Environmental Status' by 2020, so they can continue to deliver the benefits so many jobs and lives depend on. Based on this we should also aim to protect the whole of marine environment from the majestic top predators right down to the smallest vital single cell microorganism. This could be done by reducing pressures from human activities such as underwater noise, contamination by dangerous substances, marine litters *etc*., Apart from this, care should also be taken to the sustainable usage of marine resources for the well being of marine environment.

There is little time left to reach our goal of healthy oceans and seas. I hope this seminar is an opportunity to assess what we have achieved so far and rekindle our efforts and commit to combine them to save our marine resources. The high quality of papers and discussions represent the thinking and experience of men and women with reference to marine resources. This proceeding will furnish to scientific groups world over an excellent reference book. I also believe that this will be an impetus to stimulate further study and research.

Hence I wish the seminar a great success.

REGISTRAR

ANNAMALAI UNIVERSITY
Centre of Advanced Study in Marine Biology
Faculty of Marine Sciences
Parangipettai - 608 502
Tamil Nadu, India

Phone : +91 4144 243 223 Extn. 210 (work)
: +91 4144 238 419 (Home)
Fax : +91 4144 243 555 (Office)
Mobile : +91 9442068003
E-mail :kathiresan57@gmail.com
: kathirsun@rediffmail.com

Prof. Dr. K. KATHIRESAN M.Sc., Ph.D., D.Sc., F.N.I.E.
Dean, Director & Member Syndicate
Tamil Nadu Scientist Awardee
International "NAGA-2001" Awardee

FOREWORD

I am delighted to know that a National Seminar on Marine Resources is organized in the prestigious M.D.T. Hindu College, Tirunelveli, during March 2016.

India is gifted with a great wealth of non-living and living resources. The country has a coastline of about 8,000 km that span 13 maritime States and Union Territories, surrounded by the Indian Ocean, Arabian Sea and the Bay of Bengal. The country has an Exclusive Economic Zone of 2.02 million km^2 adjoining the continental regions, offshore islands and a variety of coastal ecosystems such as estuaries, lagoons, mangroves, backwaters, salt marshes, rocky coasts, and coral reefs.

The country is biological diverse with record of exceeding 20,000 species due to the habitat diversity. The country has most spectacular natural treasures; to cite a few, Inter-tidal mudflats teeming with migratory birds in winter, dense mangrove forests inhabited by the endangered tiger, seagrass meadows favoured by the seacow (dugong), most beautiful corals colonized with ornamental fishes, sandy coast with the world's largest nesting site of sea turtles, and rough sea migrated with the largest whale shark fish.

The coastal and marine ecosystems are extremely important ecological and economic resources, as they provide a wide range of ecosystem goods and services to the entire country. Nearly 250 million people live within a distance of 50 km from the coast and a large proportion of them are in urban centers such as Mumbai, Chennai and Kolkata.

The increased population pressure has led to resource depletion, and environmental degradation due to pollution and disposal of agricultural, domestic and industrial wastes. Additionally, climate change associated with global warming, sea level rise and intensified cyclones is expected to have a growing impact on coastal and marine ecosystems. There is a pressuring need for conservation of diversity in marine resources.

My hearty appreciations are due to Dr. V. Saravanan and S. Elango Subramanian for all their efforts in organising the seminar.

I wish the seminar a grand success.

K. Kathiresan

PREFACE

Ocean covers nearly 71% of earth and hence earth is called “water planet”. It is endowed with various ecosystem ranging from terrestrial to oceanic ecosystem including estuaries, mangroves mudflats, sandy, rocky, and muddy shores *etc.*, The marine environment is so peculiar than the terrestrial environment because of several physical and chemical properties the includes waves, tides, dissolved salts and gases and minerals.

India is bestowed with an intensive coastline of over 8129 km., 0.5million sq km of continental shelf and 2.02million sq. km of exclusive economic zone [EEZ], with an estimated annual marine fishery potential of 3.9 million tons. The Indian marine fisheries sector plays a very vital role in supplying protein rich food to the exploding population and also in employment generation and foreign exchange earnings.

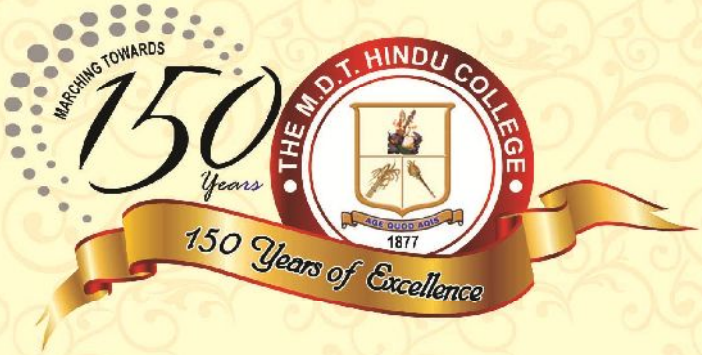
The ocean that covers most of the surface of the earth is the planet’s largest factory of organic matter. The ocean is one of the earth’s most valuable natural resources. It provides food in the form of fish, shell fish and plants about 200 million pounds each year. The ocean is an increasingly important source of biomedical organisms with enormous potential for fighting diseases. Three products agar, algin and carrageenin have been extracted from seaweeds and are used in a variety of ways. Physical marine natural resources include products from the ocean as well as the ocean itself. Sand, gravel, salt, minerals, oil and gas are physical products taken from the ocean. Ocean water itself is a natural resource and can be used to make fresh water as a coolant or for the production of energy. It is used for transportation. The ocean plays a vital role in removing carbon from the atmosphere and providing oxygen. Both ocean and atmosphere work together to form complex weather phenomena like the north Atlantic Oscillation and El Nino.

The continuous exploitation of marine resources and habitat increases because of human population explosion and its level of socio-economic development. Exploring the marine resources at a greater level is important to understand why we must keep the ocean healthy for future generations. We must work hard with an objective to attain sustainable use of marine resources while preventing stock declines.

A population of environmental perturbation and anthropogenic pressures are contributing to challenging changes in the sustainable and profitable use of marine ecosystem services and the resources of the bountiful sea are in danger of collapse. There is an urgency to broaden and promote stakeholders’ concerns, aspiration, knowledge and participation realizing imperativeness of preserving this biodiversity treasure for posterity and prosperity. Based on the above said facts this National seminar on Marine Resources (NSMR’16) may be an eye opener for the younger generation of our Nation.

S. Elango Subramanian — Associate Editor

Dr. V. Saravanan — Editor & Organising Secretary



ISBN 978 - 81 - 931973 - 9 - 4

Keynote Address

MARINE RESOURCES – SIGNIFICANCE AND CHALLENGES

A.JOHN DE BRITTO

Registrar, Manonmaniam Sundaranar University, Tirunelveli – 627 012.

Marine Resources of the world:1

The oceans cover 70% of the planet's surface area, and marine and coastal environments contain diverse habitats that support an abundance of marine life. Marine fish and invertebrates are among the rich sources of wild food on the planet, providing over 2.6 billion people with at least 20% of their average per capita protein intake. Moreover, the world's oceans host 32 of the 34 known phyla on Earth and contain somewhere between 500,000 and 10 million marine species.

Marine Biodiversity of India:

India's coastline extends to around 8,000 km and an Exclusive Economic Zone (EEZ) of 2.02 million sq km adjoining the continental regions and offshore islands. The wide range of important ecosystems characterizing the coast includes estuaries, lagoons, mangroves, backwaters, salt marshes, rocky coasts, sandy stretches, and coral reefs.
India's total mangrove cover is approximately 700,000 hectares. There are 59 mangrove species belonging to 41 genera and 29 families present along the entire coast. The Indian Ocean region harbours some of the most diverse and extensive reefs, many of which are among the least scientifically known. The total area of coral reefs in India is estimated to be 2,374.9 sq km.

Marine mammals include members of five different groups categorized under cetaceans (whales, dolphins, porpoises) and sirenians (manatees and dugongs). Of the 78 cetaceans and five sirenian species identified globally, 29 and one, respectively, exist in India.

Marine reptiles found in coastal and marine areas of India include sea turtles, crocodiles and sea snakes. Of the eight species of sea turtle, five are found along the Indian coastline. Twenty-five species of sea snakes, belonging to three families and five sub-families, have been documented in Indian waters.

Birds both feed and breed near the sea. There are two main groups of birds along the Indian coast -- sea birds and water birds. About 177 bird species are found in the mangrove forests of India. Kingfishers, herons, storks, sea eagles, kites, etc, are the dominant species observed in these systems.

A total of 2,546 fish species belonging to 969 genera, 254 families and 40 orders have been recorded so far. Indian fish populations represent 11.72% of the world's species, 23.96% of genera, 57% of families, and 80% of orders.

Significance of Marine Resources:

Food

The seas and oceans contain vast natural resources that are increasingly available to humans as technology and scientific understanding improve. Fish have historically played

highly significant roles in satisfying the protein requirements of large fractions of humanity since the earliest periods of recorded history.

Hydrocarbons

Natural oil and gas found in rocks beneath the seabed give us the fuel we need for cooking and heating in our homes, for power stations, motor vehicles and airplanes. Oil is also used to make all sorts of plastic products from bottles to mobile telephones, and for chemicals used in factories and farming. In short, our modern-day society relies heavily on a steady supply of oil and gas, generally known as hydrocarbons or fossil fuels.

Methane hydrates

Gas hydrates are naturally occurring ice-like crystals that form at high pressure and low temperature in marine sediments. They occur at water depths greater than 300 meters, wherever there is sufficient methane and water in the sediments.

Biofuels from marine algae

One promising source of biofuels has been identified as marine algae which could be harvested and turned into a carbon natural fuel source.

Marine Renewable Energy

The burning of fossil fuels such as coal, oil and natural gas produces a large amount of the carbon released into the atmosphere by man. The seas have the potential to provide a large amount of renewable energy from three main sources:

Tidal energy

The large tidal resources around the coast can be used to make electricity.

Wind energy

The movement of air across the Earth's source is a large source of kinetic energy, which has been harnessed by windmills for many centuries and nowadays by modern wind turbines. Offshore wind turbines can generate up to 25% more energy than their onshore counterparts, since the wind blows faster over the sea than over land.

Wave energy

The large waves that are found off the coast can be used to generate electricity.

Minerals and products

The non-living resources of the deep ocean floor are increasingly attractive for the mineral industry. The major mineral resource potential is held in iron-manganese nodules, cobalt-rich polymetallic crust, and polymetallic sulphides. Four elemental metals are the main components of value in manganese nodules and cobalt crust: manganese, copper, nickel and cobalt.

Challenges of conservation of Marine Resources:

Marine and coastal ecosystems are some of the most dynamic and complex systems. Furthermore, they encompass multiple-use land, adding to their complexity.

Coastal ecosystems face several conservation and management challenges:

***Habitat destruction*:**

The driving force behind coastal degradation has been large development and infrastructure projects along the coast as well as unplanned and unregulated growth in coastal areas. Ecosystems and critical habitats that are constantly being challenged are mangrove

forests, estuaries, mud-flats, coral reefs, small island ecosystems, coastal headlands and cliffs, coastal wetlands, sand dunes, etc.

***Ineffective fisheries management*:**

Large-scale mechanization in the fisheries sector, introduced nearly 50 years ago, has had a huge impact on fish resources.

***Over-exploitation of bio-resources*:**

Living bio-resources found in the coastal zone are heavily exploited, and often the exploitation is unsustainable. This includes banned species such as sea cucumbers, molluscs and sea horses.

Pollution:

The coastal zone receives waste generated by a number of point and non-point sources, especially sewage, industrial effluents, sediment, and agricultural chemicals, notably fertilizers and pesticides. These contribute to the degradation of the quality of coastal waters.

Weak implementation of laws:

Over 25 amendments were made to the Coastal Regulation Zone (CRZ) notification, most of which have considerably undermined its efficacy resulting in threats to coastal biodiversity and habitats.

***Knowledge and awareness*:**

There are huge gaps in our knowledge and understanding of many aspects of marine and coastal biodiversity such as sea grasses, corals, impacts of climate change, etc. There are also gaps in documentation of the anthropological, socio–economic, indigenous knowledge and practices of coastal communities. Moreover, there is no single stakeholder or platform that provides coordination and knowledge-networking.

The way ahead

Several urgent requirements that stem from the above-mentioned issues need to be met to address the challenges of marine and coastal management.

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The information contained in this article has been summarized from the National Capacity Self-Assessment Thematic Assessment Report on Biodiversity Final Report, 2007, prepared by ATREE, UNDP and MoEF).

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INVITED LECTURES

ASSESSMENT OF SEAGRASS BIODIVERSITY ALONG THE COAST OF PALK STRAIT

R. Gokulakrishnan, *S. Ravikumar and S. Prasannakumar

School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa university, Thondi Campus 623 409, Ramnad District, Tamil Nadu, E.mail: ravibiotech201320@yahoo.com ; Mobile: 9003306959.

*** Corresponding author**

1. Introduction

Ecology is often referred as the study of distribution and abundance of an organisms or group of organisms. Field survey is essential to identify the habitats present in a study area and to locate representative areas of each feature. Seagrass are difficult to see or identify in turbid water. The survey data is used to ensure that all restoration and mitigation requirements are required. Generally, the survey provides the details about the percent cover, shoot counts, height of the leaf and species composition etc. Of these, shoot density is a key parameter which is responsible for the meadows health (Duarte, 1989). Several factors have affected the seagrass meadows including anthropogenic activity. For the seagrass recovery, several monitoring and surveying strategies have been properly assessed all over the world especially in Portugal, Spain, Australia, the Galapagos Islands, Costa Rica, Puerto Rico and the United States for the restoration and mitigation of seagrass loss.

1.1. What are seagrass?

Seagrass are marine flowering plants that are capable of completing their life cycles even when covered by saline water. Although they superficially resemble true grasses, they are not members of the family poaceae, but belong to related families of monocots such as hydrocharitaceae and potamogetonaceae. Seagrass often grow in meadows that resemble grasslands and undergo pollination when they are submerged in water. Like terrestrial plants, they undergo photosynthesis and as a result they bound in the inter-tidal zone, the near-shore areas and shallow waters where penetration by sunlight is high.

About 60 species of seagrass have been described and they are distributed across 13 genera. Among them, six of which are found in temperate seas (*Amphibolis, Heterozostera, Phyllospadix, Posidonia, Pseudalthenia* and *Zostera*) and the rest are tropical in nature (*Cymodocea, Enhalus, Halodule, Halophila, Syringodium, Thalassia* and *Thalassodentron*). The maximum diversity of seagrass species is in tropical and sub tropical seas.

1.2. Functions of seagrass ecosystem

Seagrass ecosystem is one of the most common and productive marine habitats which play an important role in the overall health of coastal ecosystems (Ferguson *et al.*, 1993). High standing crop produces large amounts of dissolved and particulate detritus which form the basis of important food chains both within the seagrass ecosystem and shore ward and offshore as the materials are washed away from the seagrass. The leaves and erect shoot surfaces are home for epibiotic organisms. This increases both primary and secondary productivity, as well as providing a large amount of food sources for fish and invertebrates. Because, the seagrass are rooted in their substrate and produce shoots with leaf bundles, they stabilize their habitat. The leaves form a baffle, which slows and retards current and wave

activity, which promotes sedimentation of particles as well as inhibiting re-suspension of organic and inorganic materials. Seagrass creates an active environment for nutrient cycle (Wood *et al.,* 1969). Overall, seagrass ecosystems enhance the ecological function of coastal zones by increasing the productivity and biomass of the region (Heck *et al.,* 2003).

1.3. Distribution of seagrass-Global scenario

Seagrass are found along most of the coastlines of all countries except in the waters of North Arctic circle and South Antarctic circle (Green and Short, 2003; Phillips and Durako, 2000). The distribution patterns of seagrass may change quickly due to local environmental changes. Moreover, the shifts in species composition will likely occur rapidly in response to global climate change. However, it is difficult to detect whether species composition shifts are caused by climate change impacts or by other human impacts. It has been reported that, the global seagrass coverage can presently be estimated to exceed 177,000 km^2 (Green and Short, 2003). A more exact determination of the global extent of seagrass is difficult because most seagrass meadows have not been mapped due to the cost of comprehensive mapping is high.

The distribution of seagrass has been defined into six global bioregions (Short *et al.,* 2007). The tropical Indo-Pacific is the region of the highest seagrass biodiversity in the world, with many species often found in mixed meadows that have no clear dominant species. High species diversity is also found in the tropical Atlantic bioregion, with *Thalassia testudinum* often dominating in clear waters. The three distinct temperate bioregions are: the temperate North Atlantic, the temperate North Pacific and the temperate Southern oceans, with the Mediterranean bioregion having both tropical and temperate species. The North Atlantic Ocean has low seagrass diversity, with eelgrass, *Zostera marina*, being the dominant species. The temperate North Pacific is also dominated by several *Zostera* species as well as *Phyllospadix* species in the surf zone. The Southern oceans bioregion is a circum global area including the temperate coastlines of Australia, Africa and South America, where extensive meadows of low to high diversity temperate seagrass species are found. The clear waters of the Mediterranean Sea are dominated by *Posidonia oceanica* growing in vast meadows, but this bioregion also supports other temperate and several tropical seagrass.

In both the Northern and Southern hemisphere, the global distribution of seagrass is remarkably consistent, with both hemispheres containing 10 genera and only one unique genus in each hemisphere. However, some genera have more species than others, as evident in the multispecies genus *Halophila*. There are about the same number of species in tropical and temperate bioregions. The most widely distributed seagrass is *Ruppia maritima*, which occurs in both tropical and temperate bioregions and in waters from fresh to hypersaline. Seagrass bioregions at the scale of ocean basins are identified based on species distributions which are supported by genetic patterns of diversity. Seagrass bioregions provide a useful framework for interpreting ecological, physiological and genetic results collected in specific locations or from particular species.

Seagrass globally have five centers of high diversity all of which occur in the Eastern hemisphere and four of which occur in the Tropical Indo Pacific bioregion; the fifth, South-Western Australia, occurs in the adjacent Temperate Southern Oceans bioregion. The first and largest of these, with by far the greatest number of seagrass species lies over insular South-East Asia and extends across North tropical Australia, including the Great Barrier Reef; all but two of the species, *Z. muelleri* and *Z. japonica*, contributing to this regions have high diversity are tropical seagrass.

A second, much smaller center of diversity is found in South-Eastern India, represented by 13 all tropical species. The third center having high diversity globally, located in Eastern Africa, Southern Japan and South-Western Australia, obtains this designation by being located at or near a bioregional interface, encompassing both tropical and temperate seagrass species. East Africa, with 12 species, has only one temperate species, *Z. capensis*, contributing to its mix of mostly tropical species. Southern Japan also has 12 species, with *Z. japonica* the one temperate species that contributes to the diversity of this tropical region. In the temperate Southern Oceans bioregion, South-Western Australia with 13 species has 4 tropical species contributing to its high diversity. Looking at diversity patterns in more detail and also at the individual species ranges that underpin them (Green and Short, 2003), seagrass bioregions are discussed from greatest to least seagrass diversity.

Seagrass continue to be the dominant biological community in Florida Bay approximately 97% (Durako *et al.,* 2001). In North-Eastern Florida Bay, *Thalassia* was present at 75.9% and *Halodule* was present at 69%. The entire South Florida coastal zone, including the areas of West Florida Bay and within the Florida Keys National Marine Sanctuary, is dominated by seagrass habitats. Fourqurean *et al.* (2001) assessed seagrass species composition and density at 1207 sites distributed across 19,402 km^2 of near shore marine and estuarine environments in South Florida. *Thalassia testudinum* (Turtle Grass) was the most commonly encountered species, being found at 898 sites. *Halodule wrightii* (Shoal Grass) was the second most commonly encountered species, occurring at 459 sites; followed by *Syringodium filiforme* (Manatee Grass, 239 sites), *Halophila decipiens* (Paddle Grass, 96 sites), *Ruppia maritime* (Widgeon Grass, 41 sites) and *Halophila engelmannii* (Star Grass, 28 sites) were recorded around the Florida bay.

1.4. Distribution of seagrass- Indian scenario

India has several seagrass floral diversity which consists of 15 species belonging to 7 genera and accounts for 30.61% of the total seagrass reported in the world. In India, seagrass habitats are mainly limited to mudflats and sandy regions in the lower intertidal zone at a depth between 10–15 m along the open shores and in the lagoons around islands (Jagtap, 1991; Ramamurthy *et al.,* 1992). The major seagrass meadows in India occur along the SouthEast coast particularly Gulf of Mannar, Palk Bay and Andaman and Nicobar (Bay of Bengal) and the islands of Lakshadweep (Arabian Sea). It has been reported that, the Gulf of Mannar and Palk Bay, the maximum extent of around 3000 ha of seagrass (Jagtap and Inamdar, 1991). In Lakshadweep islands, there are 112 ha of seagrass has been recorded. However, a total of 830 ha of seagrass have been covered in Andaman and Nicobar islands (Jagtap, 1992; Das, 1996). The seagrass formations have been reported to be either in long or broken stretches or small to large patches (Jagtap and Inamdar, 1991; Das, 1996; Jagtap, 1996).

The seagrass cover varies from different regions which can be generally identified by using aerial photographs, ground survey and naval hydrographic charts. However, standing crop was calculated by using biomass data with the help of either line transect or quadrat methods. The maximum seagrass cover, abundance and species richness are generally found in the sandy regions along the seashores and in the lagoons of islands, where salinity of overlying water remains 33 PSU throughout the year.

1.5. Economic importance of seagrass

Seagrass meadows are the nursery grounds for many commercial fishes and crustacean species. The juveniles come into the seagrass meadows for protection against

predators, to feed on the epiphytes growing on seagrass plants and to feed on the organic detrital rain that falls into the meadow from the water above. The juvenile tiger prawns (*Penaeus esculentus* and *P. semiscatus*) and endeavour prawns (*Metapenaeus ensis* and *M. endeavouri*) have seagrass meadows as their nursery grounds in Gulf of Carpentaria, the East coast of Queensland (Staples *et al.,* 1985; Poiner *et al.,* 1987; Coles *et al.,* 1993). Post-larvae of both tiger and endeavour prawns settle from the water column into the shallow inshore seagrass meadows and they move into the deeper meadows after getting the matured growth. The juveniles of the Western rock lobster forage in seagrass meadows close to the reefs in which they shelter (Joll and Phillips, 1984). This meadow is helpful to improve the diversity of most of the organisms which are economically important. Seagrass meadows reduce the speed and change the pattern of currents. This process results in depositional environments (Fonseca, 1986).

Few larger animals possess the ability to actually digest seagrass leaves (dugong, turtle, geese, brants and some herbivorous fish). Seagrass leaves often harbour a multitude of organisms such as algae and invertebrates, which serve as food for transient fish, as well as the permanent fauna within the seagrass meadow. Moreover, adult fish migrate from adjacent habitats, like coral reefs and mangrove areas, to the seagrass meadows at night to feed on the rich food sources within the seagrass meadows. Many small subsistence fishing practices, such as those practiced in Zanzibar (Tanzania), are totally depend on seagrass meadows for their fishing grounds. The coastal populations in such areas receive most of their protein from fishing within seagrass meadows (Torre-Castro and Ronnback, 2004).

The leaf canopy and the network of rhizome and root fix and stabilize the sediment over which seagrass grow, and reduce the re-suspension of the sediment by currents and waves. This role is driven by reduced water motion due to canopy friction and by the structural frame that rhizomes and roots provide to the sediments. Sediments vegetated by seagrass are less likely to be mobilized by waves and currents, so that seagrass can reduce the erosion of the coastline. Detached seagrass leaves, which are lost either at the end of their life or earlier due to waves and storms, and their accumulation in the beaches, represent another way by which seagrass has a role in the protection of the shoreline. Large accumulation of leaves, such as those of *Posidonia oceanica* in the Mediterranean and Eelgrass in Northern Europe, dissipate wave energy and directly protect beach sediments from the impact of waves. Seagrass are important elements of coastal protection through the sediments being eroded. In the Mediterranean, the particles that constitute the sediment have in many cases a biological origin being fragments of the skeletons, shells or spines of marine animals or being the calcareous remains of benthic algae. As seagrass harbor a large diversity of marine organisms, the meadows can be considered a net source of new sediment. Biogenic particles can be the main component of sediment in coastlines with no rivers or with low fluxes of particulate matter from land to the sea. In such areas sediment produced by seagrass meadows may contribute significantly to feed the beaches, further contributing to curb coastal erosion (Koch *et al.,* 2001).

Seagrass play a very important role as basic land builders and shore stabilizers, similar to that of sand dune and mangrove vegetation. Seagrass, although of limited direct economic profit, have been used for various purposes in different parts of the world (Fortes, 1990). Coastal people use rhizomes of *Cymodocea* sp. (nicknamed as sea sugarcane) as food, for the preparation of salad. Seagrass are also used as raw materials in paper industry and in the production of fertilizer, fodder and feed. Most of the seagrass are used extensively as soil fertilizer for coconut and other plantations. A variety of medicines and chemicals are also prepared from them. Agar like substance and zosterin is extracted from *Zostera* sp.

1.6. Traditional uses of seagrass

Traditionally, seagrass have many uses (Terrados *et al.,* 2004). Seagrass are used to prepare the baskets, extracted for soda salt and used as minor fuel, stuffing, insulating and packing material, fertilizers, etc. In addition to that, they have been used as a sewage filters, coastal stabilizers, paper manufacture, fodder and compost. Further, it can be utilized for roof covering and after removing the excess salts in the leaf, it can be used as house insulation. Moreover, in the Mediterranean and in Africa, it is also used as a traditional medicine against skin diseases (Torre-Castro and Ronnback, 2004). Seagrass seeds of several species are used as a food source. Seagrass leaves from several species *viz., Z. marina, T. ciliatum, E. acoroides, P. oceanica, P. iwatensis* and *P. torreyi* are gathered from the wrack line or cut above the surface of the sediment, dried and used for thatch, animal bedding, mattress and pillow stuffing and cordage (Coles *et al.,* 2003). The seeds (raw) and rhizomes (ground into flour) of *E. acoroides* are also consumed (Bandeira and Gell, 2003; Ochieng and Erftemeijer, 2003) as food. *H. ovata* leaves are principal ingredient in a paste used to treat various skin ailments.

Evidence suggests that, the particular seagrass species had value even before the development of market based economies. In the Channel Islands of the coast of California in the United States (34° N; 120° W), the coastal chumash began to fashion cordage, thatch and footwear from the leaves of *Phyllospadix torreyi* (Salls, 1988; Connolly *et al.,* 1995). Coastal people used the thin and silicate strengthened leaves of this plant as raw material to weave fishing line and thatch shelters for thousands of years. While it is more difficult to demonstrate the intrinsic value of these wild plants in agricultural and industrial societies. There is tangible evidence that the splendour and function of seagrass species enhances both poetry and art within these cultures (Standing *et al.,* 1975; Whitt, 1988).

1.7. Threats to Seagrass

The abundance of seagrass is being destroyed due to the several activities particularly anthropogenic activity. Direct human impacts to seagrass which includes; fishing and aquaculture, introduced exotic species, boating and anchoring, and habitat alteration *viz.,* dredging, reclamation and coastal construction, etc. Fishing methods such as dredging and trawling may significantly affect seagrass by direct removal. Damage to *Zostera marina* by scallop dredging reduces shoot density and plant biomass and digging for clams can also exert extensive damage. Many of these impacts remain un-quantified as yet and their long term effects are poorly known. Most of the coastal waters are now being used in economic activities such as eco-tourism, fish caging and docking area and recreation areas. The multiple uses of these water bodies greatly receive the high pressure from the human population. Resulting impact ranges from siltation, oil spills and pollution causing fish killing and other environmental damage.

The exploitation of marine resources and the use of certain types of fishing gear like bottom trawls have detrimental effects on seagrass beds. Mussel harvest in the Dutch Wadden sea is believed to be a major factor in the loss of *Z. marina* and *Z. noltii*. Moreover, the use of dynamite poison (Cyanide) which contribute to the rapid destruction of the seagrass habitat. The impact of dynamite on the reproductive capacity of fish will surely lead to a decline in fish population in a certain habitat. Likewise, the residual effects of cyanide are irreversible or it may take several years to recover the seagrass ecosystem. In addition to that, the large scale loss of seagrass that occurred on both sides of the North Atlantic Ocean in the early 1930s, a result of "eelgrass wasting disease" had many effects on the ecosystem (Rasmussen,

1977). Associated with this loss were a collapse of scallop fisheries and dramatic reductions in waterfowl populations. In addition, it resulted extinction of a marine gastropod (Carlton *et al.,* 1991).

Direct boat propeller damage to seagrass communities has been recorded, particularly in the Florida Keys. Boat anchoring is one of the major problems which thrives the scars in the seagrass beds especially, *Posidonia oceanica* landscapes. Return of large temperate meadow forming seagrass to mooring scars may take decades. Boating may also be associated with organic inputs in areas where boats do not have holding tanks. Dredging and reclamation of marine environments, either for extraction of sediments or as part of coastal engineering or construction, can remove seagrass. Filling of shallow coastal areas, known as reclamation, can directly eliminate seagrass habitat and results in hardening of the shoreline, which further eliminates productive seagrass habitat, as seen throughout Tokyo Bay. Groynes alter sediment transport in the nearshore zone.

Dredging removes seagrass habitat as well as the underlying sediment, leaving bare sand at greater depth, resulting in changes to the biological, chemical and physical habitat values that seagrass support. Beach replenishment may have impact on adjacent seagrass by delivering sediment that may shade or bury the seagrass. Beach nourishment can also impact seagrass growing in areas where sediments are collected, often at depths < 30m.

Increased nutrient inputs, causing eutrophication is a major component to seagrass loss. Increased siltation of coastal waters is also a major human impact on seagrass ecosystems, which derives from changes in land use leading to increased erosion rates and silt export from watersheds. Siltation is particularly an acute problem in other regions of the world, such as South East Asian coastal waters, which receive the highest sediment delivery in the world as a result of high soil erosion rates derived from extensive deforestation and other changes in land use, and may be important in European waters adjacent to deforested watersheds.

Siltation severely have impact on seagrass meadows through increased light attenuation and burial which leads to seagrass loss and, where less intense siltation occurs, a decline in seagrass diversity, biomass and production. Large scale coastal engineering often alters circulation and salinity distributions, leading to seagrass loss. Hence, seagrass meadows, previously abundant in Dutch coastal areas, are now much reduced in surface, partially related to shifts of coastal waters from marine to brackish or freshwater regimes. Pollution, other than that of nutrients and organic inputs, may be an additional source of human impacts on seagrass ecosystems. Although, seagrass appears to be rather resistant to pollution by organic and heavy metal contaminants. These substances may possibly harm some components of the seagrass ecosystem, although such responses have not been examined to a significant extent. Biodiversity of seagrass meadows is greater than in adjacent un-vegetated areas and higher faunal densities are present inside the meadows. It can be used as a shelter for most of the valuable marine resource. Keeping this view in mind, the present study made an attempt to explore the biodiversity of along Palk Strait coast. The surveys are accomplished using a variety of monitoring tools including quadrats, line transects, video transect and photo interpretation. Among them, quadrat method is widely used which is economically feasible. This method is appropriate for estimating the abundance of plants and other organisms and this approach allows estimation of absolute density.

Seagrass biomass is the prime factor influencing the organization of marine macro-faunal communities (Coles, 1986). They also control the habitat complexity, species diversity and abundance of associated invertebrates and shaping the structure of marine communities.

Seagrass contribute significantly to the productivity of coastal areas of both temperate and tropical waters (Phillips and McRoy, 1980). Several authors assessed the live seagrass distribution in Gulf of mannar (Rajeshwari and Kamala, 1987; Jagtap, 1996), Andaman and Nicobar (Jagtap, 1991) and such studies are still lacking in the Palk Strait region. Hence, the present study made an attempt to survey the live and dead seagrass biomass along Palk Strait coast of South India.

2.0 Materials and Methods

2.1. Description of the survey area

The Palk Strait is situated in the Southern part of the peninsular India. The study area *viz.,* lies in the Palk Strait region. The rainfall in Palk Strait region is mainly due to the North East and South West monsoon. The present study has been surveyed along the coastline of 168Kms long which crossed five districts namely Nagapattinam, Thiruvarur, Thanjavur, Pudukottai and Ramanathapuram respectively.

2.3. Survey of offshore seagrass biomass

Monthly samplings were carried out from eight different stations *viz.,* Kodiyakarai (Lat. 10° 16' N; Long. 79° 49' E), Mallipattinam (Lat.10°16'N; Long.79°19'E), Manora (Lat.10°15'N; Long.79°18'E), Manamelkudi (Lat.10°02'N; Long.79°15'E), Kottaipattinam (Lat.09°59'N; Long.79°13'E), Mimisal (Lat.09°54'N; Long.79°08'E), S.P.Pattinam (Lat.09°51'N; Long.79°05'E) and Thondi (Lat.09°44'N; Long.79°01'E) along Palk Strait from September-2011 to August-2012 for the assessment of seagrass biomass. For the estimation, the seagrass were collected during the lowtide from the intertidal and subtidal regions. About 1M×1M size quadrat were placed around ten different places randomly at each site. The quadrats were divided into 16 squares and each square consists of 0.25 m^2. The seagrass which found inside the single square were collected by hand and the substratum should be excavated well so as to enable to collect all the underground parts. Then, they were washed thoroughly with the seawater for the removal of debris and stored in the previously unused polythene bags. After that, the collected seagrass were treated with 10% v/v orthophosphoric acid to remove the epiphytes and calcareous substances. Further, they were washed 5-10 times with tap water for the removal of acid and the moisture content was removed using the blotting papers.

Finally, the samples were oven dried at 60°C for 48 hrs. After 48 hrs, the samples were weighed for the estimation of total biomass. In addition that, the above ground biomass, below ground biomass, shoot density, canopy height, above and below ratio and the percentage of seagrass were also calculated.

(a) Above ground biomass :

Leaves, shoot and reproductive parts were considered as the above ground biomass.

(b) Below ground biomass :

The rhizome and roots were considered as the below ground biomass.

(c) Total biomass : The mean of ten quadrat samples were considered for the seagrass biomass per square meter.

(d) Shoot density : Shoot density was determined by counting all the shoots with in the square and the unit was expressed as shoots/m^2.

(e) Canopy height : Canopy height can also determined as, measure the longest leaves of the necessary number of shoots using the ruler and the unit was expressed as 'cm'.

(f) Above and below ratio : $\frac{\text{Above ground biomass}}{\text{Below ground biomass}}$

(g) Percentage of seagrass : Biomass of individual species can also be calculated by using the following formula.

$$\text{Percentage biomass of individual species} = \left\{ \frac{\text{Weight of the species individual}}{\text{Total weight in the quadrat}} \times 100 \right.$$

2.4. Survey of onshore seagrass biomass deposition

The distribution of seagrass accumulation was also estimated along the Palk Strait. The quadrat (1M×1M) was thrown 5 times randomly within each 50 meters interval. Then the samples were collected from the single square and carefully washed with seawater and transported to the laboratory. After that, the collected samples were washed with tap water and separated based on the morphology. Finally, the samples were dried at room temperature to 72 hrs for the complete removal of water. The amount of seagrass accumulation was calculated with standard deviation and expressed as kg.dryweight/m^2.

Seagrass deposition : The mean of five quadrat samples were considered for the seagrass biomass per square meter.

3.0. Results

The above ground biomass results suggested that, the four seagrass species, *Cymodocea serrulata* (166.4 ± 36.72 g.dry wt/m^2), *Syringodium isoetifolium* (84.8 ± 22.46 g.dry wt/m^2), *Halodule pinifolia* (68.8 ± 13.93 g.dry wt/m^2) and *Halophila ovalis* (62.4 ± 17.45 g.dry wt/m^2) were exhibited maximum biomass at Mimisal, S.P. Pattinam, Manora and Manamelkudi during the month of November-2011. However, the *Cymodocea serrulata* (16.0±16.0 g.dry wt/m^2), *Syringodium isoetifolium* (16±14.6 g.dry wt/m^2), *Halodule pinifolia* (11.2±2.8 g.dry wt/m^2) were recorded minimum biomass during the month of May-2012 at Manora, Mimisal and Kottaipattinam. But, the minimum biomass of *Halophila ovalis* (11.2±9.9 g.dry wt/m^2) was observed at Manamelkudi during the month of April and May-2012.

The maximum (406.4 ± 114.5 g.dry wt/m^2) below ground biomass of *Cymodocea serrulata* was recorded at Thondi during the month of December-2011 and the minimum (33.6 ± 20.63 g.dry wt/m^2) was recorded during the month of April-2012 at Manora. The maximum below ground level biomass of *Syringodium isoetifolium* was recorded (156.8 ± 37.37 g.dry wt/m^2) at Thondi during the month of December-2011 and the minimum (24 ± 21.81 g.dry wt/m^2) was recorded at Mimisal during the month of May-2012. The below ground biomass of *Halodule pinifolia* suggested that, the maximum level (80 ± 22.5 g.dry wt/m^2) was recorded during the month of November-2011 and December-2011 at Manamelkudi and the minimum value (11.2 ± 16.2 g.dry wt/m^2) was recorded at Kottaipattinam during the month of April-2012. The *Halophila ovalis* showed maximum (80

± 22.9 g.dry wt/m^2) and minimum (14.4 ± 22.9 g.dry wt/m^2) below ground biomass level in Manamelkudi during the month of December-2011 and May-2012 respectively.

The present study also made an attempt to identify the total biomass of the various seagrass species at selected coastal areas. Of the selected four seagrass species, the maximum biomass were recorded with *Cymodocea serrulata* (571.2 ± 78.94 g.dry wt/m^2), *Syringodium isoetifolium* (240 ± 24.92 g.dry wt/m^2), *Halodule pinifolia* (145.6 ± 57.41 g.dry wt/m^2) and *Halophila ovalis* (142.4 ± 21.83 g.dry wt/m^2) respectively during the month of December-2011 at Thondi and Manamelkudi stations. But, the minimum biomass were recorded in *Cymodocea serrulata* (52.8 ± 8.92 g.dry wt/m^2), *Syringodium isoetifolium* (49.6 ± 21.83 g.dry wt/m^2), *Halodule pinifolia* (22.4 ± 6.27 g.dry wt/m^2) and *Halophila ovalis* (25.6 ± 7.42 g.dry wt/m^2) at Manora, Mimisal, Kottaipattinam and Manamelkudi stations respectively during the month of May-2012. The minimum (22.4 ± 6.27 g.dry wt/m^2) total biomass of *Halodule pinifolia* was recorded during the month of April-2012 at Kottaipattinam.

The present study also made an attempt to identify the shoot density of the C*ymodocea serrulata*, *Syringodium isoetifolium*, *Halodule pinifolia* and *Halophila ovalis* seagrass species at different coastal areas. Of the four different species, *Cymodocea serrulata* (1068.8 ± 46.09 shoots/m^2), *Syringodium isoetifolium* (488 ± 12.09 shoots/m^2) and *Halophila ovalis* (427.2 ± 24.82 shoots/m^2) showed the maximum shoot density at Thondi and Manamelkudi during the month of December-2011. The maximum (387.2 ± 34.62 shoots/m^2) level of shoot density of *Halodule pinifolia* was recorded at Manamelkudi during the month of January-2012. But, the minimum shoot density was recorded in *Cymodocea serrulata* (57.6 ± 3.45 shoots/m^2), *Syringodium isoetifolium* (33.6 ± 1.45 shoots/m^2) and *Halophila ovalis* (56 ± 3.73 shoots/m^2) at Manora, Mimisal and Manamelkudi during the month of May-2012. The minimum level (51.2 ± 4.55 shoots/m^2) of *Halodule pinifolia* was recorded at Kottaipattinam during the month of April-2012.

The present study was also made an attempt to identify the canopy height of the seagrass species. The canopy height of the *Cymodocea serrulata* suggested that, the maximum (27.3 ± 0.89 cm) canopy height was identified at Thondi during the month of December-2011 and minimum (10.6 ± 0.82 cm) was observed during the month of March-2012 at Mallipattinam. The results of canopy height of the *Syringodium isoetifolium* suggested that, the maximum (34.7 ± 1.94 cm) canopy height was recorded at S.P.Pattinam during the month of October-2011, but the minimum (15.7 ± 0.52 cm) canopy height was recorded at Manamelkudi during the months of July and August-2012.

The canopy height of the *Halodule pinifolia* revealed that, the maximum (9.4 ± 0.61cm) and minimum (2.5 ± 0.04 cm) canopy height were recorded at Kottaipattinam during the month of December-2011 and November-2011. Similarly, the *Halophila ovalis* canopy height was found maximum by 7.1 ± 1.2 cm and minimum by 2.3 ± 0.7 cm at Manamelkudi during the months of March-2012 and September-2011.Of the four seagrass species, the maximum above and below ground biomass ratio of *Cymodocea serrulata* (0.83±0.l) was recorded at Manora in January-2012, the *Syringodium isoetifolium* (0.81 ± 0.08) at Manamelkudi during December-2011 and the *Halodule pinifolia* (1±0.1) was recorded at Kottaipattinam during April-2012 and the *Halophila ovalis* (0.91 ± 0.09) was recorded at Thondi during March-2012. The percentage occurrence of *Cymodocea serrulata* revealed that, the maximum (100%) was recorded at Mallipattinam throughout the year. But, the minimum (27.69%) was recorded at Manamelkudi during the month of September-2011.

The percentage occurrence of *Syringodium isoetifolium* revealed that, the maximum (52.76%) percentage was recorded at S.P.Pattinam during the month of September-2011. But, the minimum (15.37%) was recorded at Thondi during the month of January-2012. Similarly, the percentage occurrence of *Halodule pinifolia* was found maximum (48.94%) during the month of August-2012 at Manora. But, the minimum (9.74%) percentage occurrence was recorded during the month of February at Thondi. The percentage occurrence of *Halophila ovalis* revealed that, the maximum (21.98%) percentage was recorded at Manamelkudi during the month of October-2011 but the minimum (11.19%) percentage was recorded at Thondi during the month of January-2012.

The present study also made an attempt to identify the deposition of *Cymodocea serrulata* and *Syringodium isoetifolium* at the selected coastal areas. Of the selected different coastal areas, the maximum deposition of *Cymodocea serrulata* (10.73±0.08 Kg.drywt/m^2) and *Syringodium isoetifolium* (7.93±0.03 Kg.drywt/m^2) were recorded at Thondi coastal area during the month of May-2012. But, the minimum deposition of *Cymodocea serrulata* (0.624±0.002 Kg.drywt/m^2) and *Syringodium isoetifolium* (0.688±0.001 Kg.drywt/m^2) were recorded at Mimisal and Thondi coastal areas during the month of November-2011. In addition, none of the coastal area showed the seagrass deposition during the month of December-2011. But, the deposition of *Syringodium isoetifolium* was not recorded at Mallipattinam and Manora coastal areas throughout the year. In addition, neither seagrass standing crop nor deposition was recorded throughout the year in Kodiyakarai.

The result of the coastal landforms reveals that, the beach is short lined deposits of find and medium size sands on the shore. It covers the sea shore between the high and low water level of the tides. The sandy beaches were observed in all the study area. Sand dunes were poorly distributed in most of the site. The sand dunes were observed in Kodiyakarai and Mimisal. The spit growth was observed in Kottaipattinam, Manamelkudi and Mimisal. The spit length was varied from place to place. The beach ridges were observed in Kodiyakarai. The shallow stretch of sea water behind the barrier is called lagoon. The coastal lagoons were observed in Manamelkudi and S.P.Pattinam. Black colour sand was deposited in the shore line indicates the presence of heavy minerals. It was highly observed in Kodiyakarai, Mimisal and S.P.Pattinam. Small rivers namely, Puthu aaru and Pambaru were observed in Kodiyakarai and Mimisal respectively.

The average rainfall, wind speed and wind direction was also recorded by the present study. The maximum rainfall (433.5 mm) was recorded during the month of November-2011 and the minimum (1.2 mm) was recorded during the month of February-2012. However, no rainfall was recorded in January-2012 and June-2012 respectively. In addition to that, the maximum 12/06 (KmPH/Knots) wind speed was recorded during the month of May-2012 and the direction was Southern wind. The minimum wind speed was recorded during the month of November-2011. The speed was 01 KmPH/00 Knots and the wind direction was North East.

4.0 **Discussion**

The ocean covers more than 70% of the earth surface. The marine environment is frequently recognized as the largest potential sources of biodiversity such as seaweeds, seagrass, invertebrates and microbes which proved their potential in several fields. Among the biological sources, seagrasses are one of the rich biological resources which furnish the protection to the several marine organisms and used in many ways to human being. They also control the habitat complexity, species diversity, abundance of associated invertebrates and

thereby shaping the structure of marine communities. Moreover, they contribute significantly to the coastal productivity of both temperate and tropical waters. Several activities are responsible for the seagrass detachment from the substratum. The accumulation of beach cast is a result of the interaction between dense near shore seagrass meadows and physical factors such as; wind, tide and currents. The accumulations of beach cast create the unpleasant odour along the coastal areas and also give the anoxic condition to the marine organisms.

The present study also made an attempt to find out the total biomass of the seagrasses along the Palk Strait region throughout the year from September 2011-August 2012. The maximum biomass was recorded during the month of December-2011 at Thondi and Manamelkudi which might be due to the sedimentation pattern and environmental parameters of the study sites. Clara *et al.* (2001) reported that, the higher biomass in Puerto vargas could be attributed to the sediment structure and environmental condition. Estaction and Fortes (1988) reported that, higher biomass are due to the presence of liquid mud. However, the total biomass was not recorded in Kodiyakarai and this might be due to the coarse sand. This is agreeing with the previous results of Estaction and Fortes (1988).

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Acknowledgements

The authors gratefully thanks to the authorities of Alagappa University for providing facilities and also the UGC, New Delhi for providing financial support.

DNA BARCODING - AN INNOVATIVE TOOL TO IDENTIFY AND AUTHENTICATE MARINE FIN FISH RESOURCES

Dr. V. Ravitchandirane
Associate Professor, Department of Zoology
Tagore Arts College
Government of Puducherry, Puducherry-605008

Fin fishes are one among the 10-15 million species inhabiting the planet 'earth'. Fishes are important animal protein sources for human beings, and they are frequently used in complementary and traditional/ alternative medicine. The delimitation and identification of

fish species is not only of interest for taxonomy and systematic, but also a requirement in management of fisheries, authentication of food products, and identification of bio-active compounds. Most of us are familiar with the word barcode, a small thin and thick black stripe with numbers below that appear on the back cover page of books or commercial products, known as UPC label or Universal Product Code, used to scan them at the book shop or super market to check a price or to get a rebate. Imagine if the DNA of any organism is converted into a small thin and thick stripe and made available as DNA barcode, store it as reference library, used to match them to an unidentified DNA of organisms for species identification, to confirm authenticity and unravel adulteration.

It has long been recognized that DNA sequence diversity can be used to discriminate species, but different sequences have been used for different taxonomic groups and in different research laboratories. Recently, Hebert *et al* (2003) proposed that a single gene sequence would be sufficient to differentiate animal species, and recommended the use of the mitochondrial DNA. To develop a unifying identification system for eukaryotic animal species, an innovative universal marker has been proposed to serve as a so-called "DNA barcode". This DNA barcode is the sequence of the "Folmer fragment" (Folmer *et al,* 1994), a polymorphic part of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) as a global bio identification system for animals. It is clear that the mitochondrial genome of animals is a better target for analysis than the nuclear genome because of its lack of introns, its limited exposure to recombination and its haploid mode of inheritance: since mitochondrial DNA is maternally inherited (Saccone *et al,*1999). The 13 protein –coding genes in the animal mitochondrial genomes are better targets because indels are rare since most lead to a shift in the reading frame. There is no compelling *a priori* reason to focus analysis on a specific gene, but the cytochrome *c* oxidase I gene (COI) does have two important advantages. First, the universal primers for this gene are very robust, enabling recovery of its 5' end from representatives of most, if not all, animal phyla (Zhang and Hewitt, 1997). Second, COI appears to possess a greater range of phylogenetic signal than any other mitochondrial gene. In common with other protein coding genes, its third position-nucleotides show a high incidence of base substitutions, leading to a rate of molecular evolution that is about three times greater than that of 12S or 16S rDNA (Knowlton and Weigt, 1998).

DNA barcoding technology has proven to be a rapid and cost-effective method for precise identification of biological specimens where specialist knowledge may be unavailable (Ondrejicka *et al.,* 2014) and currently adopted by International Barcode of Life (iBoL). DNA barcoding works under the principle that inter-species variations are greater than the intra-species variations, allowing one to distinguish and authenticate the species using nucleotide sequences. About 655 nucleotide bases of the 5' cytochrome *C* oxidase subunit I gene (COI) have been accepted as a universal barcode to delineate animal life of this planet. This technique will speed up the discovery of many species yet to be identified. Thus this technology will provide a vital innovative new tool for appreciating and managing the mother earths immense and changing biodiversity. This genomic system of identification will overcome the deficits of morphological approaches to species discrimination: the bounds of intra-specific diversity will be quantifiable, sibling species will be recognizable, taxonomic decisions will be objective and all life stages will be identifiable. Also, the generation of COI profiles will provide a partial solution to the problem of the thinning ranks of morphological taxonomists by enabling a crystallization of their knowledge before they leave the field.

DNA barcoding works for all stages in the life cycle of fish and fish products, so it will help us to identify eggs, larval fish to adults, medicinally important fish species and it

can positively identify fishery products like fish fillets, incidence of retail substitution of fish species, estimate the fraud prevailing on the commercial fish market, assist in managing fisheries for long-term sustainability and improve ecosystem research and conservation.

PROSPECTS OF MOLECULAR MARKERS IN MARINE BIODIVERSITY STUDIES

M. Nagarajan*Vandana R Prabhu and R. Kamalakkannan
Department of Genomic Science, School of Biological Sciences
Central University of Kerala, Kasaragod-671314, Kerala
Email: nagarajan@cukerala.ac.in

Introduction

Oceans cover nearly a major portion of earth's surface and are very rich in terms of biodiversity. It serves as a habitat for nearly 97% of the flora and fauna. The marine biodiversity imparts wide range of services and resources to the humans and besides, play a substantial role in regulation of climate as well as nutrient recycling. Ocean's diversity is crucial as it produces plant biomass that fulfills the food requirements of allthe lives existing in the ocean ranging from simple planktons to large marine mammals, and on the land. The biodiversity of oceans are enriching, as more and more new species are being discovered and classified systematically. Selection, genetic drift, mutation etc. are the major driving forces that lead to genetic variation among individuals resulting in speciation and higher order taxonomic groups as a means of adaptation to a changing environment. Such variations are best studied using molecular genetic markers.

Molecular markers are biomolecules carrying heritable traits that can be used to study variation in organisms or population and has emerged during the last 2 decades.With the advancement in DNA marker technology, genomics research has provided enormous number of genetic markers such as randomly amplified polymorphic DNA (RAPD),restriction fragment length polymorphism (RFLP), microsatellite, single nucleotide polymorphism (SNP), amplified fragment length polymorphism (AFLP),expressed sequence tag (EST) and other gene markers.These markers play a significant role inaquaculture investigations unveiling the genetic variability among species (Ferguson 1998).This review article deals with the basic principles, requirements and advantagesof the most widely used molecular markers developed during the last two decades to assess marine diversity.

Molecular markers

Based on the evolutionary studies, molecular markers have been broadly categorized into two classes, nuclear DNA and mtDNA markers (Park and Moran 1994). Unlike mtDNAmarkers that shows maternal inheritance, nuclear DNA markers are biparently inherited and RAPDs, AFLPs, microsatellites and SNPs serve as a few examples for nuclear DNA markers. In contrast to nuclear DNA markers, mitochondrial DNA exhibits high rates of mutation and no recombining and has unique characteristics due to maternal transmissions. These key features make it extremely suitable for variability studies,phylogenetic relationships, pedigree analysis etc.

Random Amplified Polymorphic DNA (RAPD)

RAPD technique was developed in 1990 (Welsh 1990; Williams etal. 1990) using Polymerase Chain Reaction (PCR) in order to randomly amplify anonymous segments of

nuclear DNA with an identical pair of primers (8-10bp in length).It has several advantages and has been quite widely employed in marine fisheries. The simple, rapid, cheap, requirement of small amount of DNA, no need for molecular hybridization and most importantly, no prior knowledge of the genetic make-up of the organism makes it useful for variation studies (Hadrysetal. 1992).As the primers are free to bind somewhere in the sequence, no prior knowledge is required regarding the target genome which makes the method well liked to compare and analyze different DNA samples. The procedure specifically depends on large, intact DNA template sequence and therefore fails to analyze degraded DNA samples (Williams 1990). RAPD has also been employed to characterize and trace, the evolutionary backgroundof variousmarine species. It has been used to evaluate genetic diversity for species, subspecies and population/stock identification in brown trout and Atlantic salmon (Eloet al. 1997), largemouth bass (Williams et al. 1998) and Ictaluridcatfishes (Liu and Dunham 1998).

Amplified Fragment Length Polymorphism (AFLP)

AFLPs are dominant biallelic markers. Variations at many loci can be arrayed simultaneously to detect single nucleotide variations of unknown genomic regions, in which a given mutation may be frequently present in undetermined functional genes. PCR based AFLP markers had been developed in the early1990s that exploits restriction endonucleases to cleave the DNA, subsequently ligating the adaptors to the overhangs of the restriction fragments in order to work as primer binding sites for PCR amplification. Complementary primers to the adapter and restriction site sequence are recruited to amplify a subset of ligated fragments. The presence of polymorphisms is detected by the presence or absence of DNA fragments by running the samples on polyacrylamide gels (Blears et al. 1998). However, a disadvantage is that they show a dominant mode of inheritance, which reduces their significance in population genetic analyses of within-breed diversity and inbreeding. Nevertheless, AFLP profiles are highly informative in assessing the relationship between species.

Microsatellite

Microsatellites are one of themost prominent and versatile genetic markersthat have greater application in the field of population genetics, evolutionary biology and conservation biology.These co-dominant markers are highly polymorphic, and easily typed and exhibit Mendelianpattern of inheritancemaking them reliable for studying the population genetics and pedigree analysis and investigating variations among closely related species.In addition, microsatellites have a wide distribution in the genome and can be efficiently identified, which is essential in studies about genetic variability of populations. Microsatellites have become the marker of choice for application in fish population genetic studies (Beckmann and Soller 1990). They have multiple alleles, which are highly polymorphic among individuals. The polymorphism obtained with microsatellite markers has provided powerful information to be considered in the management of fish stocks,population analysis and biodiversity conservation (Alam and Islam 2005).

Microsatellites are highly abundant in various eukaryotic genomes including all aquaculture species studied to date. In most of the vertebrate genomes, microsatellites make up a few percent of the genomes in terms of the involved base pairs, depending on the compactness of the genomes.In fish, one microsatellite was found every 1.87kb of DNA, in human genome, one microsatellite was found every 6 kb of DNA.The presence of high numbers of alleles per locus, are exclusively useful in parent-offspring identification in mixed

populations, where as the microsatellites with lower numbers of alleles are well suited for population genetics and phylogenetic analysis (Atiyatet al. 2012).

16S rRNA

The 16SrRNA marker is the important toolused to study phylogenetic and evolutionary relationship among archaebacteria, bacteria and eukaryotic organisms.16S rRNA gene is about 1550 bp long and contains the marine microbial genome, which carries hypervariable region flanked by conserved regions on both sides. This repeats of conserved and hypervariable structure in the 16 sRNA is exploited by designing primers targeting the conserved region, which would lead to amplification of the variable region.GenBankcontains over 20 million deposited sequences in whichmore than 90,000 consistof 16S rRNA gene. The 16S and 23S rRNA mainly contributed to the backbone of bacterial taxonomy, especially for identification of non-culturable bacteria (Clarridge 2004). The variable region are species specific, hence, determination of the variable sequence could lead to identification of the host. The major advantage of this approach includes the reduction in manual labor and cost. It has enabled researchers to study unculturable microbial communities. In the marine environment, 16s rRNA has been widely used for the studies of cyanobacteria (Niclas et al. 2010), marine sponges (Janine et al. 2010), marine sediments (Aravindrajaet al. 2013).

Single Nucleotide Polymorphism

Single Nucleotide Polymorphism (SNP) is defined as polymorphism caused by point mutations, which give rise to different alleles for a particular nucleotide position in a locus. They are more popularamong molecular markersas they presentabundantly in any organism's genome. Several approaches have been used for SNP detection such SSCP analysis, DNA sequencing, SNP array etc., However DNA sequencing has been widely used for SNP detection. SNP arrays have been used to study the population genetic structure of salmon (Bourret et al. 2013).The population structure and history of the coral *Acroporadigitifera* was analyzed using genome-wide SNP analyses (Shinzato et al. 2015).

Mitochondrial DNA markers (mtDNA)

The Mitochondrion is one of the major components of eukaryotic cell. It is small, circular and double-stranded molecule, which comprises 38 genes in vertebrate species. A direct maternal ancestor can be traced using mtDNA as its transmission is uniparental, mother to child. The transmission occurs with very little recombination or mutation. Apart from its application in phylogenetic studies they play a key role in cellular respiration in all cell types. Mitochondrial DNA is prominent and widely used tool for studying population genetic structure and domestication history of species.The mtDNA marker is very sensitive marker used todiscern and analyze population structure of marine species (Norman 1994). As they show maternal inheritance they are widely and exclusively used for tracing maternal lineages and havebeen in use over the last 25 years in population geneticsas well as evolutionary biology.

Cytochrome oxidase c subunit 1 (CO1)

Cytochrome c oxidase subunit I is a mitochondrial DNA (mtDNA) encoded subunit (MT-CO1, MT-CO2, MT-CO3) of respiratory complex IV and is the third and final enzyme of the electron transport chain of mitochondrial oxidative phosphorylation.The mitochondrial cytochrome oxidase c subunit 1 (COI) gene is a remarkable marker used for population genetics and phylogeographic analysis. DNA barcoding targeting cytochrome oxidase I

(COI) gene is an efficient, less time consuming and inexpensive technique to systematize the diversity of biological systems (Novotny 2002).

Cytochrome *b* gene

The control region of the mitochondrial genome is a non coding segment and is highly variable in many vertebrate species. Due to its exceptional variability, the control region has often been used to measure the population divergence, gene flow,effective population size, and population expansion in natural populations. While nuclear ribosomal rRNA gene and complete mitochondrial DNA (mtDNA) are used to estimate the evolutionary relationships involving ancient divergences. Majority of research on marine species rely on cytochrome *b* (1,143 bp) gene to know the phylogenetic relationships of closely related taxa but has poor resolution at deeper nodes. The speciation pattern and evolution of traits in the ocean species especially baleen whale were investigated using sequences of the mitochondrial cytochrome *b* gene and its control region (Gilloolyet al. 2005).

D-Loop

Mitochondrial DNA consists of a non-coding control region called D-loop due to its role in replication and transcription of mtDNA. Mutation rate of mtDNA control region is five to ten folds higher than that of single copy nuclear genes, which makes this region extremely suitable for variability studies. The region is even very useful in identifying variation among individuals of a species. Analysis of D-loop region has been proven to be successful in unraveling the genetic diversity of marine species and its conservation (Cecconiet al. 1995).

Application of Molecular Markers

There are many essential applications of molecular marker in marine biodiversity studies.

1. Species identification.
2. Genetic stock assessment.
3. Assessment of genetic variation and population structure in natural populations.
4. Assessment of migration pattern in natural population.
5. Marker assisted selective breeding program.
6. Conservation of endangered Species.
7. Molecular phylogeny and systematic study
8. Genetic quarantine.

Conclusion

More than two third of earth is covered by ocean and assessment of marine biodiversity is a difficult task. However, there is a tendency to exploit marine resources for food, energy and other purposes due to the increase in global population. Therefore, there is a prerequisite for the assessment of marine biodiversity and prioritizing conservation strategies. Molecular markers can play a vital role in assessment and conservation of biodiversity in the diverse marine ecosystem.

Acknowledgements

The authors acknowledge the Science and Engineering Research Board (SERB), Department of Science and technology, Government of India, New Delhi for the financial Assistance (No.SR/FT/LS-40/2010) to the corresponding author.

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SEAWEEDS AND THEIR USES

P. ANANTHARAMAN and C. PERIYASAMY
CAS in Marine Biology, Faculty of Marine Sciences,
Annamalai University, Parangipettai, Tamil Nadu, India
Email: paraman_cas@yahoo.co.in, periyasamy.c@live.com

Introduction

Plants are the primary producers for the precious life to run on earth. They are the basic direct foods for the herbivores and indirectly to the carnivores and hence part of the foundation of food web. Plants are mainly classified into Algae, Fungi, Bryophytes, Pteridophytes, Gymnosperms and Angiosperms. Algae are aquatic and they grow in various types of waters. Among the plants, algae belong to the primitive group of plants, which evolved first in the universe. Algae can be microscopic and macroscopic respectively known microalgae and macroalgae. Algae are present and grown both in freshwater as well as in marine water. In the marine ecosystem, the algae are the major primary producers. Macroalgae are bigger, having simplest structure with thallus and without true leaf and roots. But they are having pseudo roots called as hold fast. They do photosynthesis through their thallus. According to Thierry **(1998),** it is impossible to give a short definition to seaweeds because this heterogeneous group is only a fraction of an even less natural assemblage, the "algae". Needless to say, the taxonomic classification of algae is still the source of constant changes and controversies, especially recently with new information provided by molecular techniques (Van den Hoek, et al, 1995). The recent study by John (1994), suggests that there are around 36000 known species of algae and they represent only about 17% of the existing species. According to Dring (1982) over 90% of the species of marine plants are algae and roughly 50% of the global photosynthesis on the plant group is algal derived (John, 1994).

Thus every second molecule of oxygen we inhale is produced by an alga, and every second molecule of carbon dioxide we exhale is reused by an alga (Melkonian, 1995).

In general benthic, macrophytic marine algae are called as seaweeds. In earlier days, usages of these aquatic plants (Marine algae) were not studied well. Hence the name was given to these marine plants as "Seaweeds". Now, the utilities of the marine plants have been studied well and we are using them (these marine plants) in our day today life in various forms. The name "Seaweeds" is already popular among the scientists and peoples. So we are unable to change the name "Seaweeds". But in scientific literature, some scientist's use the term "Marine Plants" here and there but most use as 'Seaweeds" remain. More than the name, the utilization and applications of these plants are plenty. Seaweeds are naturally growing in seawater and their growth is greater where nutrition and sunlight are more. Earth has more than 70% of marine water in its area. So we can get huge quantity of seaweeds from the sea either by natural harvest or by farming. This natural resource potential must be fully understood and utilized appreciating its applied values of varying kinds.

In India, Bay of Bengal is known for its rich living resources, particularly the Pearl oysters, Sea cucumbers, Chanks, Seaweeds and also Sea grasses. In the sea, plants include phytoplankton, cyanobacteria, seaweeds and sea grasses. Among these marine plants, seaweeds are known well for its better livelihood option provided to the coastal community. Seaweeds are one among the renewable and economically valuable marine resources. They come under the division "Thalophyta" of the plant Kingdom. Seaweeds are growing abundantly in the intertidal and sub tidal regions of the sea, shallow or near shore waters of sea, estuaries and also in the brackish water environments. They flourish wherever rocky, dead coral, rocks, stones, pebbles and any other suitable substrata are available for their attachment and nutrients for growth.

Classification and Distribution of seaweeds

Seaweeds are classified based on their pigments and coloration into four groups. They are Chlorophyceae (Green Algae, 1200 species), Phaeophyceae (Brown Algae, 2200 species), Rhodophyceae (Red Algae, 6500 species) and Cyanophyceae (Blue Green Algae) (Dhargalkar and Pereira, 2005). The other features used to classify them include cell wall composition, reproductive characteristics, and chemical nature of photosynthetic products (oil and starch). Further these four major groups of seaweeds, classified based on characteristics such as plant structure, form and shape. Green seaweeds are most commonly found in the shallow intertidal zone. Some common green seaweeds are *Ulva* (Sea lettuce), *Enteromorpha* (green string lettuce), *Chaetomorpha sp., Codium sp* and *Caulerpa sp*. Brown seaweeds live in the mid intertidal or upper subtidal zone. Some most common brown seaweeds are *Sargassum sp, Laminaria sp, Turbinaria sp* and *Dictyota sp*. In addition to chlorophyll pigments, red algae contain the pigments phycocyanin and phycoerythrin which give the red colour. However, the colour of red algae varies if the red pigment phycoerythrine is destroyed - they appear purple, brown, green and yellow. Red seaweeds grow in deep waters (Subtidal) than other algae mainly due to the presence of accessory pigments (phycocyanin and phycoerythrin). Some red algae also grow in the intertidal zone. Coralline algae (a group of red algae), are pink in colour and contain deposits of megnecium and calcium carbonate in their cell walls. These seaweeds are hard like stones, and were once thought to be animals closely related to corals (Dawson, 1966; Levring et al., 1969; Chapman and Chapman, 1980; Tseng 2001). The common red seaweeds are *Gracilaria sp., Gelidiella sp., Eucheuma sp., Ceramium sp* and *Acanthophora sp*. The blue green algae were grown in shallow water and

were grown as colony. The most common blue green algae are *Lyngbya sp, Spirulina sp* and *Oscilatorai sp*.

There are more than 20000 species of seaweeds worldwide (references). Half of them are economically important and 280 are edible. Seaweeds are mainly used as an excellent source of phycocolloids such as agar agar, alginate and carrageenan. Some others are used as food, fodder, feed and liquid seaweed fertilizers (Bio fertilizers). Worldwide, there are 42 countries with reports of commercial seaweed activity and 221 species of seaweeds are utilized commercially. Of these, about 145 species are used for food and 110 species for phycocolloid production. About 90% of seaweed production comes from culture based practices and China holds first rank in seaweed production, with *Laminaria sp.* accounting for most of its production. China is followed by North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia and USA (Sajid Khan and Satam, 2003). Taiwan and Viatmam are also doing well in seaweed production. The world production of commercial seaweeds has grown by 119 per cent since 1984 and presently, 221 species of seaweeds are utilized commercially. The global seaweed resources have been estimated at 1460 million tonnes (fresh weight) of brown algae and 261 million tonnes (fresh weight) of red algae. The total seaweed production has been estimated to be around 1721 X 10^4 tonnes (fresh weight) annually. (Michanek, 1975). World production of seaweed and other aquatic plants were 1103595 tons and 1104948 tons in 1998 and 2007 respectively. India produced 97500 tons of seaweeds in 1997 and 40,000 tons in 2001 according to official FAO estimates (2006). This declining trend must be looked into more seriously. Today the seaweed products (Phycocolloids) industry has grown rapidly and is placed at 1.5 billion US$ and the demand for seaweed and their products have been increasing, approximately at 10% per annum (Rajasekaran et al., 2006). This rapid growth is due to the wide application of seaweeds and their products in various industries such as food, Pharmaceuticals, textiles, paper, agriculture, etc. The top 10 countries producing seaweeds in the world are China, Korea, Japan, Philippines, Indonesia, Chile, Taiwan, Vietnam, Russia and Italy.

Indian Status

Seaweeds are one of the commercially important marine living and renewable resources of India. In India, commercial exploitation of its species commenced since 1966 (Oza and Zaidi, 2001). At present, 1518 tons of (dry weight) red algae and 2285 tons of (dry weight) brown algae are utilized for manufacture of agar, alginate and liquid fertilizer (Kaliaperumal,et.al, 2004). Seaweeds grow abundantly along the coastal waters of Tamilnadu, Gujarat, Andhra Pradesh, Orissa, West Bengal, Kerala, Maharastra, Lakshadeep and Andaman Nicobar Islands. There are also rich seaweed beds around Mumbai, Ratnagiri, Goa, Karwar, Vizhijzn, Pulicot and Chilka.

Among the 20,000 species of seaweeds enumerated in the world, 271 genera and 1153 species are present in India with a total standing crop of 6, 77, 309 to 6, 82, 759 tons fresh (Subba Rao and Mantri, 2006). Commercial exploitation of 844 species commenced since 1966 (Oza and Zaidi, 2001). Kaliaperumal *et al.*, (2004) reported that 1518 tons of (dry) red algae and 2285 tons of (dry) brown algae are utilized for manufacture of agar, alginate and liquid fertilizer. According to Anantharaman *et al.*, (2006), the total potential seaweed wealth is 8, 70,000 tons fresh, present natural collection is 22,000 tons fresh and through seaweed cultivation is 150 tons dry. It is a clear indication that culture of seaweeds has not been given a serious thought to supplement the natural stock and there by meeting the demands of several industries involved in this venture.

Tamilnadu ranks first in its standing stock amoung the coastal states, including Andaman and Nicobar islands. Utilizing the natural and suitable situation for seaweeds culture of seaweeds must be taken in a committed manner with proper planning and excellent execution, involving the coastal people. Gulf of Mannar Biosphere reserve is rich of seaweeds, comprising 42 species of green algae, 31 species of brown algae, 69 species of red algae and 5 species of blue green algae. About 17 economically important species from agarophytes, carragenophytes, alginophytes and edible seaweeds are recorded in the area. The total biomass of seaweed estimated in Gulf of Mannar islands, constituted 53% of seaweed biomass of Tamilnadu coast (MSSRF proceeding, 1998). The commercially important seaweeds must be produced, selecting suitable methods for culture in fitting areas/ along the coastal areas and also in the open seawater.

Uses

Seaweeds are useful as food for human, animal fodder, meal, manure, seaweed liquid fertilizers, medicines, agar agar, agarose, alginate, carrageenan, other polysaccharides, poly phenols and also carotenoids. The various uses of the seaweeds are explained for understanding their values.

As food for Human

Seaweeds are used as human food from 600 to 800 BC. In China seaweeds were used from prehistoric time. In China and Japan, seaweeds are used as a stable diet item for a very long period. Fresh, dried and processed seaweeds are utilized for human consumption. Many types of seaweeds are used as food in Japan, China, Philippines and other countries of Indopacific regions. Seaweeds are eaten as salad, curry, soup, or jam.

"Sea vegetables", as they are called, the seaweeds are rapidly moving from Asian cultures, where for centuries they have been regarded as food for kings and gods. They are also known for their natural healing (foods) and even gourmet cuisine markets of the western world (Mendocino, 1980). Seaweeds are traditionally consumed in Asia as "sea vegetables", but in the western countries, they have been used as sources of gelling or thickening agents. Western countries are only recently beginning to enjoy the taste and nutritional value of these vegetables. Seaweeds have been a staple of the Japanese diet for centuries (Escrig and Goni Combrodon, 1999). Seaweeds draw from the sea, the wealth of all mineral elements, macro elements and trace elements. The mineral fraction of some seaweeds accounts for upto 36% dry matter. Protein content in brown seaweeds is generally between 5% and 15%, where as red and green seaweeds contain 10 to 30% of protein in their dry weight. In *Palmaria palmate* (dulse), protein content is 35% and in *Porphyra tenera* (nori), the protein content is 47% dry matter. Among the Indian species, *Ulva* showed the higher range of 15 to 24 % protein.

Red seaweeds contain algal proteins called phycobiliproteins. Recent studies showed that phycobiliproteins have antioxidant properties which could be beneficial in the prevention or treatment of neuro degenerative diseases caused by oxidative stress (Alzheimer's and Parkinson's) as well as in the cases of gastric ulcers and cancers (Anantharaman et al., 2006). Seaweeds also contain poly unsaturated fatty acids called Omega 3 fatty acids and omega 6 fatty acids. These fatty acids play important role in the prevention of cardio vascular diseases, osteoarthiritis and also diabetes. Seaweeds are rich source of minerals - macro, micro and trace - coupled with biochemical components - proteins, carbohydrates, vitamins, amino acids, etc., (Darcy – Vrillon, 1993: Mabeau & Fleurence, 1993). Mineral composition of seaweeds is of 40% dry matter (Ortega – Calvo, Mazuelos, Hermosin, & Saiz – Jimenez,

1993) or more than that of land plants and animal products (Ito & Hori, 1980). The ancient tradition and everyday habit of using seaweeds as diet have made possible a large number of epidemiological researchers showing the health benefits linked to seaweed consumption (Hiqashi, Otani, & Okai, 1999; Funahashi et al., 1999; Mohamed, Hashim, & Rahman, 2012). Currently human consumption of green algae (5 percent), brown algae (66.5 percent) and red algae (33 percent) is high in Asia mainly in Japan, China and Korea (Dawes, 1998). Recently other countries such as the Republic of Korea, the United States of America, South America, Ireland, Iceland, Canada and France besides Japan, China and Korea have significantly increased the consumption, production and marketing of seaweeds. Further the demand for seaweed as food has now also extended to North America, South America and Europe. Edible seaweeds have played a significant role in economy of some nations such as Japan, Korea and China (McHugh, 2003). In world market, there are 13 algae (5 brown seaweeds, 4 red seaweeds, 2 green seaweeds and 2 micro algae) authorized as vegetables and condiments, although 152 seaweed species have been utilized for food preparations (Burtin, 2003). The nutritive value of the seaweeds is mainly due to the presence of significant quantity of protein, amino acids, minerals, lipids, vitamins, bioactive compounds, dietary fibers and antioxidants (MacArtain, Gill, Brooks, Campbell & Rowland, 2007; Subba Rao, Ganesan & Suresh Kumar, 2010; Mohamed et al., 2012). The nutrient content of the edible seaweeds vary with species, geographical location (habitat), maturity, season and temperature (environment) (Ito and Hori, 1980; Kaehler and Kennish, 1996; Fleurence, 1999 and Fleurence, Le Coeur, Mabeau, Maurice, & Landrein, 1995).

Biochemical composition of several seaweeds like *Ascophyllum nodosum, Laminaria sp, Fucus sp, Sargassum sp, Pterocladia capillacea, Himanthalia elongata, Undaria pinnitifida, Porphyra umbilicalis*, P. vietnamensis, *Palmaria palmata, Chondrus crispus, Ulva lactuca,Ulva spp and Enteromorpha(Ulva) intestinalis, Enteromorpha spp, Gracilaria verrucosa* (McHugh, 2003; Sukran, Nurhayat, Didem, Gamze & Egemen, 2003; Subba Rao, Mantri & Ganesan, 2007; MacArtain et al., 2007; Khairy & El-Shafay, 2013; Ganesan et al., 2014) and *Caulerpa recemosa, Gelidiella acerosa, Catenella repens,Acanthophora spicifera Hypnea spp Gracilaria edulis, Gracilaria crassa* and *Kappaphycua alvarezii (*Eswaran, Mairh & Subba Rao, 2002; Kaliaperumal, Ramalingam, Kalimuthu & Ezhilvalavan, 2002; Fayaz et al., 2005;Chakrabarthy & Sandra, 2008; Abhirami & Kowsalya, 2011; Baghel, Kumari, Reddy & Jha, 2014) for their use as food supplements or ingredients have been investigated . Recent studies on *Kappaphycus alvarezii* have revealed that the various solvent extracts have exhibited antioxidant activity in linoleic acid system with ferrothiocyanate reagent (Suresh Kumar, Ganesan & Subba Rao, 2008) and it also contains minerals Ca, Fe and Zn in admissible limits for its use in food or food formulations (Fayas et al., 2005). The present study was carried out to understand the chemical composition viz., protein, carbohydrate, chlorophyll *a*, carotenoids, phycobiliproteins namely phycoerythrin and phycocyanin of the cultivated *Kappaphycus alvarezii* (for one year from April 2012 to March 2013) and mineral contents (macro - Sodium, Potassium, Calcium, Magnesium, Phosphorus, and micro and trace – iron, copper, zinc, manganese, cobalt, boron, arsenic, cadmium, lead, mercury, chromium, nickel and molybdenum) for October 2012 at the three different locations (Mangadu, Munaikadu and Vedalai) of Palk Bay waters, Ramanathapuram district, Tamilnadu, Southeast coast of India.

As Fodder

In many countries, raw (fresh) seaweeds or prepared seaweeds are regularly fed to animals like cow, goat, horses, etc. In Iceland, fresh seaweeds are commonly used as food for sheep, cattle, hen and horses. In certain animals the seaweed forms almost their only food,

though it is sometimes given along with hay. Basal or youngest parts of the fronds of *Laminaria saccharina* are preferred by the horses. In Norway, Iceland and Europe, seaweeds are fed regularly to the sheep. *Pelvetia sp, Rhodymenia palmate, Alaria sp, Fucus sp, Chondra filus, Ascophyllum sp, Macrocystis sp, Palmaria sp* and *Laminaria sp* are the major genera of seaweeds used as fodder in various countries (Boney, 1965). Seaweeds are rich in protein (20 – 25%), Carbohydrates (50 – 70%), Vitamins, minerals and useful in certain medicinal applications. When used in animal feed, cows have produced more milk and chicken eggs have became better pigmented. Horse and other pet animals became healthier (White and Keleshian, 1994). Tocopheral and Vitamin E in seaweeds increased the fertility rate and birth rate of animals, when used as fooder. Milk production and fat content have been found to increase by using seaweeds as part of the diet. Feeds supplemented with seaweeds and *Spirulina* to layer chicks (White Leghorn) increased the no. of eggs, their size and colour of the yolk (Chaturvedi et al., 1985). In Japan, Germany, UK and Norway, feeding trials were reviewed in farm animals with possibility of seaweeds as supplementary animal feed by Dave et al.1977. Cattle fed with *Laminaria sp* based diet have gained more natural resistance to diseases such as foot and mouth.

Ulva lactuca, Enteromorpha compressa, Padina pavonica and *Laurencia obtusa* are potential sources of dietary protein and lipid for fishes (Wahbeh, 1997). *Kappaphycus alvarezii* and *Gracilaria heterocladia* in dry ground form diets, showed best FCR and highest survival rate in *Penaeus monodon*. The rare breed of primitive sheep on North Ronaldsay, Orkney (Schotland) survives under extreme conditions on the beach shore of North Ronaldsay with seaweed as virtually their sole feed source (Kaladharan, 2006). Seaweed treated pasture forages have increased immunity in pigs and chicks (Beas et al., 1988).

Seaweed based feeds are commercialized and popular. They are

1. Tasco 14 – a feed derived from *Ascophyllum nodusum,* benefits overall immunity of cattle.

2. Acadian – a kelp meal marketed by Mangrove Holsteins Limited proved to boost the immune system.

3. Pedigree – a carrageenan based dog feed marketed by MARS Company. Several other seaweed supplemented animal feeds such as Shrimp/ fish feed, Poultry feed, cattle feed and Holothurian feed are also used in various countries.

As Medicine

Seaweeds were considered to be of medicinal value in the orient as early as 3000 B.C. The Chinese and Japanese used them in the treatment of goiter and other glandular diseases. Romanians used the seaweeds for healing the wounds, burns and rashes. The British used *Porphyra* to prevent scurvy (Vitamin C deficiency diseases) during long voyages. *Coralline officinalis, Hypnea musciformis* and *Alsidium helminthocorton* are employed as vermifuges. *Chondrus sp., Gracilaria sp., Gelidium sp*., and *Pterocladia sp.* have been used to treat various stomach and intestinal disorders and they have also helped to relieve from constipation and other discomforts. *Laminaria* is used as a pain killer and also to distend the uterus. Some species of *Sargassum* are used for cooling and blood cleaning. *Sarconema* can be used for controlling the goiter, a disease caused by the enlargement of Thyroid gland. *Gelidiella cartilagineum* has been found to be against influenza B and mumps viruses. *Schizymenia pacifica* contained a sulfated polysaccharide in the r carrageenan family, which selectively inhibited HIV reverse transcriptase (Anantharaman et al., 2006). Seaweeds in general are used as verimifuge, for cough, stomach and chest ailments, bladder and kidney

ailments and also as Antiscorbutic. In 1965, Boney screened the seaweeds for the medicinal applications and their uses are reported

Antitumor activity was studied in *Porphyra telfairiae* and positive results are noted. Still more works are going on in antitumor activity with seaweeds in various countries. Sulfated polysaccharies such as Fucans, Xylons, etc. have several biological activities. Antibacterial activity was studied in several seaweeds and positive results are obtained in *Gracilaria cornea, Laurencia intricata, Laurencia obtusa* and *Laurencia papillosa*. The extracts of *Liagora farinose, Dasycladus vermicularis* and *Lobophora variegate* have the highest inhibition zones and a wide spectrum of antibacterial activity (Anantharaman et al., 2006). Anticoagulant substances are isolated from *Codium pugniformis* and *Codium sp* (Shanmugam et al., 2001). Antiviral activity has also been studied in various seaweeds such as *Padina sps, Dictyota sp,* etc., many positive results were obtained and more work in this direction is going on. Immunomodulating activity was well studied in seaweeds. *Hizikia fusiformis* and *Meristotheca populosa* markedly stimulated human lymphocytes to proliferate and *Eucheuma muricatum* and *Meristotheca papulosa* weakly stimulated proliferation.

Studies on isolation of immunosuppressive activity from seaweeds were made on various seaweeds. Similarly studies on anti ulcer substances from seaweeds are going on in several countries. In *Porphyra tenerakjellman* anti microbial activities of antiulcer substance were studied and positive results have been obtained. Phycocolloids such as Agar, Alginate and Carrageenan, derived from seaweeds are used in large quantities in the medical field. Alginates are used in surgical dressing. Alginate also improves wound healings. Alginate is also used in antacid formulations where the alginate gel prevents reflex of stomach acids and prevents heart burn. Agar and Carrageenan are used in various applications like pharmaceutical applications, food applications, textile applications, etc.,

Phycocolloids

The cell wall of several seaweeds contain very interesting group of complex polysaccharides called phycocolloids. The complex phycocolloids are of innumerable structural possibilities. Phycocolloids from various species have their specific applications. Among the various phycocolloids, three of them, namely Agar, Algin and Carrageenan are most important. International demands for these products are increasing day by day, because of new findings of the products and their applications and quantity of usages.

Agar Agar

Agar Agar is an important phycocolloid derived from red seaweeds. Nowadays, *Gelidium sp, Gracilaria sp., Pterocladia capillaca, Pterocladia lucida, Gelidiella sp, Ahenpeltia plicata, Acanthopheltis japonica, Ceramium hypnoides and Ceramium boydenii* are used for agar production globally in the industry. In India, *Gelidiella acerosa* and *Gracilaria sp,* are used for agar production. Agar gels are stronger and resistant at low concentrations (1 to 1.5%) with only water. It withstands even above 100^0 C (Good sterilization) and can be used in wide range of pH (5 to 8). Agar gels can be repeatedly gelled (Excellent reversibility) and melted without losing its property. The agar gels are superior to alginate because agar gels are stable, not causing precipitation in the presence of cations as happens in alginates with calcium. FAO/ WHO codex alluminates permits the use of agar as human food industry and it also accepted and authorized in countries such as United Kingdom, Germany, Russia, France, Poland, etc., Food and Drug Administration (FDA) of United States (US) assigns agar as a grading of Generally Recognized as safe (GRAS) (Anantharaman et al., 2006).

Agar is used in various applications in food industries. The following applications are very important. 1. Used in Confectionaries. 2. Used as thickening agents. 3. Agar gel cubes in fruit salad. 4. Used in Bakery 5. Fruit Jelly. 6. Applications in Yogurt. 7. Used in Meat industry. 8. Used in canned products. 9. Liquor industry to increase viscosity. 10. High concentration of agars also used in Sculpture, Archeological and dental impression moulds.

Alginate

It is yet another most important polysaccharide extracted from brown seaweeds such as *Sargassum sp* and *Turbinaria sp* in India. Algin yielding seaweeds include *Sargassum, Turbinaria, Dictyota, Padina, Cystoseira, Hormophysa, Colpomenia, Spatoglossum* and *Stoecospermum,* occurring in the Indian waters. Among these seaweeds *Sargassum* and *Turbinaria* are utilized as raw material for the manufacture of alginate in India. The alginate from these species has comparatively low viscosity. The structure of alginates from *Sargassum* and *Turbinaria* indicate that they can be very useful in applications required for the formation of strong gels. The most important algin yielding seaweeds are *Laminaria, Macrocystis* and *Ascophyllum. Laminaria* is very common and popular in Japan and Korea. In China, *Laminaria* is cultivated and produced more. In Scotland, Norway and France, *Laminaria* is collected from the natural stock. In Chile and Australia, *Durvillea lessonia* and *Ecklonia* are collected and exported to US and UK alginate industries. The polyelectrolytic property and the viscosity of alginates make them more suitable as an excellent stabilizing agent in the food industry. The Alginate (Propylene glycol alginate) has been approved as a food additive for use as emulsifier, stabilizer or thickner in USA. The Joint Expert committee of Food additives of the food and Agricultural organization of UN/ World Health Organization (WHO) has issued specifications for alginates and recommended an acceptable daily intake of 50mg/ kg body weight for alginic acid and 25mg/ kg body weight for propylene glycol alginate.

Alginate is used in several ways as follows. 1. Stabilizing and emulsifying agent. 2. Gelling agent. 3. As a film forming – binding – glazing agent. 4. Medicinal applications. 5. In Textile products. 6. Bio Engineer. 7. Food products. 8. Dairy products. 9. Paper products. 10. Rubber products. etc.,

Carrageenan

Carrageenans are complex sulphated polysaccharides. They are commercially important hydrocolloids, derived from various red seaweeds. The name carrageenan derived from a small coastal town in Ireland, where commercial harvests of *Chondrus crispus* (Irish moss) were made in 19th century. Carrageenans are divided into four types based on their properties; they are Kappa, Iota, Beta and Lambda. Kappa carrageenan is extracted from *Kappaphycus alvarezii* (cottonii of the trade). Iota carrageenan is extracted from *Eucheuma deticulatum* (Spinosum of the trade), Beta carrageenan is extracted from *Betaphycus gelatinae* (Gelatinae of the trade) and lambda carrageenan is be extracted from *Acanthophora spicifera*. The carrageenan industry depends on the major source of carrageenan from *KappaphIycus alvIarezii* and *Eucheuma spinosum. Kappaphycus alvarezii* yields kappa carrageenan and *Eucheuma spinosum* yields iota carrageenan. Carrageenan yielding seaweeds include *Chondrus, Gigartina, Iridaea, Eucheuma,* and *Hypnea,* Carrageenan makes use of their both hydrophilic and anionic range of addressing properties. Anionic property of carrageenan is influencing the hydrophilic nature. Carrageenan applications are increasing day by day due to its wide properties. More than 250 applications were identified for carragenan in different fields such as food products and processing, pharmaceutical industry,

cosmetics, coating paints and inks and other products and processes. Carrageenan is listed by the US Food and Drug Administration (FDA) as generally recognized as Safe (GRAS) (US food and Drug Administration, 1979). Carrageenan was defined as having a viscosity of not less than 5mpas at 1.5% concentration and $75A^0C$ (US Food and Nutrition Board, 1981) had been demonstrated to be safe. Eppley institute of Cancer Research demonstrated that carrageenans are not carcinogenic and carrageenans have been shown not to be teratogenic (Collins, Black and Prew, 1977, 1977, 1979).

As Manure

Seaweeds have been initially connected directly or indirectly with human beings as a source of food, fodder and manure from time immemorial (600BC) especially in densely populated countries. They are rich of micro and macro nutrients as well as growth hormones, which are essential for major agricultural crops (Thivy, 1961). So seaweeds can also be used as manure. The value of seaweeds as an agricultural fertilizer has been demonstrated, especially by coastal farmers with ready access to seaweeds (Booth, 1965). The first reference to such use appears in Roman writings from the second century AD. The potential of seaweeds is known not only for the macro nutrients such as Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Sulphur but also for its trace elements and plant growth regulators namely Auxin, Gibberellins and Cytokinins (Rengasamy, 2004). The seaweeds are used as bio- fertilizers because of their benefits as soil conditioners, fertilizers and green manure. The presence of high amount of potassium salts, micronutrients and growth substances add manurial value of the seaweeds.

In the world, brown seaweeds such as *Alaria, Ascophyllum, Durviellea, Ecklonia, Fucus, Laminaria* and *Macrocystis*, red seaweeds such as *Lithothamnion* and green seaweeds such as *Ulva lactuca* and *Enteromorpha intestinalis* have been used for their fertilizer value for wide variety of higher plants. In the recent years, liquid extracts of brown algae have appeared on the market. Two well known brands are 'Maxi crop' (A alkaline hydrolysis mainly from Fucoides produces) and 'Alginure' (produced from seaweeds).

Seaweed fertilizers are now commercially available in several trade names such as Algit, Algifert, Algigert 25, Cytex, Goemar GA 14, Kelpak 66, Maxicorp, Maxicorp original, Maxicorp trible, Seaspray, Seasol, SM 3, GYFA 17 and seacrop, etc., in different countries. In India, the use of seaweed as manure for the growth and better yield of vegetables and crops was first reported by the Central Marine Fisheries Research Institute (CMFRI) in 1960. CMFRI and Central Salt and Marine Chemical Research Institute (CSMCRI) scientists have done lot of work on biofertilizers from Indian seaweeds. Major genera of fertilizer seaweeds have been identified and tabulated (Boney, 1965). They include brown seaweeds such as *Ascophyllum, Macrocystis, Laminaria, Ecklonia, Durvillaea, Cabophyllum, Himanthalia, Sargassum* and *Turbinaria* as well as red Seaweeds namely *Pachymenia, Lithothamnion, Phymatolithon*, etc.,

Seaweed fertilizers were found to be superior to chemical fertilizers because of the high level of organic matter, which aids in retaining moisture and minerals in the upper soil level available to the roots (Wallen Kemp, 1955). Seaweed fertilizers in various concentrations from various seaweeds on various crops have been studied. The results have shown advantages of increased yields. In Bhendi yield increased when applied with *Hypnea* compost with cowdung and ash (Thivy, 1958 & 1960). Seaweeds directly used as fertilizer on coconut, palms and coco plants have resulted in better yields (Richardson, 1958). Seaweed extracts have been used in sweet corn, tomato, okra and sweet potato (Aitken and Senn, 1964), peanuts and sweet potatoes (Tseng, 1973), green chilies and turnip (Dhargalkar and

Untawale, 1980). Recently, several studies have been made on *Kappaphycus* seaweed extract by scientists of Central Salt and Marine Chemical Research Institute (CSMCRI) on various crops of commercial value. They have got international patent in recognition of the work done by them. Patent entitled "Integrated method for production of carrageenan and liquid fertilizer from fresh seaweeds". Karuppanan Eswaran, Pushpito Kumar Ghosh, **Arup Kumar Siddhanta,** Jinalal Shambhubhai Patolia, Chellaiah Periyasamy, Aditya Shantibhai Mehta, Kalpana Haresh Mody, Bharatkumar Kalidas Ramavat, Kamalesh Prasad, Mahesh Rameshchandra Rajyaguru, Singaram Kulandaivel Chennur Radhakrishna Reddy, Jayant Batukrai Pandya and Akhilesh Tewari are the group of scientists, whose research work resulted in the production of the much needed biofertilizer.

Now various seaweed biofertilizers are in the market such as Aqua Sap from *Kappaphycus alvarezii* (Aquagri processing private Limited, New Delhi), Organic 6 from *Sargassum sp* (SNAP Alginate, Ranipet), PHYCOLINN from *Sargassum*, *Turbinaria* and *Kappaphycus* (Linn Plantae Private limited, Madurai), etc., There is an urgent need to provide organic foods all kinds of farming systems, keeping in mind the health of the consumers. Also, the soil health and water quality are to be protected. Compared to the chemical fertilizers and their ill impacts, the diversified benefits of biofertilizers are great and hence, greater awareness must be made among the farmers to use biofertilizers for better food, soil health and preservation of water quality. More than the farmers, the decision makers of the country and those planning and implementing stages must understand the values of seaweeds, needs of seaweed biofertilizers, including their special benefits and merits and put the end to chemical fertilizers, supporting biofertilizers, and establishment of biofertilizer plants.

In India

Of the 2.02 million km^2 of Exclusive Economic Zone (EEZ) of India, seaweed can be farmed in 200,000 hectares or 0.001% of the EEZ area (Krishnamurthy, 2005). The rocky beaches, mudflats, estuaries and lagoons alone of the coasts offer ideal habitats for seaweed farming. The coast is characterized by narrow inter-tidal regions and mixed tides. The area presently under farming is not encouraging. This scenario must be changed to harvest the benefits of seaweeds by farming in the natural systems. With a view to develop suitable technology on commercial scale cultivation for augmenting supply of raw material to agar industries, CMFRI and CMCRI and related organizations since 1964, have attempted experimental cultivation of agar yielding seaweeds *Gelidiella* and *Gracilaria* and also carrageenophytes and edible seaweeds such as *Hypnea, Sargassum, Turbinaria, Cystoseira, Hormophysa, Caulerpa, Ulva, Enteromorpha* and *Acanthophora* in different field environments using various culture techniques (Kaliaperumal, 2004). These experiments revealed that *Gelidiella acerosa* can be successfully cultivated on dead coral stones and *Gracilaria edulis, Hypnea musciformis, Acanthophora* and *Entreromorphia flexuosa* on long line ropes and nets. Commercial cultivation of *Kappaphycus alvarezii* cultivation is going on successfully in the coastal waters of Tamilnadu, Gujarath and Andhra Pradesh (Subba Rao et al., 2008; Periyasamy et al, 2014, 2015 & 2016). Seaweeds have wide range of potentials and have multi applications but the commercial products in India are very few. So work has to be needed for the introduction of useful products is the need of the hour. To meet the need for the production of the raw materials, selected potential species must be cultured more and India should atleast play its rational role seasonally well to protect the environment and also to improve the socio economic conditions of the coastal areas.

Acknowledgement

The authors wish to thank Dr. K. Kathiresan, Dean and Director, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Cuddalore (District), Tamilnadu, India for his encouragement, interest and the facilities provided. Thanks are due to the higher authorities of Annamalai University, Chidambaram, Tamilnadu, India for rendering timely help in the execution of the work.

SEAWEED DIVERSITY, RESOURCES AND THEIR AUGMENTATION

P .V. Subba Rao FNASc[1] and C.Periyasamy
Aquaculture Foundation of India, Madurai, Tamil Nadu/Guntur, Andhra Pradesh
[1]Former Deputy Director and Group leader, Marine Biotechnology and Ecology .Discipline
Central Salt and Marine Chemicals Research Institute (CSIR), Bhavnagar, Gujarat.
& Former Scientist-in Charge, CSMCRI-Marine algal Research Station,
Mandapam Camp, Tamil Nadu Email: raokappaphycus@gmail.com

India ($08^0 04'$ $37^0 06'$ N and $68^0 07'$ $97^0 25'$ E), a tropical South Asian country has a stretch of about 7516 Km coastline, excluding its island territories with 2 million Km^2 Exclusive Economic Zone (EEZ) and with nine maritime states viz., Gujarat, Maharashtra, Goa, Karnataka and Kerala on the west and Tamilnadu, Andhra Pradesh, Odhissa, and West Bengal on the east (Subba Rao, 2000).

Seaweeds otherwise known as Marine algae are primitive and non - flowering large benthic multicellular, macrothallic forms and differentiated from most algae that are of microscopic (Smith, 1944). They inhabit in the intertidal and sub-tidal regions of seas and oceans that occupy seventy one percent (71%) of the globe. Moreover the seas and oceans form the large ecosystem sustaining in the world marine environment. Seaweed ecosystem is one of the three important components of the marine ecosystem, the other two being coral reef ecosystem and sea grass ecosystem (Subba Rao, 2012). Seaweeds have been used for food, feed and fodder, besides a source of phytochemicals, *viz*; agar, alginate and carrageenan (Chapman and Chapman, 1980; Levring *et al.* 1969). They also form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial. Reports on the uses of seaweeds have been cited as early as 2500 years ago in Chinese literature (Tseng 2004).

Seaweed flora of India is highly diversified and comprises mostly of tropical species, but boreal, temperate and subtropical elements have also been reported. Prof. M.O.P. Iyengar (1927) was the first Indian algologist who gave a detailed descriptive account of Indian marine algae occurring on the southeast coast, especially at Kurusadai Island and he is the "Father of Indian Algology / Phycology" However Børgesan (1933, 1934, 1935, 1937 & 1938) contributed much to the taxonomic account on Indian marine algae. The coasts of Tamilnadu and Gujarat represent very high seaweed diversity and considered to be seaweed hot spots **(**Subba Rao, 2007**).** India is one of the 12 mega biodiversity countries of the world and accounts for 7.8% of the recorded species of the World with only 2.5% of the land area (Subba Rao, 2012**).** In all 1153 species of seaweeds, including forms and varieties have been reported from Indian waters (Subba Rao and Mantri, 2006) and forms only 12.81% of 9000 species reported in the World (Khan *et al.* 2009). The total seaweed collections during 1978 to 2003 varied from 1173 to 6417 tons (dry). Agarophytes (*Gelidiella acerosa, Gracilaria*

edulis, G. crassa, G. folifera and *G.verrucosa*) varied from 240 to 1518 tons (dry) and alginophytes (species of *Sargassum* and *Turbinaria*) from 651 to 5534 tons (dry) (Kaliaperumal and Kalimuthtu, 1997 and Kaliaperumal *et al*. 2004).Indian seaweed resources are estimated to be 6.77 to 6.83 lakh tons(dry) till 2002-2003 (Subba Rao and Mantri, 2006) and forms 3.4% of world seaweed resources 20,05,459 tons (dry wt.) recorded during 1994—1995 (Zemke – White and Ohno, 1999). Of the 221 species of seaweeds utilized commercially in the world, 145 species are used for food and 110 species for phycocolloid production (agar, algin and carrageenan). In order to meet the demand both for food and phycocolloid production eight seaweeds viz; *Laminaria, Undaria, Porphyra, Gracilaria, Eucheuma, Kappaphycus, Monostroma, and Enteromorpha* have been widely cultivated in the worldwide in 47 countries (Zemke-White and Ohno, 1999) and major red seaweeds cultivated included *Kappaphycus* and *Eucheuma* for carrageenan, *Gracilaria* for agar and *Porphyra* for human consumption (FAO, 2013) In all 30 countries have introduced carrageenan farming seaweeds (*Eucheuma, Kappaphycus, Chondrus, Sarcothalia* ,and *Griffithsia*) to evaluate their potential biomass production (Neish, 2003). However, warm water *Eucheuma* seaweeds *viz*; *Kappaphycus alvarezii*, and *Eucheuma denticulatum*, have been cultivated substantially and commercially (FAO, 2013). *Kappaphycus alvarezii* alone has been introduced in 26 countries (Mandal *et al*. 2010) and 183000 tons (dry) in the world are produced through aquaculture (Bixler and Porse, 2011), while 4210 tons (wet) are obtained in India till 2010 (Krishnan and Narayanakumar, 2010) .In India seaweed cultivation has been initiated by Central Salt and Marine Chemical Research Institute, Bhavnagar, a National Laboratory of Council of Scientific and Industrial Research, New Delhi. Viable cultivation technologies have been developed for the agarophytes *Gelidiella acerosa* and *Gracilaria edulis* as well as for the Carrageenophyte *Kappaphycus alvarezii*, *Gelidiella acerosa* gives a crop yield of 4 tones (dry)/ha/yr .while *Gracilaria edulis* yields a crop yield of 20 tones (dry)/ha/yr using long line rope method and 30 tones (dry)/ha/yr using SRFT (Single Rope Floating Raft Technique) method (Subba Rao *et al*. 2004). *Kappaphycus alvarezii* gives a crop yield of 40 tones (dry)/ha/yr (SubbaRao and Mantri, 2006). Among all the technologies developed *Kappaphycus alvarezii* cultivation technology has been commercialized providing self-employment for tens of thousands of costal fisher folk in Tamil Nadu earning Rs.15000—16000/--per person per month (Periyasamy *et al*. 2014). In India Prof Krishnamurthy is the "Father of Seaweed Cultivation" while Dr P V Subba Rao is the"Father of *Kappaphycus* Cultivation".

Acknowledgement

The authors would like to thank Dr. M.Sakthivel, President, Aquaculture Foundation of India, Madurai, Tamilnadu and Former Chairman Marine Products Export Development Authority (MPEDA), Cochin, for his encouragement and moral support. Thanks are also due to Dr. V. Sundaraj, President Dr V. Sundaraj Foundation of Aquaculture (VSFA), Chennai, and Former Dean, Fisheries College and Research Institute, Tuticorin for his valuable suggestions.

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MARINE THERAPEUTICS

Dr. S. T. Somasundaram
Associate Professor
Centre of Advanced Study in Marine Biology
Faculty of Marine Sciences, Annamalai University
Parangipettai, TN

Oceans cover almost 70% of earth's surface and harbor more than 3,00,000 species of flora and fauna. Marine organisms, which thrive in such complex and highly competitive habitats, have evolved to synthesize unique defense molecules with potent activities. The exceptional chemical diversity offers an unlimited source of biologically active compounds. Manifold chemical entities like proteins, lipids, polysaccharides, vitamins, minerals, carotenoids, acid amines, etc., with various biomedical properties have been found to occur in the marine environment. They have been found to possess several activities like antioxidant, antiallergic, antimicrobial, anti-inflammatory, antidiabetic, anticancer, etc. In spite of their vast potential, marine organisms are the least explored source of pharmaceuticals. However, during the last few decades, several novel bioactive compounds have been isolated from marine sources like microorganisms, mollusks, echinoderms, tunicates, algae, corals, sponges and fishes. Currently, there are seven FDA approved marine derived drugs in the marine pharmaceutical pipeline and more than 25 marine compounds in different phases of clinical pipeline. Furthermore, several hundreds of marine derived compounds are in preclinical trials. The potentiality of marine compounds has increased the impetus of ongoing investigations and marine environment is considered as a huge source of structurally unique biomolecules for the development of novel pharmaceuticals and nutraceuticals.

MARINE MAMMALS: UNIQUE CHARACTERISTICS, ECOLOGICAL SIGNIFICANCE AND THE NEED FOR THEIR CONSERVATION

Dr. A. Murugan
Marine Ecology and Conservation Lab
PG & Research Department of Zoology
V.O. Chidambaram College, Tuticorin-628 008
Mobile: 9443256358 E-mail: muruganrsa@gmail.com

Introduction

Marine mammals, consisting of 120 extant species, are warm-blooded vertebrates evolved from land animals millions of years ago. In India, 30 species of cetaceans and one species of sirenia have been reported. Though adapted to marine environment, they still retain certain characteristic features of land mammals like presence of lungs, air breathing, giving birth and nourish young ones etc. They spend most or all of their lives in the ocean. They are the top-level predators that help keep our ocean ecosystem in balance.

Marine mammals are grouped into Cetaceans (whales, dolphins and porpoises), Pinnipeds (seals, sea lions, fur seals and walruses) and Sirenians (dugongs and manatees). Polar bears have also been included under marine mammals. Among them, the Cetaceans are the largest and most diverse marine mammals grouped into Odontoceti (toothed whales) and Mysteceti (baleen or rorqual whales). Cetaceans are also ecologically very diverse as their habitats range from coastal to pelagic, tropical to polar and from marine to fresh water. Their food includes planktonic crustaceans, fish, squids and marine mammals (Milinkovitch and Lambert, 2006). The blue whale is the largest animal ever known to have lived on Earth.

The Pinnipeds include sea lions, fur seals, true seals, walruses, sea otters and Polar bears. Sea lions and fur seals have visible external ears and can walk on all four flippers by rotating their rear flippers forward under their body. The mammalian order Sirenia, or sea cows, includes two extant families, the Trichechidae (manatees) and the Dugongidae (the dugong). Proboscideans (elephants) are usually considered the closest living relatives of sirenians. Manatees include three living species and are known from the early Miocene (15 Ma) to the Recent in the New World tropics. The dugong is represented by a single extant species, *Dugong dugon*.

Unique characteristics

Marine mammals have specialized adaptations to survive in the marine environment. They possess streamlined bodies to help them swim faster. Since they have to come to the surface to breathe, they possess special adaptive characters to absorb and store oxygen for use in deep diving. Consequently, the concentration of myoglobin in the locomotor muscles of diving vertebrates is 10 to 30 times greater than in their terrestrial relatives (Costa, 2007). Myoglobin is an oxygen binding pigment that facilitates oxygen transport and storage of oxygen in the muscle. They also have the ability to overcome decompression sickness and nitrogen narcosis during deep diving. Also, the blood volume of diving mammals is greater than terrestrial animals, ranging from 10 to 20% depending on the species (Costa, 2007). They possess a thick layer of blubber (or fat) to avoid heat loss and to keep warm in the ocean. Sperm whales can dive for over an hour, to depths over 1000 m.

Mysticetes (baleen whales) have no teeth. Instead, they possess unique structures in their mouths called baleen. These whales undertake yearly round-trip migration of hundreds to thousands of kilometers between the cold polar waters for food to warmer waters for

breeding. For example, Gray whales are known for longest annual migrations as they travel up to 12,000 miles between breeding and feeding grounds each year.

Odontocetes use echolocation or biosonar to detect, track and discriminate their prey, as well as to negotiate their environment. Toothed whales emit a focused beam of high frequency clicks, individually or in a series. Toothed whales, which evolved before baleen whales, have complex social systems and live in structured social groups called pods or herds. Toothed whales can use vocalizations to maintain social bonds.

Sirenians are unique among living marine mammals in having a strictly herbivorous diet, which is reflected in the morphology of their teeth and digestive system. Manatees can be readily distinguished from the strongly downturned snout of dugongs by their less-pronounced deflection of the snout, which enables them to feed at any level in the water column rather than being obligate bottom feeders, like the dugong.

Ecological significance

Marine mammals are believed to influence the structure and function of some aquatic communities (Katona and Whitehead 1988; Bowen, 1997). As top level predators, they may also play an important role in shaping the behavior and life history traits of prey species and competitors, in nutrient storage and recycling and in modifying benthic habitats (Katona and Whitehead 1988). Experimental studies have shown that sea otter, *Enhydra lutris*, which feed primarily on sea urchins, have strong influence on kelp forest. Likewise, the structure of benthic invertebrate communities is affected by Gray whale *Eschrichtius robustus* and walrus *Odobenus rosmarus* feeding. The dugong *Dugong dugon*, which feeds on seagrass, may influence the seagrass community. There is a speculation that some cetaceans may have an important ecological role in the recycling of nutrients by feeding at depth and then defecating in the euphotic zone (Kanwisher and Ridgway, 1983). Large cetaceans may continue to play an important ecological role even after death through the downward transfer of nutrients to benthic communities (Katona and Whitehead 1988).

Threats and Stranding

Marine mammals are increasingly threatened by interactions with fishing gear, ocean noise, pollution, direct harvest, ship traffic, coastal development etc. The alteration of marine ecosystems due to climate change may have an influence on the migratory patterns of whales as food becomes harder to find in their regular feeding habitats.

Marine mammal stranding is a complex phenomenon and several reasons could be attributed. Underwater shallow seaquakes, underwater acoustic noise, underwater volcanic explosions, climate change, pollution, diseases etc are a few of the factors which could contribute to their stranding. The sudden pressure related diving injury lead to sinus barotrauma, effectively disabling biosonar and knocking out their navigational ability to sense direction and location of prey. Ultimately, they swim along with the current until shoreward current directs them to the beach. The pilot whales, for example, are highly social and live in groups called pods. There is also a contention that distress call from an injured member of the pod makes other individuals to rush in for help which lead to stranding of the entire group. This phenomenon has not yet been proved.

Recent Stranding of Short-finned Pilot whales in Tuticorin district

On 12th January 2016, around 79 short-finned Pilot Whales *Globicephala macrorhynchus* got stranded along Tiruchendur to Manapad coast off Tuticorin district. It was the fourth such incident involving the same species in India. In the year 1852, stranding

of many pilot whales was reported in Salt Lakes near Calcutta (Blyth, 1852). Incidentally, along the same stretch of Tuticorin district from Kulasekarapattnam to Manapad, 147 short-finned pilot whales got stranded on 14th January 1973 (Alagarswami et al., 1973). The authors ruled out the possibility of stranding due to pollution after detailed analysis and reported that the stomach and intestine were empty in the stranded whales. The authors could not able to ascertain the causative factor for the stranding of pilot whales. Another mass stranding of 40 *G. macrorhynchus* was reported along Elizabeth Bay, North Andaman coast on 21 October 2012 (Raghunathan et al., 2013). The authors postulated that the magnetic waves generated by the undersea earthquake with a magnitude of 4.7 on the Ritcher scale which struck the Andaman sea region on 21 October 2012 at a depth of 30 km might have altered the navigational path of whales and propelled them towards the shallow waters of the Elizabeth Bay.

In the present stranding of short-finned pilot whales (personal observation), no marked injuries were observed and the stomach of few examined whales was empty. The stranded animals were found to be healthy. The stranded group included sub-adults and a few calves. The tidal amplitude was high with a strong shoreward wind on the day of stranding. This could be evidenced by the presence of the animals in the backwaters in Manapad linked to the sea through a narrow channel. The few animals rescued back to the sea by the local fisher folk in Kallamozhi on the night of stranding, beached at Manapad coast. Though the exact reason for the stranding could not be ascertained for their stranding, it seemed that they lost their navigational ability. The cause for disorientation could be linked to shallow earth quake near Java province of Indonesia a few days earlier and the possible drifting of the pods along with the current.

Conservation

The loss marine biodiversity in recent decades, mostly owing to anthropogenic activity, is a cause for worry. According to Pompa et al. (2011), at least three marine mammals, Caribbean monk seal (*Monachus tropicalis)*, Atlantic gray whale (*Eschrichtius robustus*) and the Steller's sea cow (*Hydrodamalis gigas*) became extinct because of hunting for their fur, blubber, and meat during the 19th and 20th centuries. Still, many marine mammals become victim to fisheries activity, ship traffic, entanglement in ghost nets, ingestion of plastics, commercial hunting, habitat loss, pollution including oil spill and acoustic sound, etc. Though most of the marine mammals have been included under wildlife act, IUCN Red List and CITES, the lack of data on most of the species due their deep water dwelling nature makes the conservation efforts complicated. However, since many of the marine mammals have been shown to have an ecological role, they need to be protected by all means.

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CASH FROM TRASH : SUSTAINABLE UTILISATION OF LOW VALUE MARINE TRASH FISH RESOURCES – A GREEN PERSPECTIVE ON BLUE ECONOMY

Dr.S.M.Raffi
Kerala University of Fisheries and Ocean Studies
Kochi-682 506, Kerala email: raffi_cas@yahoo.co.in

The agricultural based food products generated are not sufficient for catering the needs of burgeoning human populace. Adding to this, the availability of land for agriculture is alarmingly decreasing at a faster pace. The Indian marine fisheries sector plays a prominent role in supplying protein rich food and is providing ample employment generation and foreign exchange earnings. But the present day marine fisheries scenario is so pathetic in terms of declined catch which might be due to multiple anthropogenic factors such as over exploitation, irrational fishing of juveniles and brooders, coupled with the havocs of pollution and habitat destruction. An alarming situation like this will surely pave way to the commercial extinction of many target fish species in near future. This situation warrants rational management; hence focus should be directed on all aspects of fisheries with special emphasis on the effective utilisation of low value marine bycatch resources. Understanding this negative state, the Food and Agricultural Oganisation (FAO) Code of Conduct of Responsible Fisheries emphasised that the maritime nations should establish principles, policies and criteria for the sustainable utilisation of bycatch fish resources for responsible conservation of fisheries resources and management.

What are marine trash fish or bycatch resources?

The term 'bycatch' is defined as the catch which is retained and sold but which is not of the target species of the fishery. In other terms, they are the associated catch of fishes fished out in trawl net operations along with the fish species targeted and are often termed as 'trash fishes'. These are either thrown back to the sea or land and it has been estimated that nearly 23% of total fisheries catch around the world are discarded every year which amounts to 20 million tonnes. For instance, the Indian marine fish landings for the year 2010 revealed that bycatch species were caught to the tune of over 50000 tons a miscellaneous among the pelagic fish captured, about 25000 tons among demersel finfish, followed by 37000 tons of

stomatopods and also more or less equal quantum of other crustaceans and around 2000 tons of molluscs apart from cephalopods (CMFRI, 2011). Discard of bycatch species either to sea or land has several negative biological, ecological and economical impacts. Thus, there is an imperative need to utilise these resources, than merely discard so as to cater the protein requirements of exploding populace and for income generation.

Shall we make use of these bycatch or trashfish resources?

In this prelude, the reply to the quibble of making use of bycatch fishes is manifold. The various possibilities and avenues for the sustainable and judicious use of these bycatch or trashfish resources were broadly catagorised as follows:

1. Bycatch as alternative food species
2. Bycatch resources as export items
3. Valuable and value added products from bycatch resources
    a) Fish body oil and liver oil
    b) Fish Protein Concentrate (FPC)
    c) Fish Silage
    d) Fish pickles, surimi, sausages, breaded & battered products
    e) Fish collagen
    f) Chitin and chitosan
    g) Commercially viable products
4. Augment the utilisation of bycatch as fish meal
5. Bycatch for Ornamental purpose
6. Production of bio-fuel from bycatch and offal

1. **Bycatch as alternative food species**

As the population of target fish species is declining in a faster pace, it is inevitable to promote alternate species as food species so as to satisfy the demand. There exist quite a lot of species that are viable round the year in considerable quantities, with significant proximate composition; which can be considered or converted as edible ones. For instance, stomatopods (*Squilla* spp.) which are landed in bulk quantities form a chief bycatch component of shrimp fisheries. Majority of the species of this group are highly rich in biochemical composition, but are reluctant for human consumption due to its appearance and difficulty in extracting the meat. They can be considered as a candidate alternate food species which will create a sigh of relief to the already overexploited target fish species group.

2. Bycatch resources as export items

Certain marine species which we consider as trash has immense demand in other countries. For instance, jelly fish and puffer fishes are landed in considerable proportions during certain seasons in Indian coast are considered as trashfish; but forms an esteemed palatable food for Japanese and Chinese, that fetch high market price. These items will be processed by adopting viable technologies and can be converted to export grade items.

3. Valuable and value added products from bycatch resources

The bycatch resources can be effectively utilised and converted for the production of various valuable nutraceuticals like fish body oil and liver oil, Fish Protein Concentrate (FPC), Silage etc. The fish oil capsules available in the market are made out from commercially important forms like cod fishes. Low value clupeoid fishes like sardines are enriched with poly unsaturated fatty acids (PUFA) and can be utilised as a candidate species for the production fish oil, ensures various coronary and health benefits. FPC is a stable fish preparation, intended for human consumption are produced by hydrolising fish protein by

enzymatic or chemical treatment. FPC is superior to all other protein source as the quality of the protein is high because the aminoacids present in it are at right proportion for human nutrition. Special emphasis should be directed to the commercial level manufacturing of FPC from bycatch fishes which will solve the problem of malnutrition to a greater extent.

Bycatch fishes can be successfully converted into more palatable items like fish pickles, surimi, sausages, breaded & battered products that fetch higher price tags. Women self help groups can take this as a better choice of income generation as the raw materials are of low cost. As a prelude to this, it is to keep in mind that the products that are manufactured in hygienic conditions ensure a good shelf life and high market demand. The packaging materials employed should be sufficiently strong and durable to withstand stress during handling, storage and distribution.

Bycatch organisms having tremendous prospects for the production of diversified products like pearl essence, isinglass, fish leather, glue, gelatine etc. which are having immense industrial and market value. The existing technologies for the production of these products are to be modernised so to get more fruitful results.

4. Augment the utilisation of bycatch as fish meal

Fish meal produced throughout the world is a very cheap potential form of FPC, but is not intended for human consumption. Steps should be taken to enhance the present level of exploitation of bycatch resources for the preparation of fodder, poultry and shrimp feed by giving stress to its value addition.

5. Bycatch for Ornamental purpose

Substantial quantities of molluscan forms (gastropods & bivalves) landed as bycatch along with trawl catch is having aesthetic value as ornamental articles. Shells of the species, *Conus, Oliva* spp. etc. have excellent demand in international market. Sacred chank *Xancus pyrum*, matter of veneration for hindus is also a trawl bycatch. Special emphasis should be directed on this line to augment the proper exploitation of these resources. This acts as an alternate and additional livelihood option for fishers during lean months of fishing.

Way forward…

The aforementioned practices are to be imparted for the sustainable utilisation of bycatch resources. Though bycatch will not solve the world's food problem, but it provides new impetus to satisfy the protein requirements of the burgeoning population, if utilised in a proper manner. The role of research institutes is imperative for developing and popularising proper methodologies for the sustainable utilisation of bycatch resources. Moreover, the techniques implemented should be economically affordable to fishers with less capital investment. Considering the vast potential of effective utilisation of bycatch resources, there is an urgent and imperative need for enhancing research on this perspective, so as to achieve the goal of generating **"Cash from Trash : Wealth from Waste".**

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SEAWEED FARMING: CURRENT STATUS

C. Periyasamy, P. Anantharaman and P.V. Subba Rao*
CAS in Marine Biology, Annamalai University, Parangipettai, Tamilnadu, India
* Aquaculture Foundation of India, Madurai, Tamil Nadu/Guntur, Andhra Pradesh
Email: periyasamy.c@live.com, panatharaman@gmail.com

Introduction

Seaweeds are marine benthic, primitive and non flowering macrophytic marine algae occurring in inter tidal and subtidal regions of the seas and oceans of the world and are used as food for humans, as fodder for animals, as feed for birds and also as manure and liquid fertilizer, besides forming a source of phytochemicals like agar, alginate and carrageenan (Levring *et al.*, 1969; Chapman and Chapman, 1980).Worldwide, there are 47 countries with reports of commercial seaweed activity including China, North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia, USA, Taiwan and Vietnam and has gained prominence mainly for the manufacture of phycocolloids such as agar, alginate and carrageenan and for food purpose (Zemke White and Ohno, 1999).Among the 9900 seaweed species reported in the world (Khan *et al.*, 2009), 221 species are utilized commercially, 145 for food and 110 for phycocolloids production (Zemke White and Ohno, 1999). About 90% of seaweed production comes from culture based practices and China holds first rank in seaweed production, with *Laminaria sp.* accounting for most of its production. China is followed by North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia and USA. Taiwan and Viatmam have also succeeded in seaweed production. Among the seaweeds cultivated worldwide four genera such as *Laminaria, Porphyra, Undaria* and *Gracilaria* accounted for 93% of aquaculture production (Zemke White and Ohno, 1999 and Mc Hugh, 2003) and they are known as Marine Crop plants (Tseng, 1981). The world market of seaweeds or their products is more than 10 billion dollars (Zemke White and Ohno, 1999). The contribution of cultured seaweeds is 15% of global aquaculture (45,715,559 tons). The seaweeds mostly exploited for culture are the brown algae with 4,906,280 tons (71% of total production) followed by the red algae (1,927,917 tons) and green algae (33,700 tons) (Khan and Satam, 2003).

Seaweed Farming - World Scenario

Commercial farming of seaweed has long history. At present 10 algae are intensively cultivated such as two brown algae *Laminaria japonica* and *Undaria pinnatifida*, four red algae *Porphyra, Eucheuma, Kappaphycus* and *Gracilaria* and two green algae *Monostroma* and *Enteromorpha* and two blue green algae (microalgae) *Dunaliella* and *Spirulina* (Wikfors and Ohno, 2001). According to the latest world aquaculture production in 2012, the production of aquatic algae (mostly seaweeds) from 33 countries has been found to be 23.78 million tons wet (FAO, 2014). A few Asian countries dominated total algal production with China (53.97%) and Indonesia (27.40%) accounting for 81.37% of the total. Other countries included Philippines (7.36%), Republic of Korea (4.3%), Japan (1.85%), Malaysia (1.39%), Zanzibar (0.63%), Salmon Island (0.05%) and rest of the world (3.03%). All farmed aquatic algae (seaweeds) could be categorized into seven groups according to their nature and intended uses *viz*; *Kappaphycus* and *Eucheuma* (more than 8 million tons wet), Japanese kelp (*Laminaria japonica* – more than 5.5 million tons wet), *Gracilaria* sp (more than 2.5 million

tons wet), seaweed species not identified (more than 2.5 million tons wet), Wakame (*Undaria pinnatifida* – more than 2 million tons wet), *Porphyra* spp (more than 1.5 million tons wet) and other seaweeds and micro algae (less than 0.5 million tons wet) (FAO, 2015).

Seaweed Farming - Indian Scenario

The length of coastline of Indian peninsula ($08^0$04'- $37^0$06'N and $68^0$07'- $97^0$25'E) is 7516 km excluding its island territories (1256 islands) with 2 million kilometer square Exclusive Economic Zone (EEZ) (Subba Rao, 2000). Indian coastline is a part of the Arabian Sea in the west, Bay of Bengal in the east, and Indian Ocean in the south. It has been estimated that seaweed can be farmed in around 200 thousand ha or 0.001 percent of the EEZ (Krishnamurthy, 2005). Seaweeds grow abundantly along the coasts of Tamilnadu, Andhra Pradesh, Odhisa and West Bengal on east coast and Kerala, Karnataka, Goa, Maharashtra and Gujarat on west coast and also on the coasts of Lakshadweep and Andaman Nicobar Islands. Among the 9000 species of seaweeds enumerated in the world (Khan *et al.*, 2009), 1153 species are present in India with a total standing crop of 6, 77, 309 to 6, 82, 759 tons fresh (Subba Rao and Mantri, 2006). Commercial exploitation of seaweeds commenced since 1966 (Oza and Zaidi, 2001) and about 1518 tons of (dry) red seaweeds and 2285 tons of (dry) brown seaweeds were harvested from their natural habitats from southeast coast of India for manufacture of agar, alginate and liquid fertilizer (Kaliaperumal *et al*., 2004). Lack of sufficient raw material and paucity in supply of the same led the closure of many phycocolloid based industries (Thirupathi and Subba Rao, 2004) which had become a mandatory for development of cultivation technologies for the economic seaweeds .The rocky beaches, mudflats, estuaries and lagoons along the coast offer ideal habitats for seaweed farming. One of the CSIR (Council of Scientific and Industrial Research, New Delhi) laboratory, Central Salt and Marine Chemicals Research Institute (CSMCRI) at its field center Marine Algal Research Station (MARS), Mandapam camp initiated development of cultivation technologies for economic seaweeds such as *Gelidiella acerosa and Gracilaria edulis* during 1970s and during the subsequent years cultivation technologies for *Hypnea*, *Sargassum, Ulva*, and *Enteromorpha*, were also developed (Subba Rao *et al.*, 2004 and Rama Rao *et al.*, 1985). An laboratory of Indian council of Agricultural Research (ICAR), Central Marine Fisheries Research Institute (CMFRI) at its regional center at Mandapam Camp since 1970s also ventured to contribute its might towards cultivation technology of seaweeds like Gracilaria edulis and Acanthophora spicifera (Kaliaperumal *et al*., 2004**).** The cultivation of agar yielding seaweeds *Gelidiella acerosa* (Subbaramaiah *et al*., 1975; Patel *et al*., 1986), *Gracilaria* edulis (Raju and Thomas, 1971), carrageenophyte *Hypnea sp* (Rama Rao *et al*., 1985 and Ganesan *et al*.,2006), alginophyte *Sargassum sp* (Subba Rao *et al*., 1984), and edible seaweeds *Ulva fasciata* and *Enteromorpha compressa* (Subba Rao and Mantri, 2006) at different locations on Northwest and Southeast coast of India using various culture techniques were of note worthy.. These experiments revealed that *Gelidiella acerosa* could be successfully cultivated on dead corals and hallow cylindrical cement blocks and *Gracilaria edulis* and *Hypnea musciformis* on long line ropes and *Ulva fasciata* and *Enteromorpha compressa* on nets (Subba Rao *et al*., 2004 and Subba Rao and Mantri, 2006).

Kappaphycus alvarezii was introduced in September 1995 at Thonithurai (Mandapam near Pamban Bridge), in Gulf of Mannar waters, Tamilnadu by Dr P.V.Subba Rao, the Scientist-in-Charge of CSMCRI, Marine Algal Research Station, Mandapan Camp, Tamilnadu as such he is the "Father of *Kappaphycus* cultivation in India". The cultivation technology of this seaweed has been commercialized and the same has been awarded CSIR

Technology Award for the year 2001 in the area of Biological Sciences and Technology Development and this is the first Technology Award for the Institute. This seaweed was initially cultivated by different methods *viz*; Polythene Bag method, Net Bag method, Net enclosed Open Culture method and Raft method. Among these methods, raft method had been found to be the best one for commercial feasibility and finally this method is being now adopted by the fisher folk in Tamilnadu. M/S PepsiCo India Holdings Private Limited, Gurgaon took up this cultivation after getting the technology transferred in 2001 from Central Salt and Marine Chemicals Research Institute (CSMCRI), Bhavnagar and expanded the same on Palk Bay side of Bay of Bengal in the Mandapam region, Southeast coast of India. From 2006 onwards this cultivation was further taken up by Self Help Groups (SHGs) for their livelihood. Aquaculture Foundation of India (AFI) was instrumental to introduce Self Help Groups (five members in each group) through a project sanctioned by Department of Biotechnology (DBT), New Delhi to rehabilitate tsunami affected families. Cultivation of this seaweed generated self employment for hundreds of thousands of fisher folk in some coastal districts of Tamilnadu *viz.*, Ramanathapuram, Pudukkottai, Tanjore, Tuticorin and Kanniyakumari districts earning Rs**.** 15000/- to Rs. 16000/- per person per month (Periyasamy *et al*., 2014a; 2015). The feasibility of cultivation of this seaweed was successfully established on Okha Mandal coast at Mithapur, Okha and Beyt Dwaraka on Northwest coast of India by Subba Rao *et al* (2008) and this is first international publication from India. Subsequently cultivation of this seaweed was carried out at different locations on Indian coast: Vellar estuary, Tamilnadu (Thirumaran and Anantharaman, 2009), Palk Bay waters of Ramanathapuram, Tamilnadu (Periyasamy *et al.,* 2014a, 2014b and 2015), Vizhinjam, Kerala (Bindu, 2010), Saurashtra coast (Gunalan *et al*., 2010; Kotiya *et al*., 2011; Sureshkumar *et al*., 2015) **.** This alga gave a crop yield of 25 tons (dry)/ ha/ yr for net bag method, 40 tons (dry)/ ha/ yr for raft method and 45 tons (dry)/ ha/ yr for open culture method in eight harvests in the Mandapam region of Southeastren coast of India whereas at Okha, Northwest coast of India it yielded 22 tons (dry)/ha/yr in five harvests for the raft method (Subba Rao and Mantri, 2006).

Although economically feasible cultivation technologies are available for *Gelidiella acerosa* and *Gelidiella edulis* (Subba Rao *et al*., 2004), as of now *Kappaphycus alvarezii* alone has been taken up for seaweed farming by self help groups along Tamilnadu coast .Bottle necks for commercial cultivation of other promising seaweeds such as *Gracilaria edulis, Gelidiella acerosa, Enteromorpha compressa, Gracilaria dura, etc*. need to addressed.. Moreover seaweeds could be farmed/ cultivated in 200,000 hectares or 0.001% of the 2 million km^2 of Exclusive Economic Zone (EEZ) (Krishnamurthy, 2005) providing a lot self employment opportunities for the coastal people. It has been estimated that India has the potential to produce 1 million tons of dried seaweed, *Kappaphycus alvarezii* although its production was only 4210 tons wet (Krishnan and Narayanakumar, 2013).

Conclusion

As a whole the seaweed cultivation is not complicated but very simple and easy. Capital investment for this cultivation is less than any other aquaculture practices. It does not involve any inputs that are harmful to the environment and on the other hand it is beneficial to the ecosystem. Though seaweed farming is labour intensive, it offers lucrative earning as the market for seaweed products are diversified in the areas of food formulations, pharmaceutical and other industrial sectors. Today seaweed farming is a viable alternative source of income for small scale fishermen.

Acknowledgement

The authors wish to thank Dr. K. Kathiresan, Dean and Director, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Cuddalore (District), Tamilnadu, India and the higher authorities of Annamalai University, Chidambaram, Tamilnadu, India. The aurhors also thank the funding agency - funded through CMLRE Project, Ministry of Earth Science, New Delhi.

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OCCURRENCE OF MARINE ACTINOBACTERIA IN THE SUBTROPICAL FRONT SEA WATER, INDIAN OCEAN

*Sivakumar, K., C. Aarthi, P.V. Bhaskar[1] and N. Anilkumar[1]
CAS in Marine Biology, Faculty of Marine Sciences,
Annamalai University, Tamilnadu.
[1]National Antarctic Ocean Research Centre, Ministry of Earth Sciences, Goa.

*** Corresponding author:** oceanactino@gmail.com

1. Introduction

Marine environment is the largest environment on the planet and the oceans serve as the host for huge microbial populations whose diversity is still challenging. Among the ocean microbial populations, actinobacterial diversity is of paramount importance for natural products synthesis. Earlier works have reported the existence of marine actinobacterial genera: *Solwaraspora* (Magarvey *et al*., 2004), *Serinicoccus* (Yi *et al*., 2004), *Salinispora* (Maldonado *et al*., 2005), *Marinispora* (Kwon *et al*., 2006), *Demequina* (Yi *et al*., 2007) and still others. Present study has witnessed the occurrence of actinobacterial populations in planktonic lifestyle in the subtropical seawater where their existence is predominant in association with marine sediments or they occur as invertebrate symbionts. Present work

describes the cultural and physiological characters besides the 16S rDNA sequence analysis of the selected actinobacterial strains from the sub tropical front of the Indian Ocean.

2. Methods

2.1. Sample Collection

Water samples were collected during the 6th Indian Scientific Expedition to the Indian Ocean Sector of the Southern Ocean on board ORV *Sagar Nidhi* (Fig. 1 & 1a). The samples were collected from the stations viz., Sub tropical Front (40ºS, 58º30'E) using CTD (SEABIRD 911 plus, USA). Collected samples were transferred to sterile containers, stored in an ice box and brought to the laboratory for further analysis.

2.2. Isolation and enumeration of actinobacteria

Isolation of actinobacteria was carried out using Starch Casein Agar medium, prepared with an addition of 20mg/l of nystatin and cycloheximide (100mg/l), respectively to minimize bacterial and fungal contaminations (Kathiresan *et al*., 2005). The plates were incubated at 18°C for 15 days. The strains were sub-cultured on the Starch Casein Agar slants (medium with 100% sea water) for further investigations.

Fig. 1. Map showing the study area

Fig. 1a. ORV *Sagar Nidhi*[*]

([*]Note: ORV *Sagar Nidhi* is an ice-strengthened multidisciplinary vessel operated by the National Institute of Ocean Technology, India. The vessel is capable of carrying out geo-scientific, meteorological and oceanographic research, and is designed with blue-water capability with ranges of up to 10,000 nautical miles (19,000 km) for voyages lasting up to 45 days. She is supports the research in the Indian and Antarctic Oceans).

2.3. Phenotypic and biochemical characteristics

Cultural characteristics were determined after 3-4 weeks according to the methods given in the *International Streptomyces Project* (ISP) (Shirling and Gottlieb, 1966). All the media used were supplemented with 100% sterile seawater. The colours of the substrate and aerial mycelia and any soluble, reverse side pigments production were examined. Characteristics of the spore bearing hyphae and spore chains were determined using direct microscopic examination of the culture surface by using cover-slip culture method. Adequate magnification (400X) was used to establish the presence or absence of spore chains and to observe the nature of sporophores. Biochemical characteristics were determined by the ability of utilization of sole carbon sources by the strains, following the methods recommended in *International Streptomyces Project* (ISP).

2.4. Chemotaxonomy

Established procedures were used to determine the diagnostic isomers of DAP and the whole cell sugars (Cummins and Harris 1956; Lechevalier and Lechevalier 1970).

2.5. 16S rRNA gene sequencing

Genomic DNA was extracted from the isolates by following the procedures described by Ausubel *et al*. (1994). PCR amplification of the 16S rDNA preparations was carried out by the methods described by Karuppiah *et al*., (2011). The resultant PCR products were purified and the purified fragment was directly sequenced using an Ampli Tag FS DNA sequencing Kit (Applied Biosystem). The data were analyzed using applied biosystem DNA editing and assembly software and sequence comparisons were obtained using the Micro Seq Software.

2.6. Analysis of sequence data

Sequence similarity search was made for the 16S rDNA sequence of all isolates by applying their sequence to BLAST search of the NCBI (National Centre for Biotechnological Information, USA). Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4 (Tamura *et al*.,, 2007) after multiple alignment of data by CLUSTAL_X (Thompson *et al*., 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou and Nei (1987) from K_{nuc} values (Kimura, 1980). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

3. Results and discussion

3.1. Isolation and enumeration of actinobacteria

Bimodal distribution of actinobacteria in nearshore tropical marine environments had been reported by Jensen *et al*. (1991) and Takizawa *et al*. (1993). Existence of indigenous marine actinobacteria from oceanic sediments has been suggested from the earlier studies (Weyland and Helmke, 1988; Takizawa *et al*., 1993; Ravel *et al*. 1998). Though, there are more evidences for the presence of actinobacteria in the marine sediments, studies on the marine actinobacterial distribution in sea water is less. Ghanem *et al*. (2000) proved the existence of uneven distribution of marine actinobacteria, in accordance with the occurrence of microenvironments, providing with sites of activity, governed by intermediate factors by recording higher population density in marine sediments rather than the sea water. Present study is an attempt to explore the diversity of marine actinobacteria from the sub-tropical sea waters. Actinobacterial population density recorded from the sub-tropical sea water was

$1.2x10^{-2}$ CFU/ml. Based on the distinct colony morphology, four isolates (SSTF1, SSTF2, SSTF3 and SSTF4) were selected for further investigation.

3.2. Phenotypic and biochemical characteristics

Cultural, microscopic and biochemical characterisitics of the four strains are shown in Table 1.

3.3. Chemotaxonomy

Chemotaxonomic studies were performed to check the cell wall aminoacid type and to ensure the characteristic sugar patterns. The study revealed that the strains SSTF1 and SSTF3 possessed mesodiaminopimelic acid as the cell wall aminoacid with the characterisitic sugars viz., galactose and arabinose, indicating cell wall type IV. Whereas, the strains SSTF2 and SSTF4 possessed L-Diaminopimelic acid and glycine as the cell wall amino acids with no characteristic sugar patterns indicating cell wall type I.

3.4. Analysis of sequence data

Results of the 16S rRNA gene sequence comparison clearly demonstrated that the strains SSTF1 and SSTF3 are members of the genus *Saccharopolyspora* and the strains, SSTF2 and SSTF4 are the members of the genus *Streptomyces*. In the phylogentic tree based on the neighbor-joining algorithm, the strain SSTF1 formed a distinct subclade with *Saccharopolyspora gregorii* and the strain SSTF3 formed a distinct subclade with *Saccharopolyspora gloriosa* (Fig.2). In the same way the strain SSTF2 formed a distinct subclade with *Streptomyces flavoviridis* and the strain SSTF4 formed a distinct subclade with *Streptomyces variabilis* (Fig.2). The 16S rRNA gene sequence similarities between the strains SSTF1 and SSTF3 were 89.8 and 92.8%, respectively. Similarly, the 16S rRNA gene sequence similarities between strains SSTF2 and SSTF4 were 91.7 and 93.4%, respectively. Though the strains SSTF1 and SSTF3 formed a subclade with the respective *Saccharopolyspora* sp., the sequence similarity difference suggested that the strains belong to the genus *Saccharoployspora*. Though the strains SSTF2 and SSTF4 formed a subclade with the respective *Streptomyces* sp., the sequence similarity difference suggested that they belong to the genus *Streptomyces*.

4. Conclusion

In general, it is assumed that the occurrence of actinobacteria is largely associated with the marine sediments or they occur as symbionts in the invertebrates but their planktonic lifestyle is rare (Bull and Stach, 2007). Hence, cultivation efforts were made to get considerable actinobacterial diversity form marine samples, either from sediments or from marine organisms (Magarvey *et al*., 2004; Jensen *et al*., 2005; Moldonado *et al*., 2005; Gontang *et al*., 2007), rather than the sea water. Today, actinobacteria are consistently observed in sea water when culture-independent techniques are applied to marine samples. Application of molecular techniques has also provided with a new perspective to the diversity marine of actinobacteria (Ward and Bora, 2006), which are omnipresent and even a small portion of them occurs as bacterioplankton in sea water (Giovannoni and Stingl, 2005). In open ocean regions, proportion of actinobacteria can rise from zero to 35% of the bacterioplankton (Bull and Stach, 2007). Further, the actinobacterial diversity from sea water can be elucidated easily using the culture independent techniques.

Present study, reporting the two genera *Streptomyces* and *Saccharopolyspora* from the subtropical sea water, signifies that there is no dearth of the microbes in the sea water and it would be worth attempting to explore their diversity, using metagenomics. Such culture

independent studies may reveal and include rare and novel forms of planktonic actinobacteria in the marine realms.

Acknowledgement

The authors wish to thank the Dean, Faculty of Marine Sciences and the authorities of the Annamalai University for providing the facilities. They also thank Prof. T. Balasubramanian, Former Dean, Faculty of Marine Sciences, Annamalai University and Dr. C.T. Achuthankutty, Chief Scientist (Southern Ocean Expedition 6), NCAOR for their help and encouragement.

Table 1. Phenotypic and biochemical characteristics of four actinobacterial strains isolated from the subtropical front.

Characteristics	**SSTF1**	**SSTF2**	**SSTF3**	**SSTF4**
Spore arrangement	Hooks and spiral	RA-S	Hooks and flexous	S
Aerial mycelium	White	Grey	White	White
Reverside pigment	Whitish yellow	-	-	-
Soluble pigment	-	-	-	-
Melanoid pigment	-	-	-	-
Utilization of carbohydrates as sole carbon sources				
L-arabinose	-	+	-	+
D-galactose	-	+	+	-
D-lactose	-	+	-	-
D-maltose	+	+	+	-
D-raffinose	+	-	+	-
L-rhamnose	-	+	-	-
Sucrose	-	+	+	+
D-xylose	+	-	+	+

RA-Retinaculiaperti; S-Spiral

(+) – Positive utilization; (-) – Negative utilization

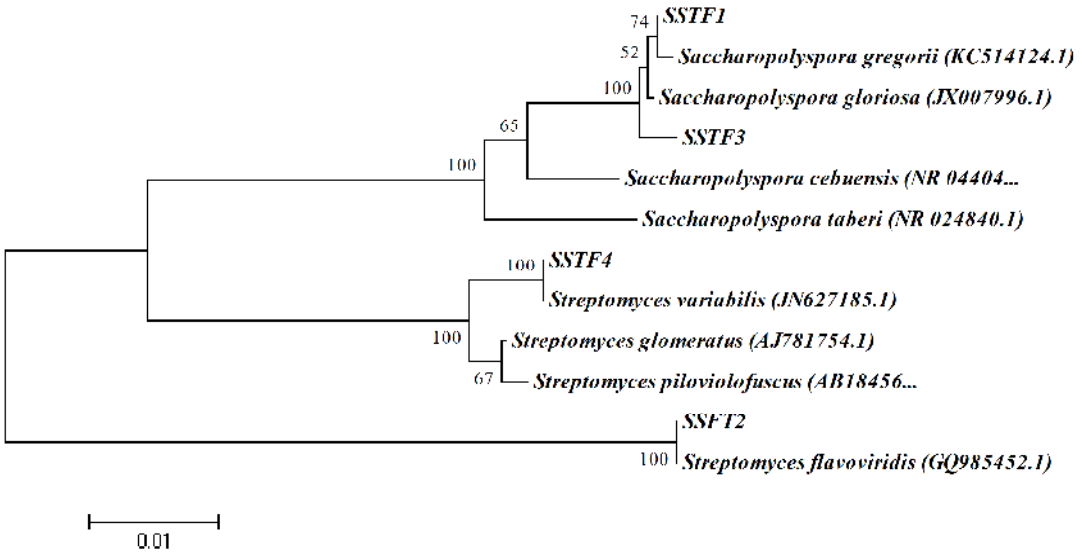

Fig. 2. Neighbour-joining phylogenetic tree, based on almost-complete 16S r RNA gene sequences, showing the relationships between the isolated strains and the type strains of recognized species of the genus *Saccharopolyspora* and *Streptomyces*. Numbers at the nodes indicate bootstrap percentages (based on a neighbor-joining analysis of 1000 resampled data sets). Bar, 0.01 substitutions per nucleotide position.

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MARINE NON-RENEWABLE RESOURCES

Dr.P.Sivasubramanian
Associate Professor in Geology
V.O.Chidambaram College, Tuticorin

1. Introduction

Natural resources are rapidly disappears from the land by over exploitation, man has turned to the oceans as his new source of supply. The world is now in short supply of many resources which have been depleted from the landmasses. Mercury, Tin, silver, cobalt etc are in short supply. There are millions and millions of dollars worth of these natural resources in ocean water and ocean floors. Yet because of the pressure and depth we can examine only the continental shelves. Shelves are areas adjacent to the continents which are less than 656 feet deep or 50 miles out from the continents, whichever is greater. Unless we develop international agreements and means of exploring the ocean floor, our source of raw materials will be limited and in short supply.

Renewable resources are those which get replenished quickly. Example. Water, soil etc. Non-renewable resources are those have a limited stock. Once the stock is exhausted it may take thousands of years to be replenished. Example. Oil and natural gas.

A scientific revolution in our understanding of the way the Earth works started in the 1960's that significantly expanded our knowledge of marine minerals while the 1982 United Nations Convention on the Law of the Sea was being formulated and adopted. The scientific revolution entailed a major change in viewing the ocean basins and continents. Before the scientific revolution, the ocean basins were viewed as big bathtubs that passively contained the oceans. The continents and ocean basins were viewed as permanent features that had remained in their present positions through most of Earth's history. The marine mineral are thought to be derived from erosion of land and carried into the ocean in particulate or dissolved form by rivers. These minerals comprised heavy metal deposits (tin, gold, etc.), gemstones (especially diamonds), sand and gravel (aggregates) deposited in sediments of continental margins, phosphorites deposited on hard rock substrates of continental margins, and polymetallic nodules (nickel, cobalt, iron, and manganese in varying concentrations) precipitated on the floor of the deep ocean from metals dissolved in seawater. Of these minerals, tin, diamonds, and sand and gravel are viable industries, with the largest annual production value attributed to sand and gravel.

The development of plate tectonics theory has changed our view of ocean basins from big bathtubs to dynamic features that open and closes on a time scale of tens to hundreds of millions of years with concomitant movement of the land areas known as continental drift. The scientific revolution recognized the ocean basins as sources of types of non-fuel mineral deposits in addition to those previously known derived from erosion of land. These newly recognized types of marine mineral resources include polymetallic massive sulphides

containing copper, iron, zinc, silver, gold and other metals in varying amounts. Polymetallic sulphides deposits are concentrated over two types of plate boundaries 1. Divergent plate boundary and 2. Convergent plate boundaries.

Another newly recognized type of marine mineral resource is cobalt-rich iron-manganese (ferromanganese) crusts that are precipitated over millions of years on the submerged flanks of inactive underwater volcanoes from metals dissolved in seawater derived from input of metals by both rivers and seafloor hot springs. None of these newly recognized types of marine mineral deposits are renewable resources, as they all require thousands to millions of years to accumulate in economically interesting grades and tonnages. They are resources for the future with no present production. We are still at an early stage in exploration of the oceans with only a few percent of the seafloor known in detail and even less known about what lies beneath the seafloor, so new discoveries will continue to be made.

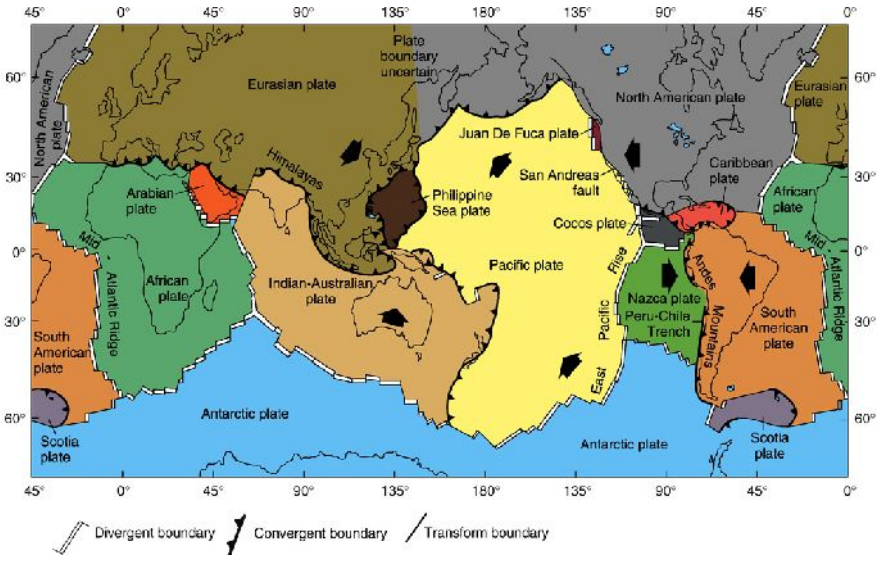
1.1 The Science of Marine Minerals: Before the Theory of Plate Tectonics

Prior to the advent of the theory of plate tectonics in the 1960s, the ocean basins were regarded as passive containers like big bathtubs that simply held the oceans and served as a repository for the rock material washed in from the continents. The ocean basins and continents were then considered to be permanent features that had remained in their present positions and retained their present shapes through much of the Earth's 4.6 billion year history.

This pre-plate tectonic view of the Earth correctly recognized those marine minerals that are derived by two processes of erosion of rocks on land and their transport into the ocean primarily by rivers.

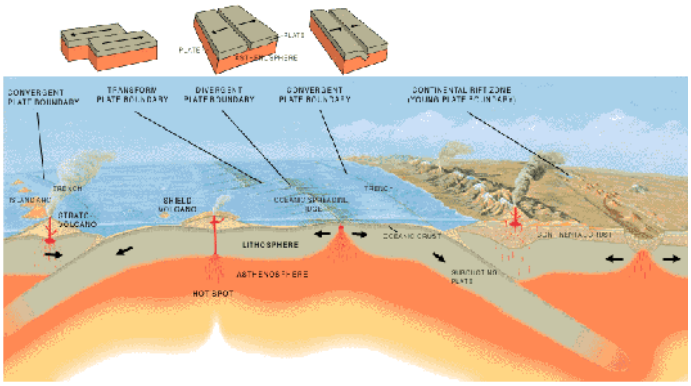
1.2 The Science of Marine Minerals: After the Theory of Plate Tectonics

The theory of plate tectonics changed our view of the ocean basins from big bathtubs that contain materials washed off the land by rivers, to active sources of materials that form types of mineral deposits different from those derived from the erosion of land. A new view of a dynamic Earth according to plate tectonics has replaced the old view of a static Earth with permanent immobile continents and ocean basins. According to this new view the outermost shell of the Earth is formed by a rigid layer about 100 kilometres thick called the "lithosphere". The lithosphere effectively floats on a more plastic underlying layer called the "asthenosphere". The lithosphere is fragmented into some twelve major plates and numerous minor plates.



These plates move interdependently in different directions. According to their moving direction, the plate boundaries are classified as 1. Divergent, 2. Convergent and 3. Transform fault boundaries. At divergent plate boundary, molten rock (magma) upwells beneath divergent plate boundaries, cools, solidifies, and forms new lithosphere. These plates moves apart from each other like two diverging conveyor belts at an average rate of centimetres or inches per year in the process of seafloor spreading.

The second type of plate boundary is called a "convergent" plate boundary because two lithospheric plates come together. One plate generally moves down beneath the other at an ocean trench. The downgoing plate descends and melts back into the Earth's interior in the process of



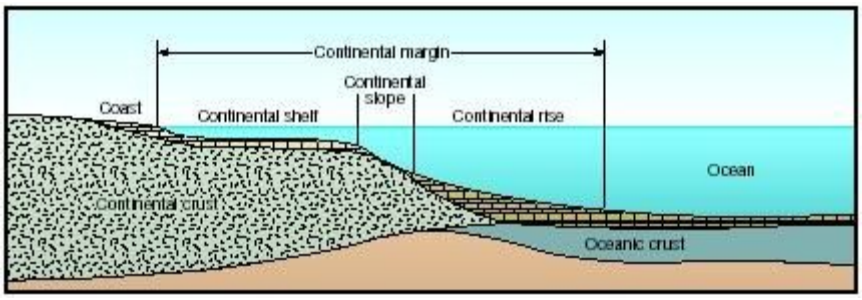
Types of Plate Boundaries

subduction. Volcanic island chains like Japan, the Marianas, the Philippines, and New Guinea that lie along the western margin of the Pacific Ocean, and volcanic mountain chains like the Andes of South America and the Cascades of North America, are formed by volcanic activity generated by melting of the lithosphere as it descends at convergent plate boundaries.

A third type of plate boundary, expressed as an offset along the length of a divergent or a convergent plate, serves to accommodate the linear boundaries to the curvature of the spherical Earth.

Plate boundaries are part of a global system of transfer of heat and chemicals between the Earth's hot interior and the oceans. The heat drives processes of mineralization and the chemicals are the source of the materials that are concentrated as mineral deposits at and away from plate boundaries. These mineral deposits include polymetallic massive sulphides deposits at sites along plate boundaries and emissions of metals that combine with those dissolved from continents to form polymetallic nodules and cobalt-rich ferromanganese crusts.

2. Seafloor Settings of Marine Mineral Deposits



Divisions of Continental Margin

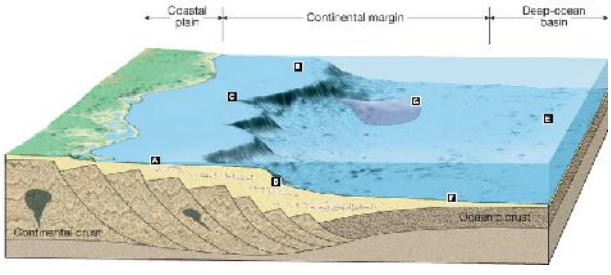
The marine minerals begins by considering the seafloor settings of both fuel and non-fuel marine minerals. The seafloor forms two broad divisions and numerous subdivisions. The two broad divisions are the margins of continents and the ocean basins. Continental margins are subdivided into passive and active types with important implications for the occurrence of non-fuel and fuel minerals (oil and gas).

2.1 Passive continental margin

A passive continental margin is where continental crust passes directly into ocean crust without any plate boundary being present. No divergent motion or subduction occurs here. Almost all of the edge of the Atlantic is passive continental margin - i.e. the east coast of N and S America and the west coast of Africa.

These margins are termed "passive" because they passively subside after breaking apart and are generally buried by layers of sediment up to tens of kilometres thick eroded from the adjacent landmass and deposited on the original rifted edges of the continents. Therefore, passive

continental margins have potential for placer-type mineral deposits of metals and gemstones eroded from the adjacent landmass and for lime and phosphorite deposits precipitated directly from seawater and/or indirectly by marine organisms. The thick accumulations of sediment generally contain organic matter primarily from the remains of tiny marine plants and animals



Passive Continental Margin

that settle out of the overlying water. The combination of pressure and heat as the organic matter is buried by sediment gradually converts it into hydrocarbons that form oil,

gas and gas hydrates. The hydrocarbons form prospective accumulations when concentrated by the coincidence of a large volume of porous and permeable sediment as a reservoir and one of a variety of structures capped by an impermeable layer as a trap.

In the case of gas hydrates, methane gas generated primarily by the decay of organic matter is incorporated into ice that forms under the high-pressure, low temperature conditions that exist within portions of the sedimentary layers underlying passive continental margins. In contrast to oil and gas, which is concentrated in natural reservoirs within the sedimentary layers, methane is disseminated in large volumes of sediment underlying large areas of continental margins.

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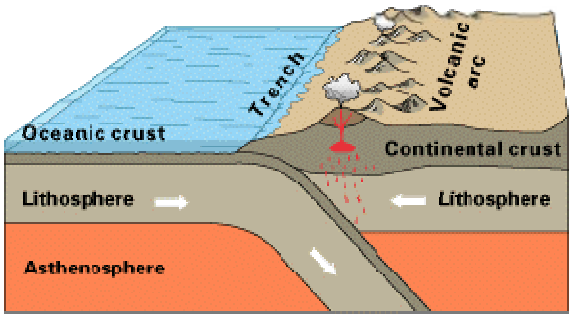
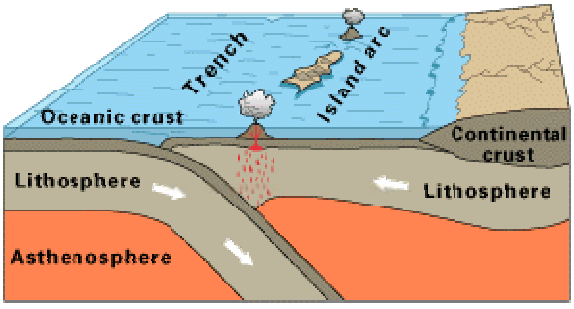
2.2 Active continental margins

Active continental margins occur at convergent plate boundaries where plates come together and the lithosphere is destroyed by descending back into the Earth's interior in the process of subduction. These margins are termed "active" because they are associated with earthquakes and volcanically active island chains like Japan and Philippines.



Oceanic-continental convergence



Oceanic-oceanic convergence

The generation of magma by the descending plate create conditions for concentration of seafloor polymetallic massive sulphides and related metallic mineral deposits. Sedimentary basins on the seaward and landward sides of the volcanic island chains have the potential for the occurrence of placer deposits and of oil, gas, and gas hydrates.

3. Sources of Marine Minerals

Non-fuel marine minerals are considered in this chapter in the following order related to their sources of origin:

a. Mineral deposits derived from land sources. These constitute beach deposits and placer mineral deposits, as well as certain deposits precipitated from seawater (lime and phosphorite).

b.Mineral deposits derived from sources in ocean basins. These comprise metalliferous sediments, polymetallic massive sulphides, and related deposits.

c.Mineral deposits derived from a combination of land and ocean basin sources. These comprise polymetallic manganese nodules and cobalt-rich ferromanganese crusts.

3.2 Marine Mineral Deposits from Land Sources

Marine mineral deposits from land sources comprise placer deposits, deposits of lime, phosphorite, and salt as well as beach deposits of continental margins.

3.2.1 Beach Placer Deposits

Placers are those deposits of metallic minerals and gemstones that are eroded from source rocks on land and transported into the ocean by rivers where they are sorted and concentrated by water motions (waves, tides, currents) by virtue of the high density of the minerals relative to surrounding sediments. Principal metals in minerals of placer deposits are barium, chromium, gold, tin, titanium, thorium, and zirconium. The principal gemstone is diamond.

3.2.2Gold

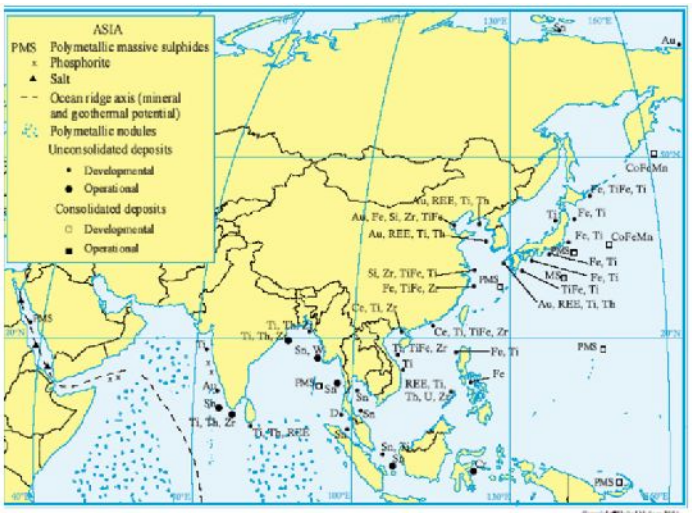
So far database contained records of 21 occurrences of offshore gold in Alaska (USA), Nova Scotia (Canada), Chile, China, India, Korea, Fiji, Philippines, Russia, Sierra Leone, Solomon Islands and Southern New Zealand. The only offshore mining operation for gold occurred in Nome (Alaska) from 1987 to 1990. The total production was 3,672 kg of gold recovered from 4,460,000 cubic metres of sediment. The deposit was identified as an ancient glacier deposits reworked by waves and tides.

3.2.3 Tin

Tin is the major placer deposit that is mined. Tin-bearing gravel is dredged from water depths up to 50 metres (about 164 feet) on the continental shelf at sites off Myanmar, Thailand, and Indonesia. It is estimated that offshore production of tin in these areas accounts for some 10 percent of world production with an annual value approaching US$100 million.

3.2.4 Thorium

Placer mineral Monazite contain up to 15% of Thorium. It is widespread in the western coast of USA, India, Brazil and Australia. Monazite is commonly associated with heavy minerals such as ilmenite, zircon, and the rutile. In India, Monazite is available in Manavalakurichi of Kanyakumari district. This monazite deposits extends up to 6 km and with an average width of 45 m. The total average content of heavy minerals is around 39 %. Of these, ilmenite forms the major constituent (24%), with rutile (1.8 %), zircon (2%), monazite (1%), sillimanite (3.5%) and garnet (5.5%). The monazite has a total of 8% $Th0_2$. IRE mines this deposits.



Off-shore mineral map of Asia

3.2.5 Titanium

Titanium is obtained from ilmenite and rutile and widely available in the beach sand. This is extensively worked out on the coasts of Australia, India, Brazil and Canada. 25% of the world ilmenite production is from Australia. Ilmenite-rich major beach and dune sand deposits occur in the coastal stretches of Kerala (Chavara), Tamil Nadu (Manavala kurichi, Midalam, Vaippar), Andhra Pradesh, Orissa and Maharashtra. The Indian ilmenite commonly contains 50-60% TiO_2 and is suitable for various process technologies.

3.2.5 Zirconium

It is extracted from zircon, a mineral which is generally found concentrated with other minerals such as ilmenite, rutile, garnet and monazite.12 million tons of resource of zircon is inferred chiefly from the beach placer sands. A reserve of 1.2 million tons in India is estimated. Australia and Sri Lanka share the major part of the world's annul production of zircon to a tune of 5,43,000 tons.

3.2.6 Diamond

Diamonds were discovered on the shores of Namibia and South Africa around 1928. The origin of the diamonds is the mouth of the Orange river from their original kimberlite Pipes. From the land, the miners went to sea, using gravel suction pumps operated by divers. During the 1970s, exploration was undertaken to determine the possibility of discovering offshore deposits in paleobeaches and paleochannels. Exploration revealed the presence of offshore deposits that extend to the south in Namaqualand (South Africa). After a prolonged period of testing, mining started in 1990 with a production of 29,000 carats (ct). In 1998, offshore production was close to 1 million ct. It decreased to 650,000-800,000 ct during subsequent years due to mechanical breakdowns. The sediment is airlifted or pumped and processed in dense media to obtain gravel concentrate. Diamond sorting is by hand or by automatic X-ray equipment.

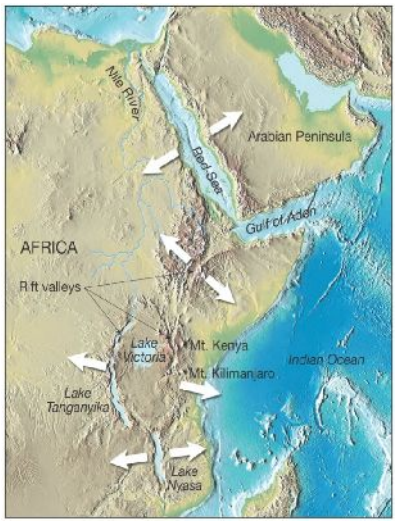
3.2.7 Lime and Phosphorite

As noted, lime, phosphorite and salt deposits of continental margins are derived by chemical weathering of continental rocks and transported by rivers in a dissolved state into the ocean where they are precipitated on continental margins under the right conditions.

Lime (calcium carbonate) may be precipitated in shallow water in subtropical and tropical climate zones or extracted from seawater by microscopic plants and animals and deposited as their remains (shells and other forms).

Marine sedimentary phosphorite deposits are naturally occurring compounds containing phosphate in the form of cement binding sediment. Phosphates extracted from phosphorites are composed of calcium phosphate. This is an important fertiliser and, therefore, agriculture mainly benefits from its use.

They are mainly found as nodule rock in a sandy deposit or as soft sediments. The phosphates lie in the top layer in a large surface area. Marine Phosphates deposits are found at various water depths that range between 0 and 2,000 metres.



Rifting of Continent and formation of new ocean

It is mainly found in off-shore of China, United States, Moracco and Western Sahara, Russia, Tunisia, Jordan, Brazil, Syria, Israel, South Africa and India.

4. Marine Mineral Deposits from Sources in Ocean Basins

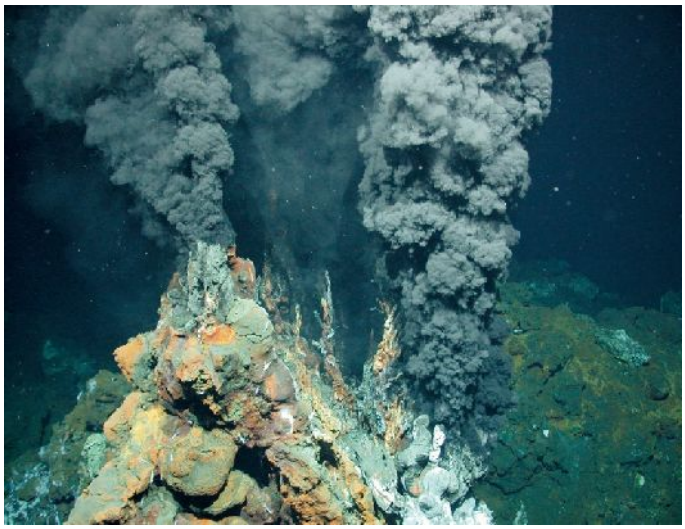
4.1 Hydrothermal Deposits: Metalliferous Sediments

The metalliferous sediments of the Atlantis II Deep in the northern Red Sea constitute the first hydrothermal deposit (a mineral deposit concentrated by hot, metal rich aqueous solutions) found at a divergent plate boundary in the ocean, and remain the most efficient ore-forming system and the largest such deposit found to date. The Atlantis II Deep is a basin roughly 10 kilometres (6.2 miles) in diameter at a water depth of 2 kilometres (1.2 miles) that lies on the divergent plate boundary that rifted Africa from the Arabian peninsula about 10 million years ago and generated lithosphere at a slow full-spreading rate (2 centimetres or 0.8 inches per year) to account for the present width of the northern Red Sea (200 kilometres or 124 miles).

The largest known sulphide occurrence is located in the Red Sea, where tectonic forces are pulling Africa and the Arabian Peninsula apart. Here, the sulphides are not associated with black smokers, but appear in the form of iron-rich ore muds with high contents of copper, zinc and gold. This occurrence, at a water depth of about 2000 metres,

was discovered in the 1960s. Because of its muddy consistency, it appears that these deposits will not prove problematic to mine, and this was successfully tested in the 1980s.

4.2 Polymetallic Massive Sulphides



In certain places of the ocean the downwelling cold and heavy seawater encounters hot or molten rocks at depths of several kilometres beneath the seafloor. The seawater is then heated, expands thermally, becomes lighter, and rises buoyantly. As the hot seawater rises through fractures in the ocean crust it reacts chemically with the surrounding volcanic rocks and dissolves metals.

assive sulphides form at black smokers

The solutions become enriched in metals as they flow upward through fractures. The metal-rich solutions discharge at high temperatures (up to 400^{o} C) from the seafloor into the water column where the remaining metals precipitate as clouds of tiny crystalline sulphides particles. These hot springs were named "black smokers" because the plume of metallic sulphides particles discharging from a chimney-like structure formed by precipitation of metallic minerals from the solutions resembles an active factory Chimney. Massive sulphides deposits are extremely localized at sites where the right combination of heat, fluid circulation pathways, metal sources and other factors focus the ore-forming systems.

The hot and molten rocks that heat the seawater circulating through the ocean crust to form massive sulphides deposits and hot springs occur at sites along the two types of plate boundaries.

- divergent plate boundaries
- convergent plate boundaries

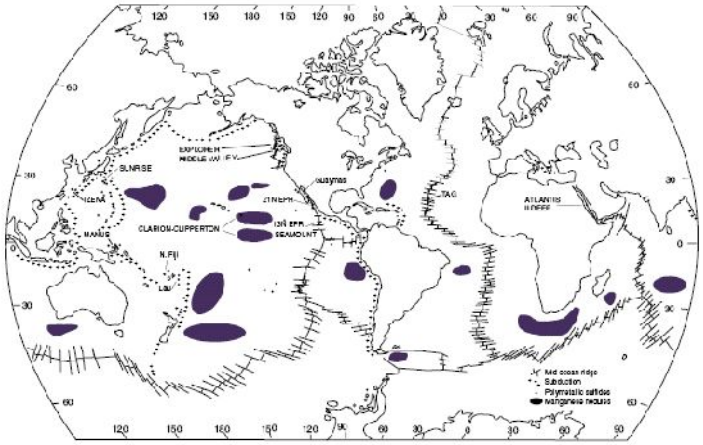
Once formed at plate boundaries, the deposits are transported away from the boundaries by the process of seafloor spreading.

4.3 Chromium, Nickel and Platinum Group Metal Deposits

Metallic mineral resources of the Earth's mantle, which underlies the ocean crust, are poorly known because of limited exposures of these deeper subseafloor rocks. The types of deposits anticipated in the upper mantle comprise chromium in the form of chromite deposits, and nickel- and platinum-rich sulphides mineral phases. These deposit types are potential future sources of chromium and platinum group metals.

5. Marine Mineral Deposits from Sources on Continents and in Ocean Basins

5.1 Polymetallic Manganese Nodules



Map showing occurrences of Manganese Nodules around the world

Polymetallic nodules typically range in size from that of a golf-ball to a tennis ball. They lie partially buried on the surface of sediments that cover vast plains on the deep seafloor (typical water depth 5 kilometres. These "abyssal" plains are the largest physiographic province on Earth covering some 70 percent of the area of ocean basins and 30 percent of the Earth's surface The upper portion of the nodules accumulates metals that are precipitated from seawater . The lower portion of the nodules partially buried in sediment accumulates metals from pore-water in the underlying sediments. The metal accumulation rates are so slow that it generally takes millions of years to form a manganese nodule. The metals come from two sources. The primary source is considered to be metals from rocks on land as part of the weathering process and transported to the ocean by rivers. The secondary source is metal-rich solutions that discharge through hot-springs at ocean ridges. Highest values of metals in nodules occur in equatorial regions of oceans where the remains of tiny plants and animals that concentrate the metals from seawater sink to the seafloor. Areas of commercial interest in the eastern equatorial Pacific (Clarion-Clipperton zone) and in the central equatorial Indian Ocean cover millions of square kilometers.

5.2 Cobalt-Rich Ferromanganese Crusts

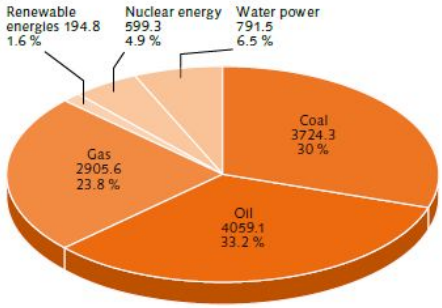
Like manganese nodules, the crusts are slowly precipitated from metals that are dissolved in seawater. The metals are derived from a combination of sources comprising dissolution from continental rocks and transport into the ocean by rivers, and discharge of metal-rich hot springs in the deep ocean. Like manganese nodules the crusts contain a suite of metals (iron, manganese, cobalt, nickel, platinum, and other metals), that varies depending on proximity to different sources. Instead of accumulating as nodules on the sediment surface of abyssal plains in the deep ocean, cobalt-rich-ferromanganese crusts accumulate as extensive layers directly on volcanic rock that forms submerged volcanic seamounts and volcanic mountain ranges. The

crusts accumulate slowly over millions of years and attain a thickness up to 25 centimetres. The large number of seamounts in the Pacific Ocean is favourable place for the occurrence of cobalt-rich ferromanganese crusts.

6. Energy Resources of Ocean

6.1 Oil and Natural Gas

There is a long tradition of offshore natural gas and oil production. The United States first coastal oil rigs were constructed in 1890. With drilling and extraction technology also becoming increasingly sophisticated, it is now possible to extract oil and gas at ever greater depths. The water depth record for oil production is currently held by an international oil company which produces oil from a well, located in the Tobago field, 2934 metres below the surface of the Gulf of Mexico. The world's growing energy hunger is driven to a large extent by population growth in Asia and ongoing industrialisation in the emerging economies. China, India and West Asian nations account for around 60 per cent of the world's growth in energy demand. Today, energy production still largely relies on the burning of fossil fuels: natural gas, oil and coal.



World energy Production

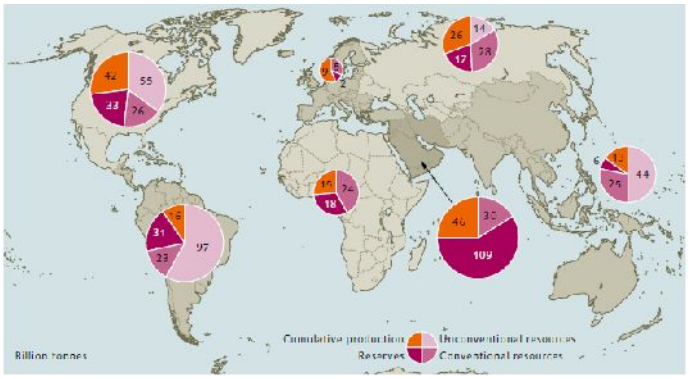
6.1.1 Formation of Fossil fuel

Gas and oil form in the sea over a period of millions of years, as the remains of animals and plants sink to the ocean floor. Combined with sand particles flushed from the land, they are buried and compressed into layers of sediment several kilometres thick on the ocean floor. Aided by the Earth's pressure and temperature conditions, bacteria convert the biomass into precursor substances from which hydrocarbons are ultimately formed. Oil reservoirs were formed whenever the upward travel of the oil was blocked by impermeable materials such as salt or clay layers. Depending on the ambient conditions, oil or natural gas develops.

6.1.2 The future of oil lies in our oceans

Since industrial oil extraction began in the mid-19th century, 147 billion tonnes of oil have been pumped from reserves around the world – half of it during the past 20 years. In 2007 alone, oil consumption worldwide reached a total of about 3.9 billion tonnes.

Currently the conventional oil reserves – i.e. those which can be recovered easily and affordably using today's technology – are estimated to be a good 157 billion tonnes. Of this amount, 26 per cent (41 billion tonnes) are to be found in offshore areas. The most productive areas are currently the North Sea and the Gulf of Mexico, the Atlantic Ocean off Brazil and West Africa, the Arabian Gulf and the seas off South East Asia.

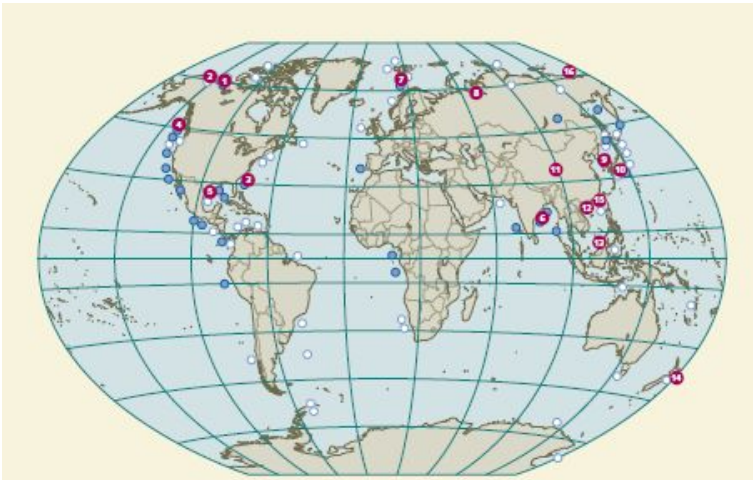


For some years now the trend has been towards drilling in deeper and deeper water. In 2007 oil was extracted from 157 fields at depths of more than 500 metres. In 2000 there were only 44 such fields. Of these, 91 per cent are situated in the so-called Golden Triangle in the Atlantic between the Gulf of Mexico, Brazil and West Africa.

Next Energy Game-changer - Methane hydrates

Methane hydrates are white, ice-like solids that consist of methane and water. The methane molecules are enclosed in microscopic cages composed of water molecules. Methane gas is primarily formed by microorganisms that live in the deep sediment layers and slowly convert organic substances to methane. These organic materials are the remains of plankton that lived in the ocean long ago, sank to the ocean floor, and were finally incorporated into the sediments.

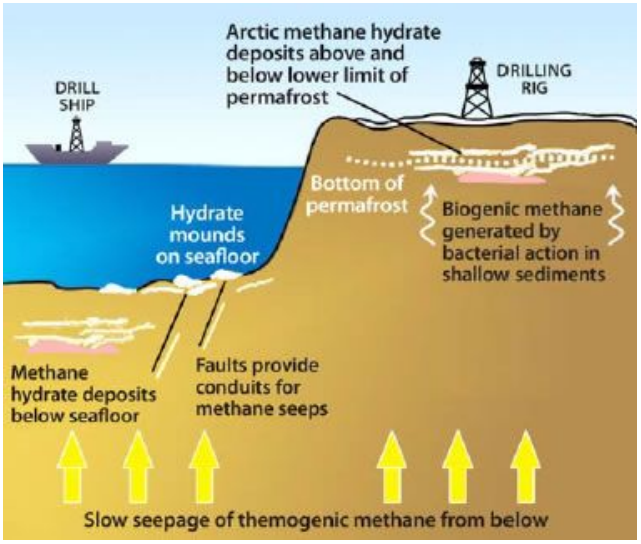
Methane hydrates are only stable under pressures in excess of 35 bar and at low temperatures. The sea floor is thus an ideal location for their formation: the bottom waters of the oceans and the deep seabed are almost uniformly cold, with temperatures from 0 to 4 degrees Celsius. In addition, below a water depth of about 350 metres, the pressure is sufficient to stabilize the hydrates. But with increasing depth into the thick sediment layers on the sea floor the temperatures begin to rise again because of the proximity to the Earth's interior, so that no methane hydrates can be deposited.



Methane hydrate occurs in all the oceans as well as some locations on land. White dots indicate occurrences identified by geophysical methods. The blue dots show occurrences proven by direct sampling. The most important research sites and areas worldwide are also highlighted with numbers.

Where Are the Methane Hydrate Deposits?

Four Earth environments have the temperature and pressure conditions suitable for the formation and stability of methane hydrate. These are: 1) sediment and sedimentary rock units below Arctic permafrost; 2) sedimentary deposits along continental margins; 3) deep-water sediments of inland lakes and seas; and, 4) under Antarctic ice. With the exception of the Antarctic deposits, methane hydrate accumulations are not very deep below Earth's surface. In most situations the methane hydrate is within a few hundred meters of the sediment surface.



Methane hydrates therefore occur mainly near the continental margins at water depths between 350 and 5000 metres. For one reason, enough organic material is deposited in the sediments there, and for another, the temperature and pressure conditions are favourable for methane to be converted to methane hydrates. The total global amount of methane carbon bound up in these hydrate deposits is in the order of 1000 to 5000 giga tonnes.

Environmental impact of Methane hydrates

At lower temperature methane hydrates are stable. But when the temperature of sea water and sea floor increases, methane hydrates does not escape directly out of the sea as methane because it is transformed into CO_2. But the formation and release of carbon dioxide are considerable. An additional problem is that the oxygen in seawater is consumed through the formation of carbon dioxide. Furthermore, the CO_2 released not only contributes to further global warming; it also leads to acidification of the oceans. Based on geological records it can be assumed that hydrates have broken down on a large scale numerous times in the Earth’s history, leading to extreme global warming and massive extinctions of organisms on the sea floor. Further investigations are necessary to determine the scale at which changes in the climate and oceans will accelerate in the future due to the release of methane gas at the sea floor.

ORAL PRESENTATION

MOLECULAR INTERACTION STUDIES BETWEEN HIV-IN AND HUMAN LEDGF

V.Anuradha[1], M.Syed Ali[2], N.Yogananth[2]
[1]PG and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai – 119.
[2]PG and Research Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai – 119.

Introduction

The interactions between proteins are important for many biological functions. The subject of protein-protein interactions represents a vast ensemble of results from biological, biochemical and biophysical studies carried out to date and cannot be treated in its entirety in any reasonable fashion.

Retroviruses are a large and diverse family of RNA viruses that synthesize a DNA copy of their RNA genome after infection of the host cell. Integration of this viral DNA into host DNA is an essential step in the replication cycle of HIV and other retroviruses. An infecting retrovirus introduces a large nucleoprotein complex into the cytoplasm of the host cell. This complex, which is derived from the core of the infecting virion, contains two copies of the viral RNA together with a number of viral proteins, including reverse transcriptase and integrase. The full length HIV-1 integrase (288 amino acids) has three domains; the catalytic core, the C-terminal, and the N-terminal domains. Although all three domains are required for integration, it is thought that the catalytic core domain contains the active site responsible for catalysis of all the reactions of integration/disintegration. The C-terminal domain confers the capacity to bind both viral and host DNA. The structures of the catalytic core and C-terminal domains have been determined separately. The structure and function of the N-terminal domain are presently unknown, but it contains a His_2Cys_2 zinc binding motif suggesting interaction with nucleic acid.

In the case of lentiviral INs, integration site targeting is in large part guided by the cellular chromatin binding protein lens epithelium derived growth factor (LEDGF)/p75, which facilitates integration into active gene bodies (1-4). Ectopically expressed HIV-1 IN accumulates in the nuclei of human cells (5,6), associates with chromatin (7), and forms a tight complex with endogenous transcriptional coactivator p75 (4,8), which is also referred to as lens epithelium-derived growth factor (LEDGF). Binding to LEDGF appears to account for the characteristic intracellular distribution of IN. RNA interference-mediated depletion of LEDGF or overexpression of a nuclear localization-defective mutant of LEDGF disrupted nuclear/chromosomal localization of HIV-1 IN (9-11). Conversely, the interaction-deficient IN mutant Q168A was excluded from condensed chromosomes (12). Furthermore, interaction with LEDGF protected HIV-1 IN from degradation through the ubiquitin-proteasome pathway (13). LEDGF binds HIV-1 IN via a small, approximate 80-residue IN-binding domain (IBD) within its C-terminal region (14, 15). The IBD is both necessary and sufficient for the interaction with HIV-1 IN (14). The three-dimensional structure of the IBD was recently determined by NMR spectroscopy, revealing an α-helical domain that is topologically similar to a pair of HEAT repeats (16). The CCD of IN possesses the main

determinants for interacting with LEDGF, although the NTD increased the affinity of the interaction (8).

Targeting the HIV integrase (HIV IN) is a clinically validated approach for designing novel anti-HIV therapies. One of the best characterized virus–host interactions, the integrase-LEDGF/p75 interface opens a range of opportunities for lentiviral vector targeting for gene therapy applications as well as for the development of novel classes of antiretroviral drugs. For structure based drug design, the molecular mechanism of interaction between the associated proteins should be explored. This has been known with protein-protein interaction analysis. Thus, the work will pave way to the design of small molecules against integrase through structure based approach.

Methodology

Step 1: Since, the project aims for the protein-protein interaction studies, the corresponding proteins in it's 3D structure was retrieved from PDB (Protein Data Bank). PDB id for HIV-1 Integrase: **1K6Y;** PDB id for Human Core P75 protein: **2B4J**

Step 2: To understand the structural basis for the interaction between HIV-1 IN and LEDGF, the proteins in their 3D structure with 3D atom co-ordinates was subjected to PATCHDOCK, freely available web server for molecular docking. The **PatchDock** method performs structure prediction of protein–protein and protein–small molecule complexes. Once the docking request is submitted, the PatchDock algorithm starts the prediction process. The user is notified when the results are ready by an email message that contains a link to a web page where the predictions are presented. On this page the user can both view specific predictions and download a compressed file of the top scoring solutions. Given two molecules, their surfaces are divided into patches according to the surface shape. These patches correspond to patterns that visually distinguish between puzzle pieces. Once the patches are identified, they can be superimposed using shape matching algorithms.

Results and Discussion:

Protein–protein interactions are critical for biological function. They directly and indirectly influence the biological systems of which they are a part. A very useful source of information about the interaction between two proteins is the 3D structure of their macromolecular complex. Using this, it is possible to easily identify such details as the residues that are directly involved with binding, the nature of the interface itself, and the conformational change undergone by the protein partners. Protein–protein docking can be defined as the determination of the complex structure between two proteins, given the coordinates of the individual proteins. Protein protein docking can be further classified as bound docking, which uses the structures from the complex structure as input, and unbound docking, which uses the structures from the individually crystallized subunits as input. Protein docking will generally output its best predictions for what the structure of the complex would look like, along with scores for these predictions. As these scores are heuristic, they are generally not energies and would not be a good criterion for determining whether or not there will be an interaction. The initial stage performs a full coarse-grained search and outputs approximately 1000–10,000 predictions. The refinement stage then improves these predictions through energy minimization, followed by a more detailed rescoring (and possibly clustering by position and score). Ideally, the top scoring prediction output from the refinement stage will be similar to the correct complex.

A) PATCHDOCK OUTPUT:

A web page that presents the top 20 solutions is automatically generated. The user receives an email message with the URL of this page (a web link). The solutions are presented in a table, a row for each solution. The geometric score, the desolvation energy, the interface area size and the actual rigid transformation of the solution are shown. A link to a PDB file that presents the docking solution is also available in each line. The user may view or download it. There is also an option to view additional, lower ranking solutions by pressing the 'next 20 solutions' button at the lower right corner of the table. In the solutions page an option to download the top scoring solutions is available. The solutions are downloaded as a compressed file in ZIP format. This compressed file contains the PDB files of the top scoring solutions. The best possible solutions in the PDB form can be viewed using any of the visualization tools. The results of patchdock and the scores predicted was summarized in Table 1. The best possible pose as given by Patchdock web server was visualized using Swiss Pdb Viewer [Figure 1]. On the basis of the above results, it could be suggested that residues Gln168, Glu170, and Thr174 in chain A of IN, Thr125, and Trp131 in chain B of IN as well as Ile365, Asp366, Phe406, and Val408 in LEDGF/p75 were responsible for their binding. These results might be helpful for discovery and design of small molecules to interrupt the interaction between HIV-1 IN and LEDGF/p75.

Table 1: Molecular Docking Algorithm Based on Shape Complementarity Principles

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	14272	2146.70	390.34	1.25147 -0.66210 -2.66680 89.77087 74.09382 42.08954	result.1.pdb
2	13226	1795.70	441.08	-0.39777 -0.04160 -1.16950 104.31302 41.19499 80.68349	result.2.pdb
3	12990	1601.40	488.92	2.71946 -0.45132 2.64833 83.29902 59.71150 61.14058	result.3.pdb
4	12458	1773.00	487.12	1.82271 0.62244 -3.03395 57.26667 62.83463 39.02953	result.4.pdb
5	12400	1477.80	341.91	0.78162 -0.33532 -1.37884 99.67933 75.57137 78.44305	result.5.pdb
6	12222	1553.30	162.17	0.72187 -0.11670 2.59491 100.77625 55.58776 76.56250	result.6.pdb
7	12150	1938.40	482.37	-2.24126 0.54928 2.18572 82.18744 72.88946 95.14201	result.7.pdb
8	12128	2139.00	347.80	2.08112 0.68837 -0.85524 71.84288 34.34216 53.97971	result.8.pdb
9	12076	1794.70	-13.49	-2.46178 0.19612 -2.50394 102.34836 49.02007 46.71833	result.9.pdb

10	11982	2065.70	-41.43	0.58934 -0.01174 2.77113 104.29948 51.22229 73.37492	result.10.pdb
11	11962	1745.60	46.17	0.78360 -0.19730 2.81296 101.22912 59.16668 77.40534	result.11.pdb
12	11880	1550.50	159.67	-1.89819 -0.72488 -2.40655 91.53472 4.03209 51.65166	result.12.pdb
13	11790	1650.00	0.88	2.47016 -0.79514 0.22135 104.60745 43.36725 52.31320	result.13.pdb
14	11710	1804.40	348.42	-2.95644 -0.32165 -1.28082 77.21461 53.66302 34.44888	result.14.pdb
15	11670	2103.00	-140.52	2.56286 -0.51083 0.40394 109.89733 48.74657 55.05887	result.15.pdb
16	11608	1856.80	349.57	1.46254 1.08582 0.08517 97.08717 31.10323 52.91979	result.16.pdb
17	11596	1598.20	214.70	-1.30979 -0.81527 2.97992 126.18111 44.68711 66.13663	result.17.pdb
18	11566	1520.10	459.07	-2.94762 1.06088 -1.98476 81.78833 31.92282 50.79675	result.18.pdb
19	11550	1535.20	328.18	1.66054 0.28526 0.10885 33.31572 25.69718 94.12911	result.19.pdb
20	11534	1572.40	359.24	3.00536 -1.00024 1.27829 118.07864 49.93076 67.36010	result.20.pdb

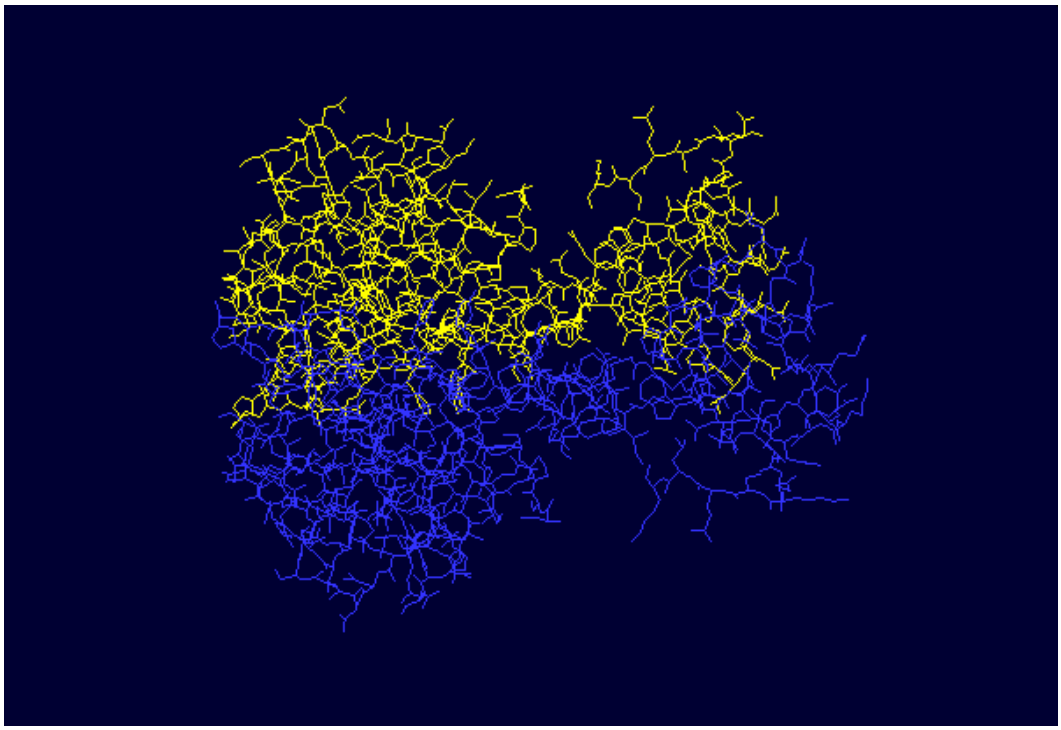


Figure 1: Best Docked Pose

Web resources:

- www.rcsb.org
- http://bioinfo3d.cs.tau.ac.il/PatchDock/
- 1nar.oxfordjournals.org/
- www.pubmedcentral.nih.gov/
- www.ncbi.nlm.nih.gov/sites/entrez

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STUDIES ON EFFICACY OF MELANIN PIGMENT FROM MARINE ACTINOMYCETES AS POTENTIAL ANTIBACTERIAL METABOLITE

*G. Ramanathan R.Renuga Devi, P.Suma Rajalakshmi
Research Department of Microbiology,
V. H. N. Senthikumara Nadar College, Virudhunagar-626 001 Tamilnadu, India
.***** Corresponding author: saranbag@gmail.com

GENERAL INTRODUCTION

Actinomycetes are aerobic, spore forming gram-positive bacteria, belonging to the order Actinomycetales characterized with substrate and aerial mycelium growth (Lechevalier and Lechevalier, 1981). The ocean cover contain over 2, 00,000 invertebrate and algal species (Mac Connell*et al.*, 1990). These organisms live in complex communities and in close association with other organisms both macro (algae, sponges,) and micro (non-filamentous bacteria, fungi and actinomycetes) organisms (Wright, 1998). Among the multitude of diverse organisms in the marine environment; marine microorganisms stand out as excellent source of many useful metabolites. Because of the diversity of marine organisms and habitats marine natural products encompass a wide variety of chemical classes. It is well understood that marine microorganisms have been largely unexplored as a source of bioactive agents for industrial applications. Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites (Berdy J, 2005). Among actinomycetes, the genus *Streptomyces* has long been recognized as a rich source of useful secondary metabolites and continues to be a major source of new bioactive molecules (Miyado, 1993; Berdy, 2005). They are the origin of a good number of marketed antibiotics which used against most resistant bacteria causing important community acquired infections include methicillin resistant *Staphylococcus aureus* (MRSA).

A critical element in a drug discovery based on microbial extracts is the isolation of unexploited groups of microorganisms that are at the same time good producers of secondary metabolites. Few of the antibiotics produced by actinomycetes are included in this review along with their activities to prove the versatility of this powerful microbial organism. Many ecological niches still remain unexplored yet which needs to be studied for a greater diversity of novel actinomycetes. Different strains of actinomycetes generally produce different compounds. For this reason intensive efforts can be increased for isolation and screening of new strains to discover new compounds. The present investigation was made an attempt to explore marine actinomycetes for their bioactive potential against fish bacterial pathogens.

MATERIALS AND METHOD

Sampling site Description

The soil sample was collected from Tirupullani coastal sand deposits in the South Eastern Coast of Tamil Nadu.

Collection and processing of Sample

The sediment and the soil samples were collected during the month of December 2014. Sediment samples taken from 15cm depth and suspended in distilled water.

Isolation of marine *actinomycetes*

Starch casein agar medium (g/l) starch-10, Casein-0.3, KNO_3-2, NaCl –2, K_2HPO_4-2, $MgSO_4.7H_2O$-0.05, $CaCO_3$-0.02, $FeSO_4.7H_2O$-0.01 and agar-18; and supplemented with cycloheximide (50 mg/l) and nalidixic acid 20 mg/l was used for the isolation of actinomycetes. Actinomycetes agar was also been used for the sediment samples were approximately diluted with seawater or saline and an aliquot of 0.1 ml was spread over the medium with a sterilized bent (L) rod and plate spinner. The inoculated plates were incubated at 30^{o}C for 7 to 10 days. The actinomycetes colonies that developed on the plates were counted and numbers were expressed in colony forming units (CFU). The colonies were picked out and purified before being stored in starch casein agar slants.

Identification and characterization of isolated cultures

Purified isolates of *actinomycetes* were identified using morphological and cultural characteristics by the methods as described in the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1970).

Extraction of pigment produced by actinomycetes

To extract pigment, 100ml of actinomycetes broth cultures were centrifuged at 8,000 rpm for 10 min at 4^{0}C. The supernatant was separated and methanol was added to the pellet and followed by centrifugation at 10,000 rpm for 10 min at 4^{0}C. Then the pellet was transferred into watch glass and allowed it to dry until the solvent evaporated and brown colored powder appeared. Extraction procedures and analysis tests were conducted in dark conditions.

Spectroscopic Analysis

The samples extracted with methanol solvent were scanned between 200nm and 500nm using UV-Visible scan Spectrophotometer (Shimadzu-1650 PC) and it shows individual peaks for respective melanin pigment with their optical densities.

TLC Analysis of melanin pigment

Qualitative analysis of melanin in the experimental sample was carried out by using Thin Layer Chromatograpy (TLC). Then, applying slurry made by silica Gel G for TLC grade and applied over the glass plate, TLC plates were made. This was dried at 60^{0}C for an hour of period. The dried plates were pre-activation base line.

After that, 3µl condensed melanin pigment samples were spotted on the baseline of the TLC plates at 1.0cm interval and then allowed to dry at room temperature. Often the sample applied on TLC plates was placed in a pre-saturated TLC chamber contains mobile phase. (Hexane: ethyl acetate the ratio of 1:9).Then the chromatogram was developed by providing the dark environment up to a distance of 15cm mark. Then the plate was taken out dried for few min. Using UV light torch, the developed spots were seen and taken out and marked. The distance travelled by each spot in baseline and relative R_f values were calculated. By comparing the standard R_f values for the chosen mobile phase, the melanin pigment present in the samples were identified.

$$R_f = \frac{\text{Compound distance from origin}}{\text{The solvent front distance from origin}}$$

HPLC analysis

The spot obtained from thin layer chromatography was redissolved in methanol and was subjected for HPLC analysis on Schimazdu, LU-10AT VP HPLC available at science instrumentation centre, ANJA College, Sivakasi. Results were recorded.

FTIR analysis

The fraction obtained from the TLC was redissolved in acetone and was subjected for FTIR analysis on FTIR Schimazdu 8400S, available at Research Department of Chemistry, VHNSN College, Virudhunagar. Results were recorded.

Extraction of antimicrobial melanin metabolites, from marine *Streptomyces sp*

Screening of antibacterial activity using well diffusion method

The antimicrobial activities of cell free pigment extracts were tested against different test organisms by using agar disc diffusion method as described by Kirby-Bauer with modification. Late exponential phase of the test bacteria were prepared by inoculating 1% (v/v) of the cultures into the fresh Muller-Hinton broth and incubating on an orbital shaker at 37 °C and 100 rpm overnight. Before using the cultures, they were standardized with a final cell density of approximately 10^8 cfu ml. Muller-Hinton agar were prepared and inoculated from the standardized cultures of the test organisms then spread as uniformly as possible throughout the entire media. Well was made in Muller-Hinton agar each seeded with test organisms. The about 100µl of melanin pigment was added to each well. The antibacterial activities of the extracts were recorded. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (mm) on the surface of plates and the results were reported.

Antibacterial activity of fish pathogens

For antibacterial assay three different concentrations of (25, 50 and 100 µl) of cell free pigment filtrate extracted with Ethylacetate was taken, by following well cut method, screened against the test pathogens, seeded on Muller-Hinton agar plates. The fish pathogens viz., *Aeromonas hydrophila*, *Vibrio harvei*, *Vibrio parahaemolyticus*, *Serratia sp* and *Bacillus subtilis* which is obtained from Centre for coastal studies. Division Marine Microbiology and Infectious medicine. Alagappa University Thondi campus, Thondi, Tamil Nadu.

RESULTS

Population diversity of melanin actinomycetes was studied in the sand deposit soils collected from Tirupullani Coastal Region. It was observed that the highest (4.7×10^6 CFU) of Streptomyces in calcareous sand deposit then compared with other isolates. (Table-1).

Table-1. Actinomycetes population at collection site

Sampling site	Sample type	CFU×$10^6$of Samples	
		Streptomyces	Other
Tirupullani coastal region	Sand deposite	3.3	2.7
	Calcareous deposites	4.7	1.3

The isolated marine *Streptomyces sp* was characterized based on morphological, cultural and physiological properties. It was observed that among the various media such as Starch casein agar, Glycerol asparagine agar, Tyrosine agar, Nutrient agar, ISP6 agar, Actinomycetes isolation agar used for the cultivation the marine *Streptomyces* exhibited good growth pattern on starch casein agar, glycerol asparagine agar and ISP6 agar respectively, poor growth was observed on Nutrient agar media. The coloration of aerial mycelium of marine *Streptomyces sp* varied from white, whitish yellow, oily brown, pink, yellow, Diffusible green

and Fluorescent green. In case of substrate mycelium whitish yellow, pink, yellow, Diffusible green, Fluorescent green and White was appeared on various media used. Dark brown pigmentation was observed in ISP6 media used for the cultivation. (Table-2).

Table-2. Cultural characteristics of *Streptomyces sp* on various Growth medium.

Culture media	Isolated strains	Aerial mycelium	Substrate mycelium
ISP6 agar	Act-1	Brown with silver shine	Brown
	Act-7	Ash	Dull brown
ISP7 agar	Act-1	Diffusible green	White
Actinomycetes isolation agar	Act-1	White	Dull white
	Act-2	Dull white	Pink
	Act-3	White	Pink
	Act-4	Pure white	White
	Act-5	Fluorescent green	Yellowish fluorescent
	Act-7	Ash	Dull white
	Act-6	Light ash	Light pink
Starch casein agar	Act-8	Yellow	Yellow
	Act-8	Yellow (Dry)	Dull Yellow
	Act-8	Yellow (Shiny)	Yellow

As part of characterization of marine actinomycetes, the sensitivity / resistant pattern with commercially available 13 antibiotics were screened against marine *Streptomyces sp.* Among the antibiotics the marine *Streptomyces sp* exhibit resistance to Ampicillin, Amikacin, Cloxacillin, Cephalexin, Ceftazidime, Cefixime, but Vancomycin, Rifampicin, Norfloxacin, kanamycin, Clindamycin, Ciproflaxacin, Bacitracin showed sensitivity to *Streptomyces sp*.(Table-3)

Table-3.Characteristics of marine *Streptomyces. sp* against commercial antibiotics.

Name of the antibiotics	Sensitivity/Resistance to antibiotics
Ampicillin	**R**
Amikacin	R
Bacitracin	S
Cloxacillin	R
Ciprofloxacin	S
Cephalexin	R
Clindamycin	S
Ceftazidima	R
Cefixime	R
Kanamycin	S
Norfloxacin	S
Rifampicin	S
Vancomycin	S

S: Sensitive; R: Resistant

The different concentration such as 25 µl, 50µl and 100µl of cell free extract of melanin pigment antimicrobial metabolite was screened against five fish bacterial pathogens viz., *Vibrio harvei, Vibrio parahaemolyticus, Aeromonas hydrophila, Serratia sp. and Bacillus subtilis* all the bacterial pathogens are susceptible to cell free melanin extract of marine *Steptomyces sp.*Among the fish pathogens *Bacillus subtilis* was found to be more susceptible to antibacterial metabolite of marine *Streptomyces sp* which showed 19mm of inhibition followed by this *Vibrio parahaemolyticus* (17mm), *Vibrio harveyi*(16mm) but less response was observed with *Serratia sp* (14mm).(Table-4)

Table-4.Antibacterial activity of cell free melanin pigment metabolite of *Streptomycetes sp* against fish pathogens

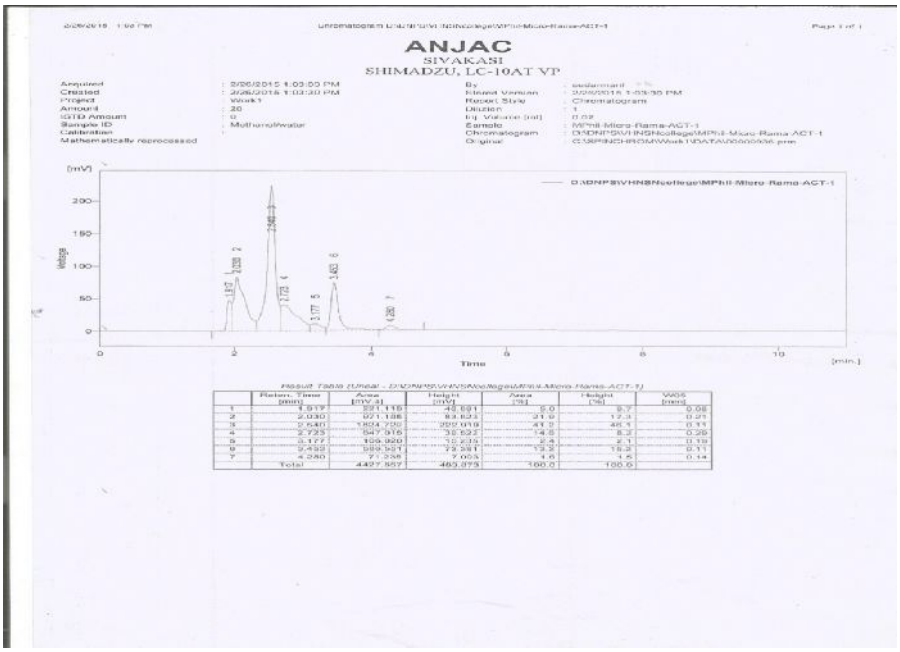
Test organisms	**Zone of inhibition (mm dia)**		
Fish pathogens	**25 µl**	**50µl**	**100µl**
Vibrio harvei	7	13	16

Vibrio parahaemolyticus	10	16	17
Aeromonas hydrophila	8	15	16
Serratia sp.	7	12	14
Bacillus subtilis	9	17	19

TLC Identification of melanin metabolite.

The extracted melanin metabolite separated by thin layer chromatography. Single separated band of pinkish color was observed in Thin Layer Chromatography. The Rf value of active melanin compound was 0.75cm in thin layer chromatography. Single separated spot which appeared as pinkish in color.

Fig.1.HPLC analysis of melanin metabolite



Single separated spot which appeared as pinkish in color corresponds to R_f value of melanin pigment through TLC analysis was further subjected for HPLC analysis to determine the Retetion time of the melanin metabolite the retention time of 3.45 indicates the presence of melanin derivatives.(Fig.1).

FTIR analysis

Melanin pigment extracted from marine Streptomyces sp was further subjected for FTIR analysis to determine major functional groups of melanin pigment. The peaks were obtained at 3402.43, 2926.01, 2854.65, 1735.93, 1633.71, 1523.76, 1460.11, 1404.18, 1234.44, 1114.86, 1076.28, 927.76, 806.25, 754.17, 719.45, 669.30, 619.15, 536.21 and 424.34.

The peaks 3402.43cm^{-1},relates to amino second group (NH), 2926.01cm^{-1},relates to Hydroxy(OH), C-H stretch bond,Methane (CH) group.1633cm^{-1},relates to amino group with NH_2 strech. 1234.44cm^{-1},relates to anhydride group (C-O),which indicates the functional

groups of melanin pigment from this result it was suspected that the isolated melanin pigment may be eumelanin because of lack of N group (Vasanthabharathi *et al.*,2011).

Spectroscopic analysis of melanin pigment

The extracted melanin pigment of Streptomyces sp was subjected for UV spectroscopic analysis. The λ max of melanin metabolite was found to be in the range of 259nm to 396nm, it indicates that the presence of melanin derivatives in the cell free pigment preparation.

DISCUSSION

Microbial diversity is one of the difficult areas of biodiversity research, estimation of microbial diversity required for understanding the biogeography, community assembly and ecological processes. Marine microorganisms which are salt tolerant, have diverse range of bioactive potential, enzymatic activity. The secondary metabolites produced by halophilic microorganism have higher therapeutic potential with less toxicity when used for therapeutic application to humans (Sabu, 2003). *Actinomycetes* are thus well adapted members of marine microbial community, have provided many important bioactive compounds of high commercial value. About 61% of all bioactive microbial metabolites were isolated from *actionmycetes* especially from *Streptomycetes* and also from rare *actinomycetes*. It has been emphasized that *actinomycetes* from marine sediment may be valuable for the isolation of novel strains of *actinomycetes* which could potentially yield useful products.

In this present investigation the diversity of the *actinomycetes* population particularly on marine *Streptomyces* was studied in Tirupullani coastal sand deposits of South Eastern Coast of Tamil Nadu. It was revealed that 10^6 dilution of calcareous deposit consist of 4.7×10^6CFU. The population dynamics are influenced by the available nutrients and pigments from the ecosystem. The occurrence and distribution of different genera of marine *actinomycetes* the frequency of the genus *Streptomycetes* was 57.4% in the Palk Stait region of Bay of Bengal. (Ramasamy Vijayakumar *et al*., 2007).

The present study made an attempt to characterize the isolated *Streptomyces sp* with reference to various media preferences and response to various commercial available antibiotics. The Streptomyces isolates ACT-1 which was luxuriantly supported by the cultivation medium ISP6, ISP7 and actinomycetes isolation agar with brown pigmentation in ISP6 agar. Various cultural characteristics were observed and recorded which are the key factors for the chemotoxonomic evaluation of the various species of *actinomycetes*. (Kavitha and Vijayalakshmi 2007).

Mahmoud marbrouk (2013) reported that among the different isolated which were isolated from different localities of Egypt subjected for growth in different cultivation media such as Tyrosine liquid medium, Shinobu's synthetic tyrosine medium, Peptone yeast extract medium, showed pigment production in only one isolate exhibit melanin and identified as *Streptomyces torulosus*.

The present study also found that the formation of melanoid pigment by the isolated marine *Streptomyces sp*. The Dark brown colour pigmentation was measured their absorbance was noted with λ max of 365 and 395 nm by UV spectroscopy. The effect of various carbon, nitrogen, pH and salinity on the pigment formation with reference to biomass was also been assessed, glycerol and the nitrogen source lysine was found to favor the pigmentation when supplemented with medium B at 1 % level.1.5 % of NaCl and neutral pH has significant impact on the melanin pigment formation and the cell mass by marine *Streptomyces sp*. The

pigmentation of *Streptomyces sp* is distinct enough to allow ready delineation in most *Streptomyces* cultures when combined with other fundamental features when complex organic media is employed. Das *et al* (2006) has reported that the production of melanin by *Streptomyces* has influenced by the supplementation of L-tyrosine as a substrate and fructose as a carbon source among the nine strains of *Streptomyces* isolated from different location of Gulbarga India.

Characterization of melanin pigment produced by marine *Streptomyces* sp was done with UV spectroscopic analysis. The absorption maximum for (λ max) melanin found in the range of 254-366nm. It was supported by the studies of Vasanthabharathi *et al.,* (2011). Brown black pigmentation of *Streptomyces* has been referred to as melanin several types of melanin has been described:eumelanin,pheomelanin,allomelanin and pyomelanin. Each of the types exhibit different absorption maximum in UV spectrum.

As part of the investigation the cell free supernatant of marine *Streptomyces sp* was screened against fish bacterial pathogens with three different concentrations. Broad inhibitory activity was observed in fish bacterial pathogens. Zone of inhibition of the melanin metabolites is based on the better diffusibility of the substances and also the chemical nature of the metabolite that exhibit biological activity. It was observed that the maximum *B. subtilis* inhibition was found against (19mm) in 100µl concentration of melanin. Most of the biologically active substances such as sapurimycin, aminocyclopentane carboxylic acid, altemidicin, 4 Phenyl 3 butenoic acid has been reported from *Streptomyces koyangenesis*. (Jee yeon *et al.,* 2005). Recently the marine *actinomycetes* was isolated from sundrbans of Bay of Bengal, India, which exhibited potent antimicrobial activity against Gram-positive and Gram-negative bacteria, moulds yeast and several multiple Drug resistant bacteria. (Saha 2005). Hayakawa *et al*. 2004 reported that Broad spectrum of antimicrobial activity against *B.subilis*, *M.luteus*, *Staphylococcus aureus*, *S.murinus*,*C.albicans* and *S. cerevisiae* and filamentous fungi *A.niger*,*A.oryzae* and antitumor activity reported from soil Streptomycetes isolate *Streptomyces albidoflavus*, *S.diasticus* and *S.chromofuscus*. Charusharma *et al.*,(2014) reported that sixty distinctly pigmented isolates wave showed antagoninstic activity against test bacterium *Staphylococcus aureus*(MTCC737),*Bacillus subtilis*(MTCC 441), *E.coli*(MTCC739). Among the isolates *Bacillus subtilis* was highly susceptible to the metabolite of isolated actinomycetes.

The extracted melanin pigment was subjected for TLC, HPLC and FTIR analysis for partial characterization. It was observed that R_f value of melanin pigment of *Streptomyces*. It was found to be 0.75. Tindall, B.J, (1993) reported that the compound extracted from *Streptomyces coelicolor* strain sub (JQ828940). Single separated band was onserved in Thin Layer Chromatography and the R_f value of active compound was 0.4cm in TLC. Melnin pigment extracted from *Streptomyces sp* was further elucidated with HPLC and FTIR analysis. It was observed that the retention time of 3.45indicates the presence of melanin derivatives. R.O. Rajesh, (2013) reported that antimicrobial metabolite of Streptomyces was characterized by HPLC showed retention time of 3.483 and 7.650 indicates antibiotic live metabolites.

The peaks 3402.43cm^{-1},relates to amino second group (NH), 2926.01cm^{-1},relates to Hydroxy(OH), C-H stretch bond, Methane (CH) group.1633cm^{-1},relates to amino group with NH_2 strech. 1234.44cm^{-1},relates to anhydride group (C-O),which indicates the functional groups of melanin pigment from this result it was suspected that the isolated melanin pigment may be eumelanin because of lack of N group (Vasanthabharathi *et al.*,2011)

CONCLUSION

The melanin pigment was extracted and partially characterized by chromatography analysis. As the melanin pigment showed potential bioactivity against fish bacterial pathogens.The conservation and utilization of biological diversity requires comprehensive knowledge about the species distribution. Recent anthropogenic interventions in marine environment have lead to the study of marine microbial diversity is of vital importance to the understanding of different processes of the ocean, which may present potent novel microorganism for screening of bioactive compounds. Actinomycetes are thus well adapted and are functional members of the marine microbial community moreover the present investigation has evolved that coastal area of Tirupullani (South East Coast of Tamilnadu) as a source of marine actionomycetes. In view of broad antibacterial spectrum with increasing of antibiotic resistance, attempts has to made to complete characterization of the bioactive metabolite produced by marine *Streptomyces sp.*

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EFFECT OF POTATO PEEL POWDER WITH BACILLUS ON GROWTH OF FISH ETROPLUS SURATENSIS

Dr.S.J.Sreeja[1], Dr.A.Palavesam[2], Dr.V.Siva Nadanam[1]
[1]Assistant Professor in Zoology, Lekshmipuram College of Arts and Science, Neyyoor.
[2]HOD, Department of Animal Science,
Manonmaniam Sundaranar University, Tirunelveli.
Email- s.jsreeja@yahoo.com

Introduction

Aquaculture is one of the fastest growing food production systems in the world, which has emerged as an industry possible to supply protein rich food throughout the world (Prasad, 1996). Presently, aquaculture is facing heavy production loss both in hatcheries and grows out systems due to disease outbreak. Use of probionts has been proposed as a measure to maintain healthy environment in aquaculture and to prevent occurrence of disease (Lipton, 1998).

Fish feed is the most expensive input in aquaculture operation. The shortage and high cost of pelleted feed severely constrained the development of low cost aquaculture systems suitable for small scale farmers in the developing countries. It would therefore be more economical to utilize plant protein in fish feeding than high cost animal protein materials. Cellulose is the major complex carbohydrate in plant cell walls. Coverings, potato peelings carry most of the cellulose. Potato peelings are, more often than not, just treated as unnecessary wastes. Use of Potato peelings will partly solve the problem of waste disposal at the same time maximize its use (Annadurai *et al*., 2002; Essien *et al*., 2005). Hence, the present study was taken up to investigate the effect of probionts, *Bacillus* and Potato peel powder on growth response and food utilization in pearl spot, *Etroplus suratensis*.

Methodology

The branded feed ingredients such as fish meal, groundnut oilcake, wheat bran, soyameal, tapioca powder, vitamin, mineral mix and cod liver oil were purchased from commercial merchants. In addition to this, probiotics, *Bacillus* was isolated from the gut of Estuarine Pearl spot *Etroplus suratensis*. Based on the suitability, different ingredients were selected for feed formulation. Three different types of diets (DietA and DietB) with 40% protein were compounded separately by mixing different ingredients with 2% potato peel powder (experimental Diets A) and 2% CMC (control Diet B) at various proportions. Then the probionts Bacillus was added as feed additives at 1% in Diet A. Diet C was used as the control, without addition of probionts.

Results and Discussion

After acclimatization, the healthy fishes were weighted individually (15.00±0.20g). They were reared at the rate of 3 numbers/ 12L water and fed ad libitum. The leftover food and feacal matters were removed and dried at 800^{o}C in an oven. Four replicates were maintained for each feed randomly. During the experiment, which lasted 91 days, water quality was maintained. During the experimental period of 91 days, the Specific Growth Rate (SGR) of *E.suratensis* fed on Diet A was high (0.73±0.27%) and low in control diet (Diet C). The consumption rate of *E.suratensis* fed on control diet was maximum (38.62±0.86mg/g/day) and minimum (29.70±0.56mg/g/day) in Diet A (Table 1). The production rate of *E.suratensis* was high in probionts Diet A fed group (14.2±0.17mg/g/day) whereas, it was low in control Diet B fed group (10.8±0.26mg/g/day). The present observation is in congruence with the findings of Paulmony (1996). He reported that the probiont yeast supplemented diet significantly influenced the growth, food conversion ratio and specific growth rate of *Cyprinus carpio*.

Parameters	**Growth responses**	
	Diet A	**Diet B**
Initial wt(g)	15.0±0.60	15.00±0.20
Final wt(g)	29.2±0.45	25.8±0.75
Production(g)	14.2±0.17	10.8±0.26
Food consumed(g)	29.70.83	38.62±0.86
FCE (%)	30.96±0.76	27.96±0.65
SGR (%)	0.73±0.27	0.59±0.32
FCR	2.09±0.45^{ab}	3.53±0.23^{a}

Table 1. Overall growth responses of *E. suratensis* fed on experimental diets (Diet A) and control diet (Diet B) during 91 days of feeding experiment.

The biochemical composition of muscle of experimental fishes such as protein, carbohydrate and lipid were analysed following the method of Lowry *et al*., 1951; Roe, 1955 and Folch *et al*., 1957 respectively. The muscle, gill and gut of *E.suratensis* after the termination of the experiment is given in Table 2. After experimental period of 91 days, the biochemical components such as protein, carbohydrate and lipid contents in the muscle, gill and gut samples of experimental fish were higher than control diet fed fishes. Only limited number of studies has been carried out on the infuence of probiotics on fish. Addition of

probionts and feed additives in the diet increased the growth rate by accelerating the secretion of certain enzymes in fishes (Das, 1975).

Biochemical composition	Fish samples					
	Muscle		Gill		Gut	
	DietA	DietB	DietA	DietB	DietA	DietB
Protein	40.96±0.14	33.46±0.20	32.45±0.14	27.15±0.12	27.36±0.17	23.36±0.14
Carbohydrate	4.73±0.01	3.06±0.01	3.42±0.01	2.00±0.01	3.12±0.02	2.25±0.01
Lipid	3.53±0.02	2.86±0.02	3.12±0.02	2.08±0.12	2.34±0.01	1.82±0.01

Table 2. Biochemical composition of muscle, gill and gut samples of *E.suratensis* fed on DietA and DietB

The present study shows considerable weight gain in *E.suratensis* fed with probiont supplemented diets than control diet. The consumption rate of experimental groups did not vary much, but the rate of production varied significantly in fishes fed with these diets. The probionts administrated through diet might choose binding sites in the intestine, preventing colonization by pathogens. So far results with probiotics to reduce disease prevalence among commercially produced finfish, have been disappointing. However, the principles behind their use remain sound and their full potential needs to be explored further.

Conclusion

The present work proved the effect of varoius bacterial probionts and vegetable waste on increased growth of *E.suratensis*. The results will be further used in aquaculture industry for large scale production of *E.suratensis* under controlled environmental conditions. Further more this work can be extended in aspect of application in various other fishes also using different sources of food waste.

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CHARACTERIZATION OF EXTREME HALOPHILIC BACTERIA HALOBACTERIUM SODOMENSE ISOLATED FROM SALTPAN

Berciyal Golda.P and Palavesam.A
Department of Animal Science, Manonmaniam Sundaranar University,
Tirunelveli-627012.

Introduction:

Solar salterns present a man-made ecosystem consisting of a discontinuous salinity gradient. Hypersaline habits constitute a typical example of extreme of environments in which relatively low microbial species diversity can be found (Brock, 1979). Hypersaline environments include water and soil habitats. Hypersaline waters derived from the concentration of seawater by evaporation are wide spread throughout the world, and they probably represent the natural habitat of halophilic bacteria (Brock.,1979). The majority of extremely halophilic bacteria are included in one the three primary phylogenetic groupings, the so called archaebacteria (Woese and Fox, 1977). The main characteristics of the family Halobacteriaceae are: Rods, cocci, a multitude of involution forms (discs, triangles etc.,). Require at least 1M NaCl for growth. Red pigmentation due to the presence of bacterioruberins (C50 carotenoids) in the cell. Lack of muramic acid–containing pedtidoglycan in the cell envelope. Membrane lipids composed of ether linked isoprenyl phosphoglycerides (Larsen, 1984).The extremely halophilic bacteria are widely distributed in brines containing very high salt concentrations.

Antibiotics produced as secondary metabolites by microorganisms are generally used as chemotherapeutic agents. Although there are thousands of antibiotics, only a few are widely used as drugs and chemotherapeutants for treatment of infectious disease caused by microbes. Resistant strains have appeared among the disease causing microbes due to the indiscriminate use of these therapeutic antibiotics. Most of these antibiotics are of microbial origin of the terrestrial environment. This has forced the scientific community and pharmaceutical industry to search for novel bioactive substance from unexplored sources. Initial studies have shown that compounds isolated from marine microorganisms exhibit antibiotic, antitumor and other pharmacological activities (Bernan et al., 1997). The main objective of this experiment is to isolate and characterize of Halophilic bacteria from saltpan.

Materials and Methods:

The water sample was collected from the crystallizer pond (Plate 1) of Thamaraikulam salt pan, Kanyakumari District, Tamilnadu (Plate 1). The temperature and salinity of the water in the saltpan was noted using Celsius Thermometer and Salinity Refractometer. The sample was transported immediately (within 4 hours of collection) to the laboratory and bacterial analysis was made. Known weight of the sample was washed with known volume of sterilized distilled water by shaking on a rotary shaker. The sample was 10 fold serially diluted and plated on Halobacteria medium (5g yeast extract, 5g casamino acids, 1g Na-glutamate, 2g KCl, 3g Na -citrate, 20g MgSO .7H O, 200g NaCl, 36mg FeCl. 4H O, 36mg MnCl. 4 H O, 1000mL distilled water, pH was adjusted to 7.0-7.2) and Nutrient broth containing 100 g/L NaCl, in shaking incubator at 25°C and 120 rpm for 20 days at 25°C. . The cell density in the culture was monitored with UV-VIS scanning spectrophotometer (UV2101 pc, Shimadzu) by measuring the absorbance at 560 and 600 nm. The bacterium which showed rapid growth in halophilic broth was then halophilic agar and incubated at 25^0c for two days. The isolated colony was identified as *Halobacterium sodomense* with the results of following biochemical tests: Gram staining, motility, acid production, catalase, starch hydrolysis, gelatin hydrolysis, nitrate reduction, indole, casein hydrolysis, lipid hydrolysis, hydrolysis H_2S production. Antibacterial activities of antibiotics were evaluated against *H. sodomense*. Antibiotic sensitivity was seen using eight antibiotics such as Amikacin, Ampicillin, Co-trimoxazole, Kanamycin, Norflaxacin, Ofloxacin, Perfloxacin and Streptomycin.The activity was higher in Kanamycin and no activity was observed in Ofloxacin and Pefloxacin.

Results:

The water sample collected from the crystallizer pond in the Thamaraikulam saltpan, Kanyakumari District, Tamilnadu contained many bacteria including Halobacterium sodomense (Plate 2). The data presented in the Table 1 shows biochemical test of H. sodomense. Growth pattern of *H. sodomense* cultured in halophilic broth medium at room temperature showed marked variation in growth pattern at 560 and 600nm (Fig.1).

The effect of antibiotics on the inhibitory activity against *Halobacterium sodomense* is presented in Table 3. Among the tested antibiotics, Kanamycin (30 mcg) showed maximum inhibitory activity with the zone of inhibition of 25mm in diameter; whereas Oflaxacin (5 mcg) and Pefloxacin (5mcg) showed no inhibitory activity against *H. sodomense.* The no inhibitory action of the above two antibiotics indicated that the halophilic bacteria *H. sodomense* has the antibiotic resistant to Oflaxacin (5 mcg) and Pefloxacin (5mcg). The other antibiotics Amikacin (30mcg), Ampicillin (10mcg), Co-trimoxazole (25mcg), Norfloxacin (10mcg) and Streptomycin (10mcg) showed inhibitory zones. The inhibitory activity and the zone of inhibition of the antibiotics are presented in the plate 3 and 4.

Plate 1

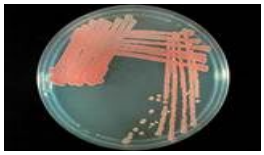

Plate 2

Plate 3

Plate 4

Discussion:

The study of halophilic bacteria in the salt work of Kanyakumari district has been under taken since 1999. Parameswari (1999) observed *H. sodomense* in the salt work of Rajakamangalam, and Melbin Kala (1999) observed *H. sodomense* in the Tamaraikulam salt work. Studies on the isolation and characterization of halophilic bacteria have been studied in different salt works and saline environment by many authors. The growth rate of the halophilic bacteria in the Dead Sea, salt works of Greece and salt pan of Israel (Oren, 1990) have been studied.

Analysis of the data obtained for the antibiotic assay of *Halobacterium* sp. exposed to different concentration of antibiotics such as Kanamycin, Ampicillin and Streptomycin revealed that, most of the halophilic bacteria are resistant to the antibiotics except the few, which are sensitive to the antibiotics. For instance, Prabha (2000) reported that *H. saccharovorum* was sensitive to antibiotics, Ampicilin and Kanamycin. Similarly Parameswari (1999), showed the sensitivity of *H. sodomense* to streptomycin. It is interesting to note that, the same concentration of streptomycin showed sensitivity to *H. sodomense*.

From table 4, it is also understood that the resistance and sensitivity to an antibiotic depends on *Halobacterium* sp. On comparing *H. saccharovorum* and *H. sodomense*, it is evident that both the species are resistant to Amikacin, Ampicillin, Kanamycin, Oflaxacin and Streptomycin. It is observed that *H. saccharovorum* is resistant and *H. sodomense* is sensitive to the antibiotics, norfloxacin and co-trimoxazole. The observation is vice versa in case of pefloxacin i.e. *H. saccharovorum* is sensitive and *H. sodomense* is resistant.

Table 1 Biochemical characteristics of *H. sodomense*

(+ : positive; - : negative)

Sl.No	Tests	*H. sodomense*	Sl.No	Tests	*H. sodomense*
1	Gram staining	-	7	Nitrate Reduction	+
2	Motility	+	8	Indole	-
3	Acid production Glucose Lactose Xylose	 + - +	9	Casein hydrolysis	-
4	Catalase	+	10	Lipid hydrolysis	+
5	Starch hydrolysis	+	11	Hydrolysis	+
6	Gelatin hydrolysis	-	12	H2S production	-

Table 2 Different characteristics of *H. sodomense*

Sl.No	Tests	*H. sodomense*
1	Cell shape	Rods
2	Cell dimensions(µm)	0.5 - 0.7 x 2.5 - 5.0
3	Presence of vacuoles	-
4	Growth at 10^{o}C	-

Fig 1 Growth of *H. sodomense* in 50, 100, 150, 200, 250, 300 and 350ppt salinity at 560 and 600nm

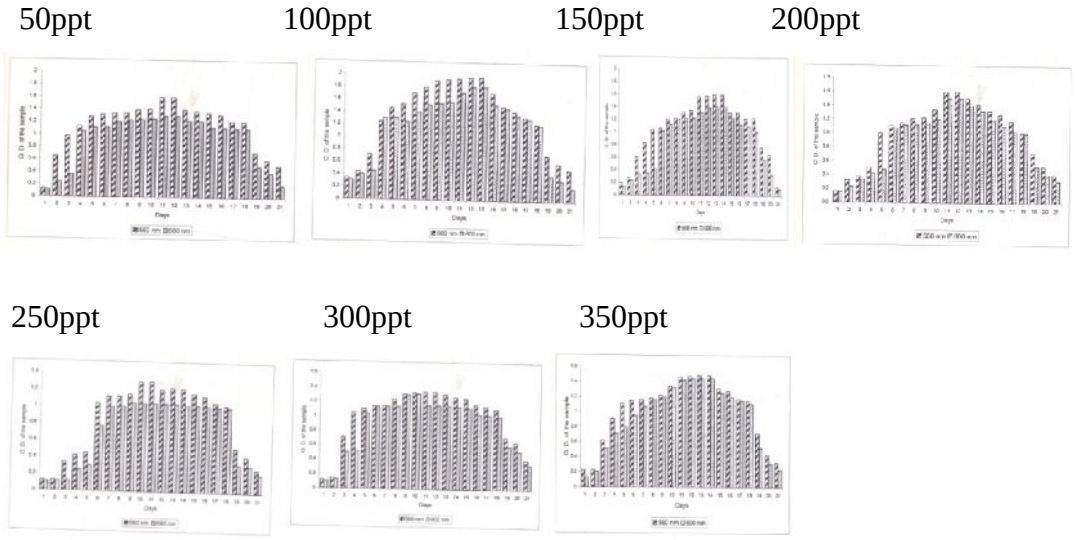

Table 3 Antibiotic resistance of *H. sodomense*

Sl.No	Name of the Antibiotic	Symbol	Concentration of Antibiotic (mcg)	Zone of Inhibition (mm)	Area of Inhibition (mm^2)
1	Amikacin	AK	30	20	314
2	Ampicillin	I	10	18	254.34
3	Co - trimoxazole	Q	25	16	200.96
4	Kanamycin	K	30	25	490.63
5	Norfloxation	NF	10	23	415.265
6	Ofloxacin	OF	5	-	0
7	Pefloxacin	PF	5	-	0
8	Streptomycin	S	10	22	379.94

Table 4 Comparison of antibiotic sensitivity in different Halophilic bacteria

Sl. No	Antibiotic	Halophilic bacteria	Activity	Reference
1	Amikacin(AK) (30 mcg)	*Halobacterium saccharovorum*	Sensitive	Prabha(2000)
		Halobacterium sodomense	Sensitive	Present study
2	Ampicillin(I) (10 mcg)	*Halobacterium salinarium*	Resistant	Elazari–Volcani(1957)
		Halococcus morrhuae	Resistant	Kocur and Hogkiss(1973)
		Haloferax volcanii	Resistant	Mullakhanbhai and Larsen(1975)
		Halobacterium saccharovorum	Resistant	Tomlinson and Hochstein(1976)
		Haloarcula vallismortis	Resistant	Gonzalez et al.,(1978)
		Haloferax menditerranei	Resistant	Rodriguez – valera et al.,(1983a)
		Halobacterium sodomense	Resistant	Oren(1983c)
		Haloarcula hispanica	Resistant	Juez et al.,(1986)
		Halobacterium lacusprofundi	Resistant	Franzmann et al.,(1988)
		Haloarcula japonica	Resistant	Takashina et al.,(1990)
		Halobacterium saccharovorum	Sensitive	Prabha (2000)
		Halobacterium sodomense	Sensitive	Present study
3	Co – trimoxazole	*Halobacterium saccharovorum*	Resistant	Prabha (2000)

	(25 mcg)			
		Halobacterium sodomense	Sensitive	Present study
4	Kanamycin(K) (30mcg)	*Halobacterium salinarium*	Resistant	Elazari-volcani(1957)
		Halococcusmorrhuae	Resistant	Kocur and Hogkiss(1973)
		Haloferax volcanii	Resistant	Mullakhanbhai and Larsen(1975)
		Halobacterium saccharovorum	Resistant	Tomlinson and Hochstein(1976)
		Haloarcula vallismortis	Resistant	Gonzalez et al.,(1978)
		Haloferax mediterranei	Resistant	Rodriguez – valera et al.,(1983a)
		Halobacterium sodomense	Resistant	Oren(1983c)
		Haloarcula hispanica	Resistant	Juez et al.,(1986)
		Haloarcula japonica	Resistant	Takashina et al.,(1990)
		Halobacterium saccharovorum	Sensitive	Prabha (2000)
		Halobacterium sodomense	Sensitive	Present study
5	Norfloxacin (N7) (10 mcg)	*Halobacterium saccharovorum*	Resistant	Prabha (2000
		Halobacterium sodomense	Sensitive	Present study

6	Ofloxacin(O 7) (5 Mcg)	*Halobacterium saccharovorum*	Resistant	Prabha (2000
		Halobacterium sodomense	Resistant	Present study
7	Pefloxacin (P7) (5 mcg)	*Halobacterium saccharovorum*	Sensitive	Prabha (2000
		Halobacterium sodomense	Resistant	Present study
8	Streptomycin (S)(10 mcg)	*Haloarcula hispanica*	Resistant	Juez et al.,(1986)
		Haloarcula japonica	Resistant	Takashina et al.,(1990)
		Haloarcula vallismortis	Resistant	Gonzalez et al.,(1978)
		Halococcus morrhuae	Resistant	Kocur and Hogkiss(1973)
		Halococcus saccharolyticus	Sensitive	Parameswari (1999)
		Halococcus saccharolyticus	Resistant	Ventosa et al.,(1990)
		Haloferax denitrificans	Resistant	Tomlinson et al.,(1986)
		Haloferax menditerranei	Resistant	Rodriguez – valera et al.,(1983a)
		Haloferax volcanii	Resistant	Mullakhanbhai and Larsen(1975)
		Halobacterium locusprofundi	Resistant	Franzmann et al.,(1988)
		Halobacterium salinarium	Resistant	Elazari-volcani(1957)

		Halococcus salinarium	Sensitive	Parameswari (1999)
		Halobacterium saccharovorum	Resistant	Tomlinson and Hochstein(1976)
		Halobacterium saccharovorum	Sensitive	Parameswari (1999)
		Halobacterium saccharovorum	Sensitive	Prabha (2000
		Halobacterium sodomense	Resistant	Oren(1983c)
		Halobacterium sodomense	Sensitive	Parameswari (1999)
		Halobacterium sodomense	Sensitive	Present study

Conclusion:

The results showed Halobacterium in the saltpan are extreme halophilic in nature. It grow in media containing salt and were found to be much resistance to antibiotics.

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BIO-STIMULATING EFFECT OF MARINE ALGAE *KAPPAPHYCUS ALVAREZII'S* SLF ON *SOLANUM LYCOPERSICUM*

Lakshmi. V
PG Department of Zoology, PMT College,
Melaneelithanallur, Tirunelveli – 627 953
South India. *Email:* moselakshmi@gmail.com

Introduction

Organic agriculture is an eco-friendly construction management system that encourages and boosts biodiversity and soil biological activity. It is centered on nominal use of farm inputs and some management practices that restore, maintain and enrich green harmony.

Seaweed fertilizers can be used as a soil management, meanwhile most plants absorb their nutrients through the leaves and applying it with a foliar method will benefit the plant. As the plant engrosses sunlight, it will also absorb the nutrients in the fertilizer.There is a long history of shoreline people using seaweeds, particularly the large brown seaweeds, to neutralize the nearby land. They have a proper content of nitrogen and potassium. The large amounts of insoluble carbohydrates in brown seaweeds act as soil conditioners and have good moisture retention properties. In the late 1960s, Dr. T. L. Senn at Clemson University found that high levels of natural plant hormones in seaweed, called cytokinin, stimulated plants, providing a growth stimulator effect.

Seaweed has been used widely in South Asian countries for multipurpose application such as food, animal feeds, fertilizers and others (Dhargalkar and Verlecar, 2009).The present study endeavor to assess the fertilizer potential of Kappaphycusalvarezii's Extract (SLF) in endorsing linear growth and yield of *Solanumlycopersicum*plant.

Materials and Methods

The freshly collected marine macro algae *Kappaphycusalvarizii* were rinsed thoroughly with fresh water for removing accumulated salt. Then the rinsed materials were homogenized at room temperature and filtered liquid was stored in an air tight container. These filtrate was taken as 100% SLF. 20% diluted SLF was used for foliage spray to plant *Solanumlycopersicum.*

Bag experiment was conducted from August to October, 2015. A mixture of red soil, black soil, sand and farmyard manure in 2:1:0.5:0.5 was used to fill bag in order to uphold substratum standardization. Certified seeds of *Solanumlycopersicum* were sorted out for uniform size and used for sowing. Two sets of experimental bags (45cm height 30cm width sized) were designed for this study. The first set of bags containing plant was treated with compound chemical fertilizer 2g/bag (CHF)and second set of plantwas treated with diluted SLF 50ml/bag at regular intervals (once in 20 days).SLF application done in early morning or in the late evening.

The SLF treated seeds were sowed in SLF bags and non-treated seeds were sowed in CHF bags. The effects of chemical fertilizer and the SLF on *Solanumlycopersicum*were analyzed from germination to yield.

Results and Discussion

Application of seaweed extract as organic bio-stimulant is a fast becoming accepted practice in horticulture due to its beneficial effects (Verkleij, 1992).To meet the increasing demand of organic fertilizer many viable options have to be explored (Chhaya, 1997) and one such option is use of seaweed extracts as fertilizer (Zodape, 2001).Soaking seed with liquid seaweed prior to planting will improve seed germination, root growth and early seedling vigor.(http://www.growgreatvegetables.com/fertilizers/liquid-seaweed-fertilizer/) and this statement was proven by this experiment (Table – 1; Picture – 1: A).

Table – 1. CHF and SLF effects on *Solanumlycopersicum*seedlings.

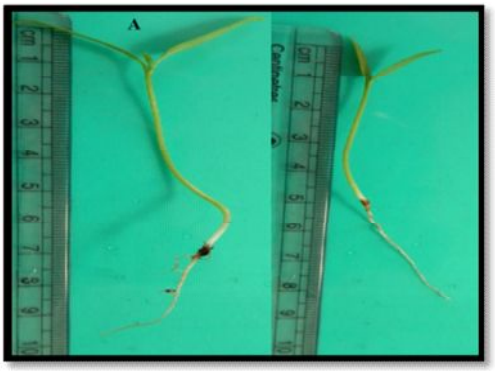
Fertilizer	Germination	Primary leaf length	Shoot length	Root length	Number of secondary roots
CHF	6th day	1.1 ± 0.05	4.3 ± 0.2	3.1 ± 0.4	2 ± 0.02
SLF	4th day	3.2 ± 0.3	5.2 ± 0.3	4.7 ± 0.3	9 ± 0.2

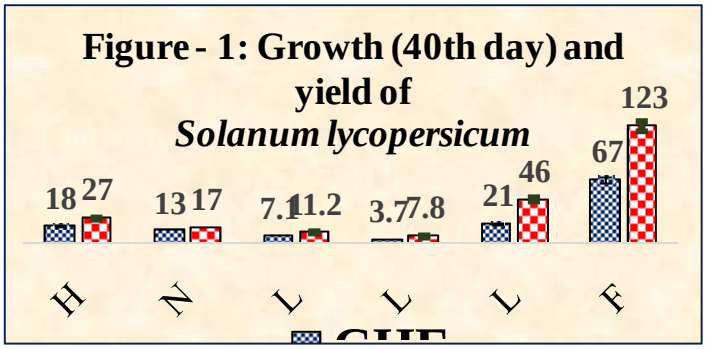
Mean = 10 ± SD; CHF – Chemical Fertilizer; SLF – Seaweed Liquid Fertilizer

Picture – 1: A. Seedlings of *Solanumlycopersicum;*

B. 50th day plant

C. Fruits of *Solanumlycopersicum*





Organic agriculture is an eco-friendly construction management system that encourages and boosts biodiversity and soil biological activity. It is centered on nominal use of farm inputs and some management practices that restore, maintain and enrich green harmony.

The seaweed extract contains micro nutrients, auxins, cytokinins and other growth promoting substances and the bio - stimulant present in seaweed extract increase the vegetative growth, Chlorophyll content, Stomata density, Photosynthetic rate and the fruit production of the plant Strawberry (Spinelli et. al., 2010). In 2008 Zodape et al., reported that treatment of seaweed extract increase length, Diameter and yield of *Abelmoschusesculentus* and alsothe similar results were obtained in the present study, it shows that the SLF sprayed plants had greater effect on growth and yield than the CHF treated plants (Picutre – 1; and Figure -1). Thivy (1960) studied the application of seaweed as fertilizer on vegetables and field crops and the performance of the seaweed manure was found to be expressively better than that of farm yard manure due to the easy decomposability of its carbonaceous matter and presence of micro nutrients. The use of seaweed manure in aggregation with the inorganic fertilizers has been found to be better than the other organic input for the growth and development of plant (Kaliaperumal, 2000).All the growth and yield related parameters were significantly higher (one way ANOVA $P<0.05$) in the SLF treated *Solanumlycopersicum*plant (Figure – 1).

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ANTICOAGULANT AND ANTIANGIOGENIC EFFECT OF FUCOIDAN FROM TURBINARIA DECURRENS (BORY DE SAINT – VINCENT, 1828)

Selvaraju Meenakshi[1], Ravichandran Saravanan[2],
Shanmugam Umayaparvathi[3], Thangavel Balasubramanian[3]
1.Sriparamakalyani Centre of Excellence in Environmental Sciences,
2.Manonmaniam Sundarnar University. Tamilnadu, India
3.Sri K.G.S Arts College, Srivaikuntam, Tamilnadu, India

1 Introduction

Marine algae, especially seaweeds are unexplored reservoir of bioactive compounds. Sulfated polysaccharide from seaweeds comprises a complex group of macromolecules like fucoidan, laminarin and alginate with a wide range of important biological properties with low toxicity (Mourao, 2007). Fucoidan represent 40% of the dry weight of isolated cell walls of brown seaweeds (Berateau and Mulloy, 2003) and it is composed of fucose, uronic acids, galactose, xylose and sulfated fucose (Leite *et al*., 1998). They are comprised of long chains of linked sugar molecules, along with sulfate groups which make them negatively charged. They composed primarily of highly branched (1→2) or (1→3) linked α-L-fucose-4-sulfate units (Ermakova *et al*., 2011). Fucoidan from algae possess important pharmacological activities such as antioxidant (Wang *et al*., 2008), anticoagulant and antithrombotic (Zoysa *et al*., 2008), antitumour and immunomodulatory (Kima *et al*., 2008), antipeptic and antiadhesive (Azevedo *et al*., 2009) hepatoprotective (Meenakshi *et al*., 2014), neuroprotective (Meenakshi *et al*., 2015), against myocardial injury (Krishnamurthy *et al*., 2012).

Anticoagulant activity is one among the most widely studied properties of sulfated polysaccharides. The anticoagulant effect of fucoidan is considered to be a complex mechanism involving direct inhibition of thrombin catalyzed fibrinogen cleavage (Grauffel *et al*., 1989) and also the enhancement of heparin cofactor II (HC 11) mediated thrombin inhibition (Church *et al*., 1989). Unfractionated heparins and low molecular weight heparins are the only sulfated polysaccharides currently used as anticoagulant drugs. However these compounds have several side effects such as bleeding and thrombocytopenia (Pereira *et al*., 2002), which is increasing the necessity to look for alternative source of anticoagulant agents.

A role of sulfated polysaccharide from algae as antiangiogenic agents has also been suggested. Angiogenesis refers to the growth of new capillaries from pre-existing capillaries and post-capillary venules. It is a tightly controlled process that rarely occurs under normal conditions, except for instances of wound healing, embryonic development and development of the corpus luteum (Bergers and Benjamin, 2003). Many diseases, however, are driven by persistent unregulated angiogenesis. To date, therapeutic benefit has been achieved with anti angiogenic therapy in the treatment of life threatening infantile hemangioma, pulmonary hemangiomatosis and in the treatment of some vascular tumours. So, there is a great interest for identifying and modulating antiangiogenic pathways and antiangiogenic drug development for therapeutic purposes (Folkman, 1995). Some algal sulfated polysaccharides have been found to inhibit angiogenesis by interfering with the binding of vascular endothelial growth factor (Koyanagi *et al*., 2003). So the study was conducted to observe the anticoagulant and antiangiogeneic effect of fucoidan from *T. decurrens*.

4.2 Materials and methods

4.2.1 Collection and extraction of fucoidan

The brown seaweed *T. decurrens* belonging to the family Phaeophyceae was collected in yervadi, India. The specimens were identified and authenticated by phycologist Dr. P.Anantharaman, Associate professor, Faculty of Marine sciences, Annamalai University, Parangipettai, India. The collected *T. decurrens* was initially washed with seawater to remove the macroscopic epiphytes and other extraneous matter, and then rinsed in distilled water. The specimen was shade dried and coarsely powdered. 100g of dried seaweed powder was depigmented with acetone followed by hot water extraction at 90-95°C for 3-4 hrs. The brown colored syrup was then filtered through Whatmann No.3 filter paper, concentrated to ¼ of the original volume, cooled and precipitated with three volumes of ethanol overnight at 4°C. The precipitate was centrifuged at 5000 rpm, dehydrated with diethyl ether and further purified in HPLC (data not shown) to get a sulphated polysaccharide, fucoidan (Krishnamurthy *et al*., 2012). The biochemical estimation of the isolated fucoidan is: Carbohydrate 59.62%, Sulfate 26.52% and Uronic acid 6.3%. All the chemicals were purchased from Sigma chemical company (St. Louis, MO) and Himedia (Mumbai, India) respectively.

4.2.2 Anticoagulant activity

4.2.2.1 Preparation of Plasma: Blood was collected from individual healthy donor through vein puncture without bleeding or thrombosis and it was mixed with 3.8% tri sodium citrate at 9:1 ratio. Further it was centrifuged for 20 min at 2400×g and the plasma was stored at -40°C until use.

4.2.2.2 Activated Partial Thromboplastin Time (APTT)

In activated partial thromboplastin time assay, citrated normal human plasma (90µl) was mixed with sample (10µl) in each concentration (25, 50, 100, 150 and 200µg/ml) and incubated for 1 minute at 37°C, followed by APTT reagent (100µl) was added to the mixture and incubated for 5 min at 37°C. Thereafter, the clotting was induced by adding 0.02M calcium chloride (100µl) and clotting time was recorded. (Method followed by Pacific hemostasis kit).

4.2.2.3 Prothrombin time (PT)

In prothrombin time, the citrated normal human plasma (90µl) was mixed with 10µl of sample in each concentration (25, 50, 100, 150 and 200µg/ml) and incubated for 10 min. Then, PT reagent (200µl) was pre-incubated for 10 min at 37°C. The pre-incubated PT reagent was added and clotting time was recorded. (Method followed by Pacific hemostasis kit).

4.2.3 Antiangiogenic activity

Chrio allontonic membrane assay

The CAM assay was performed as earlier described by Ribatti *et al*., (1997) with suitable modification. Fresh fertilized white leghorn chick eggs were purchased from farm, Parangipettai, Tamilnadu and further incubated in a humidified incubator at 37± 2°C. The eggs were divided equally into four group viz control and test groups. In day 3 of incubation, a hole was punctured on the egg shell and 2-3 ml albumin were drawn, the shell sealed with sterile scotch magic tape and the egg was placed for further incubation. In 7th day of incubation a small window was drilled in the egg shell, vascular endothelial growth factor

(VEGF) and sample was loaded on the chrio-allantonic membrane of each egg under sterile condition. In 12th day of incubation, the windows were opened and the anti-angiogenesis response was observed on the chorio-allantonic membrane. The chrio-allanonic membrane of each egg was viewed under microscope and documented.

4.3 Results

4.3.1 Anticoagulant effect of fucoidan from *T. decurrens*

In this study, the coagulation of both intrinsic and extrinsic pathways of fucoidan from *T. decurrens* in different concentration (25, 50, 100, 150 and 200µg/ml) was determined through APTT and PT assays. In APTT, the coagulation time of the control was 23.4s and in fucoidan from *T. decurrens* it prolonged to >300s at 150 and 200 µg/ml. In PT, the coagulation time of the control was 9s and in fucoidan from *T. decurrens* prolonged to 120s at 200 µg/ml (Table.1).

Table.1. Anticoagulant activity of fucoidan from *T. decurrens* using APTT and PT assays

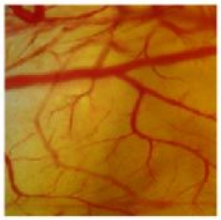
Sample (µg/ml)	APTT*	PT*
25	66.5s	55.7s
50	81.3s	78.3s
100	103.2s	95.7s
150	>300s	102s
200	>300s	120s

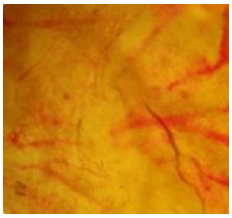
*The data are the mean values of two experiments
APTT for control without fucoidan from *T. decurrens*: 23.4s
PT for control without fucoidan from *T. decurrens*: 9s

4.3.2 Antiangiogenic effect of fucoidan from *T. decurrens*

Fucoidan exhibited strong antiangiogeneic activity in a dose dependent manner by CAM assay, which is commonly used to study the *in vivo* angiogenesis and anti angiogenesis. Between the two concentrations of the sample 100 µg (fucoidan from *T. decurrens*) /egg showed good result and blood vessel was not developed after the administration of compound around the disc. In the case of 50µg (fucoidan from *T. decurrens*)/egg the inhibition of vessel formation is less (Fig.1).

Fig.1 Antiangiogenic activity of fucoidan from *T. decurrens*

4.4 Discussion

Most of the algal species exert their anticoagulant action through the sulphated polysaccharides (Church *et al*., 1989) and some of them trigger anticoagulant activity through protein, or glycoprotein-like compounds (Yasuda *et al*., 2004). In the present study, hot water extraction method showed promising result for anticoagulant activity and it was in good agreement with Shanmugam and Mody, (2000). The sulphated polysaccharide (fucoidan) which is used in the present study can be capable of binding with proteins at several levels of specificity and exhibit high affinity for particular proteins and it is accepted that the anticoagulant activity of the sulfated polysaccharides partly results from the strong interaction between the negatively charged sulfate groups and some positively charged peptidic sequences (Azevedo *et al*., 2009).

The fucoidan stimulates tissue-type plasminogen activator-induced plasma clot lysis by protecting plasmin activity from inactivation by ct2-antiplasmin and the biological effects of fucoidan have been found to depend on the degree of sulfation and molecular size of the polysaccharide chains. (Soeda *et al*.,1992). In the present study, fucoidan from *T. decurrens* showed highest activity in both APTT (>300s) and PT (120s) assays. Similar result (>300s) was observed by Athukorala *et al*., (2007) in Ultraflo extract of *C. fragile* and *S. hornei* in the APTT activity but it was lower in hot water extract of *C. fragile* (250s). The lower activity was observed in few brown seaweeds such as, *S. siliquastrum* and *S. hornei* (170s), *Laminaria ochotensis* (150s), *S. thunbergii* (85s), *S. fulvellum* (75s), *S. coreanum* (70s), *Undaria pinnatifida* (48s) and *Padina arborescens* (37s) (Athukorala *et al*., 2007), *Gracillaria. verrucosa, G. textoria* and *Gloiopeltis furcata* (38.6s, 49.1s, and 51.7s) (Pushpamali *et al*., 2008). In fermented brown seaweed *S. fulvellum* the activity (202s in eighth week and 104s for tenth week) was low (Zoysa *et al*., 2008). This indicates that the polysaccharides have good activity than the fermented seaweeds. In PT assay, the lower activity from *C. fragile* (20s), *S. hornei* (13s) (Athukorala *et al*., 2007) and *G. verrucosa, G. textoria* and *Gloiopeltis furcata* (25.3s, 24.7s, and 31.6s) (Pushpamali *et al*., 2008) were comparatively lower than the present study. Hence, fucoidan from *T. decurrens* will be a better candidate as a anticoagulant agent.

Angiogenesis is a strictly controlled process in a normal human body and regulated by a variety of endogenous angiogenic and angiostatic factors (Folkman and Klagsbrun, 1987). The pathological angiogenesis occurs, in cancer, chronic inflammation, or atherosclerosis. Angiogenesis inhibitors are able to interfere with various steps of angiogenesis, on the other hand, angiogenesis promoters can stimulate angiogenesis occur basement destruction of blood vessels, proliferation and migration of endothelial cells. The present study demonstrates the antiangiogenic activity of fucoidan from *T. decurrens* which showed good activity even in lower concentration. In the CAM assay in chick embryos which are perhaps the most widely employed *in vivo* model for studying vessel development (Yancopoulos *et al*., 2000). An important stimulating factor in angiogenesis is vascular endothelial growth factor (VEGF), which acts on VEGF receptors. VEGF stimulates the cells to produce matrix metalloproteinases (MMPS), which degrade the basement membrane and surrounding extracellular matrix. As a result, endothelial cells proliferate and migrate towards the interstitium, where they start sporuting. Subsequently, the cells proliferate and migrate towards the newly formed sporuts and mature by forming single cell layer around the sporut (Hoeben *et al*., 2004). Fucoidan from *T. decurrens* prevent the VEGF from binding with the receptors on the surface of the endothelial cells owing to its central role in promoting tumor

growth, VEGF has become a key therapeutic target and its function can be blocked at different levels of the signaling pathways.

Cumashi *et al*., (2007) investigated the anti- angiogenic potential of nine fucoidans from brown algae. Ye *et al*., (2005) also showed antiangiogenic property of *Cladosiphon novae* in the concentration of 1 mg/ml. Dias *et al*., 2005 observed the inhibition of sulphated polysaccharide from *S. stenophylum* in the highest dose of 1500µg/plug was roughly double that achieved by hydrocortisone (156g/plug). Hence, the role of fucoidan from *T. decurrens* showed its prominent role as anti-angiogeneic compound by reducing the number of blood vessels as observed in treated eggs.

4.5. Acknowledgement

The authors would like to thank the Ministry of Earth science, India (Project entitled: Drugs from the sea) for the financial support. The first was supported by UGC-Dr. D.S. Kothari fellowship, Government of India, during production of this manuscript. The authors are grateful to the authorities of CAS in Marine Biology, Annamalai University for providing the necessary facilities.

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SEAWEEDS AS BIOFERTILIZER

C. Parthiban,[1] P. Anantharaman[2]

1. National Centre for Sustainable Coastal Management,

Ministry of Environment forest and climate change,

2. CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University,

Parangipettai - 608 502, Tamil nadu Email : panantharaman@gmail.com

pcsparthiban@gmail.com

Introduction

Nowadays there is much usage of more number of chemical fertilizers to increase the productivity of crops but it causes several damages to the ecology of the soil and their fertility content. Chemical fertilizers are harmful to human way of directly and indirectly. For health benefits, agricultural practices are being modified with organic farming. The use of bio-fertilizers is to increase the plant growth and development and it is eco-friendly to the environment. The cost of chemical fertilizers is very high and sometimes it is not available in the market for which the farmers fail to apply the chemical / inorganic fertilizers to the crop field in the optimum time. On the other hand, the organic manure is easily available to the farmers and is cost effective when compared to that of chemical fertilizers. Therefore researchers have to find out the suitable bio-fertilizers for replacing the chemical fertilizer.

Seaweed

Seaweed is the marine macroscopic algae found attached to the bottom in relatively shallow coastal waters. They grow in the intertidal, shallow and deep sea areas up to 80 meter depth, estuaries and backwaters on solid substrate such as rocks, dead corals and pebbles. The

seaweeds are totally different from higher plants as they neither have true leaves, stems and roots or vascular systems neither specialized sex organs.

There are 20,000 species of seaweeds found distributed globally. The major sources of seaweeds are the northeast, western central and southwest pacific areas. There is not much information regarding the Antarctic and Arctic regions. India, with a long coastline (7500 km), has vast resource of seaweeds along with many open coasts and estuarine areas. Along the coastline of India, the littoral and sub littoral rocky areas support good growth of different seaweeds. There is luxuriant growth of seaweeds along the Southeast coast of Tamil Nadu, from Mandapam to Kanyakumari; Gujarat coast; Lakshadweep Island and the Andaman and Nicobar Islands. Fairly rich seaweed beds are present in the vicinity of Mumbai, Ratnagiri, Goa, Karwar, Varkala, Kovalam, Vizhinjam, Visakhapatnam and few other places such as Chilka and Pulicat lakes. In India, 844 species are reported (Oza and Zaidi, 2001) including 216 species of green algae, 194 species of brown algae and 434 species of red algae

The word 'Weed' means an economically useless plant, which grows wild and mostly harmful. But man started thinking of how to make use of those useless plants for the benefit of human-kind. This has gradually lead to domestication of the weeds, and also attempted to produce food and raw materials for industries from those weeds. The importance of seaweeds as economic resources has been known for quite a long time. Their usefulness is felt both directly and indirectly. A direct benefit includes use of seaweeds as food, feed and fertilizer, as source of various products of commercial importance and as source of energy.

Seaweeds are divided into green, brown and red algae based on the types of pigments, morphological and anatomical structures. They are the only source for the production of phytochemical such as agar, carrageenan and alginates. These phytochemicals are extensively used in various industries such as food, confectionary, textile, pharmaceutical, cosmetics, dairy, liquor, canning, paint, varnish, paper etc. and as gelling, stabilizing and thickening agents. Seaweeds are also used as human food, live stock feed and fertilizer for plants in many parts of the world. They contain more than 60 trace elements, protein, iodine, bromine, vitamins and several bioactive substances of economic value and they also serve as both feeding and breeding grounds for invertebrates and fishes (Krishnamurthy, 2005).

Biofertilizers

Adequate supply of plant nutrients is necessary for proper growth of crop plants. Nitrogen and phosphorus are the most important among nutrients. Generally they are supplied to crops by applying manures and fertilizers in the soil. Depending on the nutrients they supply, biofertilizeres are grouped into broad categories; nitrogen supplying biofertilizers and phosphorus supplying biofertilizers.

There are a number of advantages in using bioferilizers. The following are the main among them and are briefly explaine. Biofertilizers can be called poor man's technology. Taking in to account the amount of nutrient supplied, biofertilizers are many times cheaper than chemical fertilizers. Biofertilizers not only supply nitrogen and phosphorus but also some micronutrients essential for plant growth. Sometimes yield is limited by micronutrients essential for plant growth. Sometimes yield is limited by micronutrients and application of nitrogenous, phosphatic and potassic fertilizer does not improve yield significantly. Organic matter is the essential component of soil. It serves as an inexhaustible source of nutrients and energy from the plants as well as for useful microorganisms. Organic matter has great impact on the physical and chemical properties of the soil. When chemical fertilizers are exclusively

and continuously used for a few years, they may create acidity or alkalinity in the soil and deteriorate the quality of the soil. Soil also becomes unresponsive to further use of similar fertilizers. Application of biofertilizers along with chemical fertilizers can avoid this problem to a great extent. Besides, large amount of organic matter supplied by the biofertilizers impart tolerance power (buffering capacity) to the soil against acidity or alkalinity. It also withholds metallic elements from entering the plant roots, thereby reducing harmful effects of pesticides. Plants also need for their growth and development, some natural complex chemical compounds called hormones in adequate amounts, azotobactor blue green algae and azolla have been found to synthesise growth hormones (e.g. indol acetic acid and vitamin B) which benefit the main crop. Sometimes, biofertilizer application gives significant response even if the soul is already rich in plant nutrients. This occurs due to the supply of growth hormones by biofertilizers to the main crop.

Seaweed as Biofertilizer

Seaweeds, one of the important marine living resources are now days considered as a promising marine bioresource. The use of seaweed as manure in farming practice is very ancient and was prevalent among the Romans. Seaweed Liquid Fertilizer (SLF) contains macronutrients, trace elements, organic substances like amino acids and plant growth regulators (Verkleij, 1992). The use of seaweeds as biofertilizers in horticulture and agriculture has increased in the recent years (Dhargalkar and Pereira, 2005). Recent researches have proved that seaweed fertilizers are preferred not only due to their nitrogen, phosphorus and potash content but also because of the presence of trace elements and metabolites similar to the plant growth regulators. Seaweed fertilizer was found to be superior to chemical fertilizer because of high level of organic matter which aids in retaining moisture and minerals in upper soil level available to roots (Wallenkemp, 1955). Seaweed liquid fertilizer (SLF) contains macro nutrients, trace elements, organic substances like amino acids and plant growth regulators such as auxin, cytokinin and gibberellins (Williams *et al.,* 1981; Nelson and Van Staden, 1984 and Ramo Rao, 1991). The seaweed liquid fertilizer (SLF) is an excellent source of major elements such as N, P, K, Ca and Mg as well as many micro nutrients, required for normal growth of plants (Aitken and Seen, 1965).

Seaweed Liquid fertilizer

The use of seaweeds as manure in farming practice is very ancient and was prevalent among the Romans and also practiced in Britain, France, Spain, Japan and China. There are also records of the culture of seaweeds for manure in Ireland and South Africa. Seaweed liquid fertilizer has a uniquely balanced mix of nutrients that will maximize its potential if these nutrients are available at the optimal levels and at the opportune moments in the plant's development. The Seaweeds are used either directly or after composting or burning is being made into a meal. In some case of seaweeds, especially species of *Sargassum* has been used in parts of coastal Kerala as manure for coconut plantation. Experiments on the use of seaweeds as manure have been carried out by Thivy (1960), who showed that seaweed promotes higher rate of growth and higher yield in crop plants. Thivy (1964) also advocated seaweeds for improving the fertility of soil in cultivable fields. Bhosle *et al.,* (1975) prepared seaweed liquid fertilizer (SLF) and studied its effect on *Phaeseolus vulgaris*. Rama Rao (1979) reported good yields of *Zizyphus rugosa* fruits, where leaf spray of SLF obtained from *Sargassum* was used. Similarly Parthiban et al., (2013) has reported good yield of *Vigno mungo* grains used as *Spatogloassum asperum*.

Seaweed extracts are known to enhance seed germination and plant growth (Bhosle *et al.,* 1975; Venkatraman Kumar *et al.,* 1993; Mohan *et al.,* 1994; Sekar *et al.,* 1995). In

agriculture and horticulture practices seaweeds are used as manure, liquid fertilizer, growth promoter and crop protestant against pest and diseases. Seaweed extracts are known to enhance seed germination and plant growth (Bhosle *et al*., 1975; Rajeshwari *et al*., 1983; Venkataraman Kumar *et al*., 1993; Mohan *et al*., 1994). In India, as a step towards the expansion of native sources of natural manures, the seaweed fertilizers application will be useful for achieving higher production. Dhargalkar and Untawale (1983) studied the effect of seaweed extracts on the growth of chillies and turnip and found that lower concentrations of SLF enhanced the rate of seed germination.

So far not much work has been done under field conditions to examine the efficiency of SLF, especially in India (Dhargalkar and Untawale, 1983). The SLF obtained from brown, red and green seaweeds are now available commercially in trade names such as Maxicorp (Sea born), Algifert (Manure), Golmar, GA 14, Kelpak 66, Seaspray, Seasol SM3, Cystex and Sea Crop 16 for use in agriculture (Jeanin *et al.,* 1991).

Conclusion

In India, agriculture making the backbone of our economy, nearly 70% of the people thrives in rural areas. The growing population is mounting tremendous pressure in food production in the country. To meet the demand, farmers use chemical fertilizer to enhance the crop production this results in vast pollution. As an alternative, SLF was found to be a good source of plant growth but further more research is need to strongly establish the mechanism of action of the seaweed extract on the plant growth. Aqueous seaweed extracts is employed extensively as fertilizer additive in foliar spray an organic and horticulture crop plants. One important explanation for the effect of seaweed concentration in promoting nutrient uptake is the increased uptake of nitrogen, potassium, calcium, manganese, magnesium, iron and zinc.

As a whole seaweeds not only improve the growth of plants but also helps to retain the nutrients lost by the soil, thus improving soil for agriculture to a great extend enabling pollution free land that also meets the growing requirement of high yield.. The use of SLF is a wise eco-friendly technique to enhance crop production. This would be beneficial to the environment also. The SLF is a boon to the agriculture and are eco-friendly with less cost effectiveness without affecting the soil and their ecosystem. Earthworms are farmer's friend which is harmed by chemical fertilizers, so replacement of the chemical fertilizers by the SLF is an adaptive way for today's world. Further, seaweed extracts are considered as an organic farm input as they are environmentally benign and safe for the health of animals and humans.

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DISTRIBUTION OF SEAWEEDS AT DIFFERENT COASTAL AREA OF KANYAKUMARI DISTRICT – A PRIMARY STUDY

M. Suresh, V. Thanappan, P. Anantharaman*

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalau University, Parangipettai – 608 502. Tamilnadu, India

Email: panantharaman@gmail.com

1. INTRODUCTION

Seaweeds are macroscopic marine algae found attached to the bottom in relatively shallow coastal waters. They grow in the intertidal, shallow and deep sea areas up to 80 meter depth, estuaries and backwaters on solid substrata such as rocks, dead corals and pebbles. Seaweed zone is one of the obvious and wide spread biotope in the shallow marine environment. The seaweeds are totally different from higher plants as they neither have true leaves, stems and roots or vascular systems neither specialized sex organs. Seaweeds have been divided into green, brown and red based on the types of pigments, morphological and anatomical structures. They are the only source for the production of phyto-chemical such a sagar, carrageenan and alginates.

Seaweed resources of the world comprise about 1460 million tones wet weight of brown algae and 261 million tonnes of red algae. The total seaweed production may be about 1721×10^{-4} tonnes wet weight annually (Michanek, 1975). The major sources are the northeast, western central and southwest pacific areas. Along the coastline of, the littoral and sublittoral rocky areas support good growth of different seaweeds. There is luxuriant growth of seaweeds along the Southeast coast of Tamilnadu, from Mandapam to Kanyakumari; Gurarath coast; Lakshadweep island and the Andaman and Nicobar Islands. Fairly rick seaweed beds are present in the vicinity of Mumbai, Ratnagiri, Goa, Karwar, Varkala, Kovalam, Vizhinjam, Vishakapattinam and few places such as Chilka and Pulicat Lakes. In India, 850 species are reported (Oza and Zaidi, 2001) and their commercial exploitation has been commenced since 1966. At present, 1518 tonnes of dry weight red algae and 2285 tonnes of dry weight brown algae were exploited for the manufacture of agar, algin, carrageenan and liquid fertilizer (Kaliaperumal *et al.,* 2004).

Distribution of economic seaweed resources along the Indian coast was first mapped by Thivy, 1958. The Tamilnadu coast was surveyed during 1972-76, covering a distance of 320km from Rameswaram and adjoining islands to Melmidalam (Colachel) by CSMCRI (Central Salt Marine Chemical Research Institute) in collaboration with Central Marine Fisheries Research Institute (CMFRI), Cochin and Department of Fisheries, Government of Tamilnadu. The total standing crop of seaweeds in the intertidal region of Tamilnadu was estimated about 22,044 tonnes (fresh weight) in a potential area of 9891.35ha of the 20,000ha total area surveyed (Anon, 1978). The evaluation of the species composition or distribution along the coast of kanyakumari is still unexplored. Hence, we have concentrated on the survey of seaweed distribution along the Kanyakumari coast in a single season.

2. MATERIALS AND METHODS

The survey on seaweeds distribution was made throughout the Kanyakumari coastal area during the post monsoon period from January to February 2016. The seaweeds were collected from Vattakottai, Arockiapuram, Leepuram, Kanyakumari, Mutton, Chinna mutton,

Manakudi, Chinnavilai, Manavalakurichi. The seaweeds were collected in the plastic container contain seawater added with 4% formalin. The fresh seaweeds were washed well in the habitat water at the respective sampling site to remove silt and debris including the phytal fauna. The collected seaweed samples were identified in species level and the biomass values were recorded as dry weight (wet) of the seaweed.

3. RESULTS

3.1. Distribution

Distribution of seaweed species were recorded in Kanyakumari district coastal area at eleven stations. Totally 34 species were recorded including 23 genera, 15 species of red algae, 10 species of Brown algae and 9 species of green algae. Maximum number of species was recorded in red algae compared to other two groups. Minimum number of species was recorded in green algae. In Vattakottai, totally 13 species were recorded and it was observed to be 7 species of brown algae and 2 species of green algae and 4 species of red algae. Next to the Vattakottai, the maximum of 9 species were recorded in Kanyakumari, which includes 4 species of green algae, 2 species of brown algae and 3 species of red algae (Table 1).

Table. 1. Distribution (Number of species) at different station

Seaweeds / Station	Green Algae	Brown Algae	Red Algae
Kanyakumari	4	2	3
Vattakottai	2	7	4
Leepuram	-	-	-
Arockiapuram	2	2	3
Manakudi	-	-	-
Chinnavilai	2	4	3
Muttom	2	3	2
Chinna Muttom	4	3	1
Manavalakurichi	1	1	2

Maximum number of green algae was recorded in Kannyakumari subsequently the maximum number of brown seaweeds and red seaweeds were recorded in Vatakottai, and Arockiyapuram respectively. The distribution of *Gracilaria corticata* and *Sargassum* sp was found to be dominant at all stations. The minimum number of species was recorded in Chinnavilai.

The brown algae, *Stoechospermum marginatum* and *Porphyra kanyakumariesnsis* was recorded only in Vatakottai. The red algae *Padina boergesenii and Padina tetrastromatica* was recorded in Kanyakumari and Arockiapuram respectively. The green algae *Ulva lactuca* and *Ulva fasciata* was only recorded at Muttom. The green alga *Valaniopsis pachynema* was recorded in the rocky area of Arockiapuram whereas it was not seen at other stations. The brown alga *Calpomenia sinuosa* was observed only at Vatakottai. The green alga *Chaetomorpha linum* was recorded in Vatakottai.

3.2. Biomass

The total biomass of the green algae was observed as 3.25 kg dry weight. The maximum biomass was recorded at Kanyakumari. Total biomass of brown algae was recorded as 6.50 kg dry weight subsequently the maximum was recorded at Vatakottai. The biomass of red algae was found as 3.10 kg dry weight. The maximum was recorded at Kanyakumari.

4. DISCUSSION

The present study investigates the distribution and biomass of seaweeds along the district of Kanyakumari coastal area. The studies on the diversity and distribution of seaweeds in Indian waters (Untawale et al., 1989; Selvaraj and Selvaraj, 1997; Mohammed *et al.,* 1999; Stella roslin *et al.,* 2001;; James et al., 2004; Kerkar, 2004; Rath and Adhikary, 2006; Satheesh and Wesley, 2012).

In the present study we have recorded habitat viz seaweed distribution during the study period. The present results are in accordance with Thakur *et al.,* 2008, had reported seaweeds are important marine and esturine plants distributed throughout the world. The biomass as well as species composition of seaweeds largely depend on season, population structure and several other ecological factors.

The distribution of seaweeds during the present study was found to be vary at different stations. It is propable that some species might have lost due to changes in the environmental conditions over a long period of time.

5. CONCLUSION

In total, 34 species of seaweeds have been recorded from the selected coastal area. The occurrences of seaweeds in the different station vary might due to the environmental conditions. Hence, the analysis of environmental parameters with rigorous statistical tools will give the real picture about the distribution of seaweeds in the respective places.

ACKNOWLEDGEMENT

The authors of this manuscript would like to thank University Grant Commission-Major Research Project for providing financial support during the study period.

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PRELIMINARY EVALUATION AND PARTIAL CHARACTERIZATION OF ANTIHELICOBACTERIAL BIOACTIVE METABOLITE FROM SEAWEEDS.

* Dr. G. Ramanathan, T. Devi Priya A. R. Vijaya Lalitha, and P.Suma Rajalakshmi
Research Department of Microbiology, V. H. N. Senthikumara Nadar College,
Virudhunagar- 626 001, TamilNadu, India.
* Corresponding author saranbag@gmail.com

INTRODUCTION

Peptic ulcer, lesion that occurs primarily in the mucous membrane of the stomach or duodenum (the upper segment of the small intestine); Between 10 and 15 percent of the world's population suffers from peptic ulcer. Duodenal ulcers, which account for 80 percent of peptic ulcers, are more common in men than in women, but stomach ulcers affect women more frequently. The symptoms of gastric and duodenal ulcer are similar and include a gnawing, burning ache and hunger like pain in the mid-upper abdomen, usually experienced

from one to three hours after meals and several hours after retiring. In the early 1980's Australian researcher, Barry Marshall challenged previous theories of ulcer development with evidence that ulcers could be caused by *H. pylori. H. pylori* exists the world over and its prevalence in the population increases with age. In developed countries, prevalence increases about 1% per year of age where it is rare in children, and reaches 70% in the seventh decade. In developing countries, more than 50% children acquire the infection by the age of 10 years, and more than 80% of the population gets infected by the age of 20 years. In asymptomatic individuals prevalence of *H. pylori* infection varies from 31%-84%. *Helicobacte*r *sp* are able to thrive in the very acidic mammalian stomach by producing large quantities of the enzyme urease, which locally raises the pH from ~2 to a more biocompatible range of 6 to 7. Bacteria belonging to this genus are usually susceptible to antibiotics such as penicillin, are microaerophilic (optimal oxygen concentration between 5 - 14%) capnophiles, and are fast-moving with their flagella. The different *Helicobacter sps* are *H. acinonychis, H. anseris, H. aurati, H. bilis, H. bizzozeronii, H. brantae, H. canadensis, H. canis, H. cetorum, H. cholecystus, H. cinaedi, H. cynogastricus. Helicobacter pylori* previously named *Campylobacter pyloridis*, is a Gram-negative, microaerophilic bacterium found in the stomach. In patients with chronic gastritis and gastric ulcers, conditions that were not previously believed to have a microbial cause. It is also linked to the development of duodenal ulcers and stomach cancer.

Seaweeds appearance somewhat resembles non arboreal terrestrial plants. Two specific environmental requirements dominate seaweed ecology. These are the presence of 10 seawater (or at least brackish water) and the presence of light sufficient to drive photosynthesis. Another common requirement is a firm attachment point. As a result, seaweeds most commonly inhabit the littoral zone and within that zone more frequently on rocky shores than on sand or shingle. Seaweeds occupy a wide range of ecological niches.

Seaweeds or marine macroalgae are the renewable living resources which are also used as food, feed and fertilizer in many parts of the world. Seaweeds are of nutritional interest as they contain low calorie food, but rich in vitamins, minerals and dietary fibres (Ito *et al.,* 1989). In addition to vitamins and minerals, seaweeds are also potentially good sources of proteins, polysaccharides and fibres (Darcy vrillon 1993). Most of the secondary metabolites produced by seaweeds have bacteriocidal or the antimicrobial compounds derived from seaweeds consist of diverse groups of bacteriostatic properties brominates phenols, oxygen heterocyclic; Terpenols, Sterols, Polysaccharides, dibutenolides peptides and proteins. Although most of the antibiotics found from terrestrial sources are used as therapeutic agents to treat various diseases, the oceans have enormous biodiversity and potential to provide novel compounds with commercial value. Seaweeds have been screened extensively to isolate lifesaving drugs or biologically active substances all over the world (Khotimchenko *et al.,* 2003). The sulfated polysaccharides, viz. fucans, carrageenans and ulvans have exhibited strong antioxidant, antitumor, immunostimulatory, anti-inflammatory, pulmonary fibrosis anticoagulant/ antithrombotic, lipid lowering, antiviral, antibacterial, antiprotozoan, hyperplasia prevention, gastrointestinal, regenerative and nanomedicine applications.

The development of safe anti-*H. pylori* compounds is therefore desirable. Studies have documented that some medicinal plant extracts have antibacterial activities, including *H. pylori* (Cowan, 1999; Isogai *et al.,* 2000; Funtogawa *et al.,* 2004; Ndip *et al.,* 2007). The course of treatment against helicobacteriosis is usually based on classic triple therapy

including proton pump inhibitors and antibacterial therapy, clarithromycin and amoxicillin. For allergic patients, amoxicillin could be replaced by metrodinazole. In cases of antibiotic resistance, the bismuth compounds can be applied (Graham *et al.,* 2008). Currently available first line anti *H. pylori* therapies may fail in up to 30% of patients leading to a significant increase of antimicrobial resistance (Bazzoli *et al.,* 2002). New antimicrobial agents such as a combination of antibiotics with plant extracts and other natural products that possess antimicrobial activity (Ndip *et al.,* 2007) are, therefore, being developed to overcome this problem.

MATERIALS AND METHODS

A total of 5 individuals (2 male; age 50-55years, and 3 female; age 23-45years). Those individuals who had previously received ulceration symptoms in stomach. Gastric juice as possible 5 to 10 ml was collected from each individuals during early morning episodes of intermittent reflux which may facilitate the passage viable organisms into the mouth.

A total *of* two clinical isolates of *H. pylori* obtained from the gastric mucosa of patients. It was placed on Egg Yolk Emulsion agar medium. Suspicious growth was noted; bacteria were sub cultured and were incubated for 4 days in a micro aerobic gas environment which was handled in candle jar method. Bacterial growth was identified as *H. pylori* on the basis of colony morphology; positive biochemical reaction for catalase, urease, and oxidase and negative Gram stain.

The inoculum was prepared from 24 hours old cultures in nutrient broth. Muller Hinton Agar plates were prepared and the inoculum was seeded by spread plating method. Antibiotic discs including Metronidazole(5µg), Amoxicillin(30µg),Nystatin(30µg), Ranitidine (10µg), Methicillin(40µg), Tetracycline(30µg), Furazolidone(50µg), Trimethoprim (45µg), Azithromycin (15µg*)*, Omeprozole (15 µg), Clotrimazole (30µg) and Clarithromycin (15µg), (HiMedia Laboratories, Pvt. Limited, Mumbai, India) were placed on the bacterial lawns and the plates were incubated at 37ºC under microaerophilic conditions for 2-5 days. After 24 hours of incubation at 37°C, the inhibition zone from the edge of the well to the inner margin of the surrounding bacterial growth was measured in mm by using graduated scale and recorded.

Seaweeds (*Gracilaria edulis, Sargassum wightii, Bryopsis sp, Jama sp, Caloroba grapes, Euchoma sp, Chondrococus sp, Padina tetrastomatica, Caloroba sp, Gracilaria sp, Halmiola sp*)

samples which were healthy and fully grown and submerged underwater from the tide pools were collected from Thondi Coastal region Ramnad District. The samples were washed with seawater and freshwater to remove salt, epiphytic microorganisms and other suspended materials. The clean algae were frozen. The dry material was stored.

Organic solvent methanol was used for extraction. Each powdered sample (5g) was soaked in about 40 ml of the solvent for three days. The resultant crude extracts were filtered and then concentrated in a rotatory evaporator at a temperature of less than 40^0C. The residual water was removed with a vacuum pump. The crude extracts were weighed and deep frozen (-20°C) until testing.

The crude extracts from chosen seaweeds were subjected for the qualitative identify of different classes of natural compounds, using the methodology of Sofowora (1982). The major pharmaceutically valuable phytochemical compounds *viz.*, alkaloids, flavanoids,

steroids, terpenoids, total sugars, total protein, tannin, phenolic compounds, anthroquinone, and saponins were investigated in the present study.

RESULTS

Helicobacter sp was isolated form gastric mucosa of stomach ulcer suspected individual using Egg Yolk Emulsion agar medium and it was characterized based on staining and biochemical parameters. (Table: 1) both the isolates were urease positive. Antibiogram for two clinical isolates of *Helicobacter sp* was carried out by using different antibiotics. Resistance and sensitivity pattern of antibiotics were noted and listed in (Table: 2). Among the *Helicobacter* isolates, isolate 2 was more susceptible to all the antibiotics screened than *Helicobacter* isolate 1. The phytochemical constituents of properties of extracts of *Gracilaria edulis, Padina tetrastomatica* and *Halmiola sp* were analyzed by following standard methods. The presence of major constituents such as sugars, phenols and alkaloids were detected. The antihelicobacterial activity of selected dried seaweeds against isolated *Helicobacter sp.* was assessed by the presence or absence of inhibition zones.

Among the seaweeds, screened methanol extract of *Gracillaria edulis* showed antihelicobacterial activity with the zone of inhibition range from 20 mm against two tested *Helicobacter sp*. Also the methanol extract of *Padina tetrastomatica* showed inhibitory effect against two tested *Helicobacter* strains in the range of 13-16 mm. The results were showed in (Table:3).

Time kill assay was performed against *H. pylori* isolated by using potential seaweed metabolites with 1x, 4x, and 16 x concentrations for 12 hours. Efficiency of reduction in bacterial population was collected in log CFU for every 3hours incubation. It was found that 16 x concentrations of all the four seaweed extracts reduced the population considerably (Fig: 1).

Retention times of all the compounds which are presented in the extracts were detected. The identification of the peaks is based on the analysis of their retention time. The Retention time of identified peaks for *Gracilaria edulis are* 1.400, 2.183, 2.303, and 2.753 and for *Padina tetrastomatica* is 1.427 which indicates the presence of halogenated / sulfated polysaccharides based on earlier reports on seaweed bioactive metabolites.

The peak value of *Gracilaria edulis* and *Padina tetrastomatica* were analyzed and shown in Fig: 2 to 3. The major peaks obtained at four different alternative scans. The mass spectral data revealed that the peaks indicated, may be the presence of phenolic compounds and sulphated polysaccharides which were compared based on earlier reports on seaweeds.

Table:1 Study subjects and sample collection

S.No	Patient Name	Age	Sex	Place	Amount of Gastric juice taken µl/ml	Symptoms
1	A.Usha rani	23	Female	VHNSN College, Virudhunagar	5	Abdomen pain
2	N.Ravi	53	Male	Railway colony, Virudhunagar	10	Stomach ulcer
3	R.Kanimozhi	24	Female	VHNSN College, Virudhunagar	5	Vomiting, Abdomen pain
4	V.Uma	45	Female	Pandian Nagar, Virudhunagar.	10	Abdomen pain, vomiting
5	S.Chella pandi	51	Male	Karuppasamy Nagar, Virudhunagar.	10	Abdomen pain

Table: 2 Antibiogram of selected Antibiotics against *Helicobacter* isolates

R- Resistance

Antibiotics	Concentration (µg/ml)	Isolate 1 (mm)	Isolate 2 (mm)
Amoxicillin	30	40	36
Tetracycline	30	48	22
Nystatin	30	R	R
Omeprozole	15	R	5
Metronidazole	5	R	R
Ranitidine	25	R	20
Azithromycin	15	45	42
Clarithromycin	15	45	43
Trimethoprim	45	R	33
Clotrimazole	30	10	12
Furazolidone	50	47	45
Methicillin	40	R	22

Table: 3 Anti-Helicobacterial activity of selected seaweed extracts

Control-7mm(-) No Zone formation

Name of the Seaweeds	Concentration (100µl)	
	Helicobacter sp Isolate 1(mm)	*Helicobacter sp* Isolate 2(mm)
Calaroba grapes	15±0.30	—
Calaroba sp	5±0.93	6±0.92
Bryopsis sp	7±0.76	8±0.93
Halimiola sp	9±0.10	12±0.95
Gracilaria sp	6±0.96	8±0.83
Gracilaria edulis	6±0.98	20±0.90
Sargasum wightii	7±0.91	—
Chondrococus sp	8±0.34	13±0.91
Jama sp	6±0.92	15±0.87
Padina tetrastomatica	13±0.90	16±0.06
Euchoma sp	—	12±0.09

Fig: 1 Time kill assay of crude metabolite of *Padina tetrastomatica* against *Helicobacter pylori*

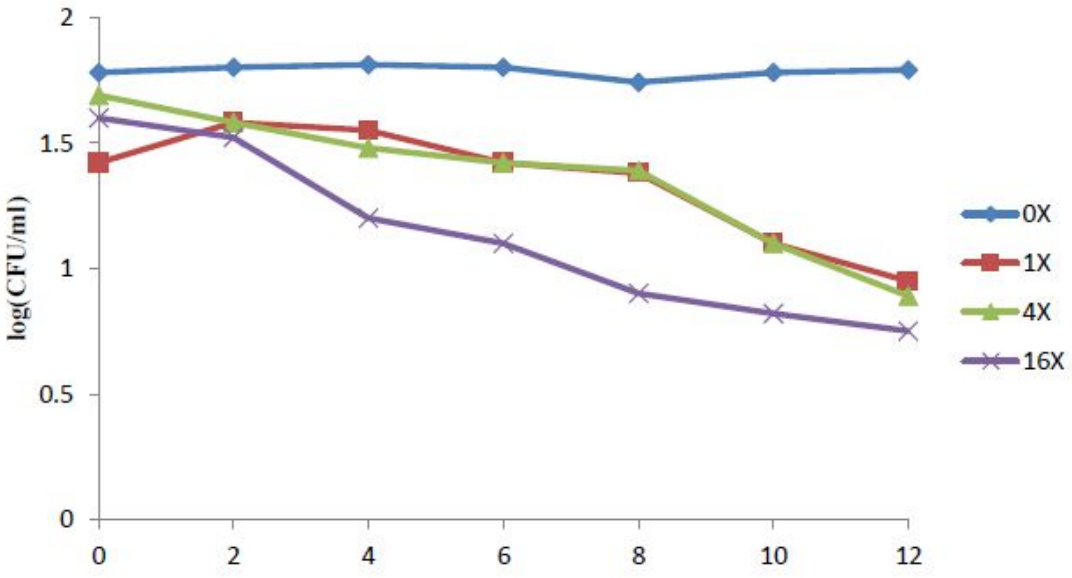


Fig: 2 LC-MS Analysis of *Gracilaria edulis*

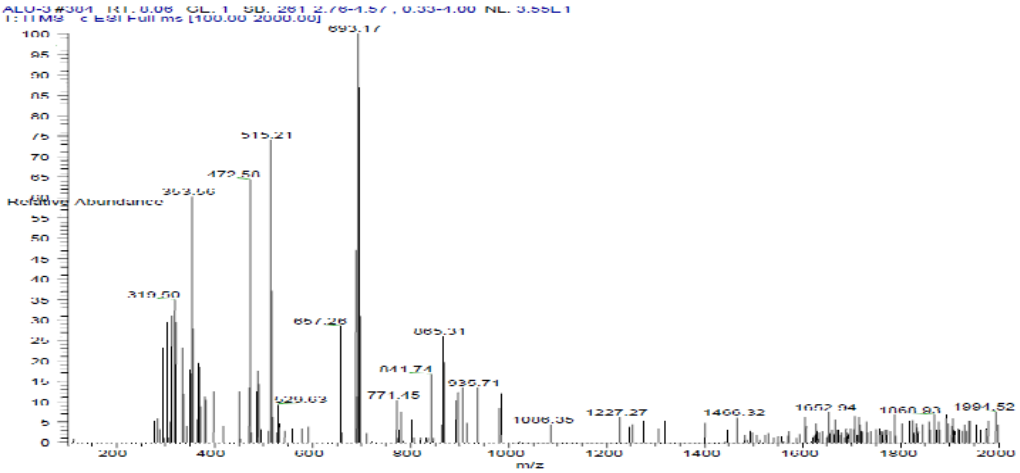
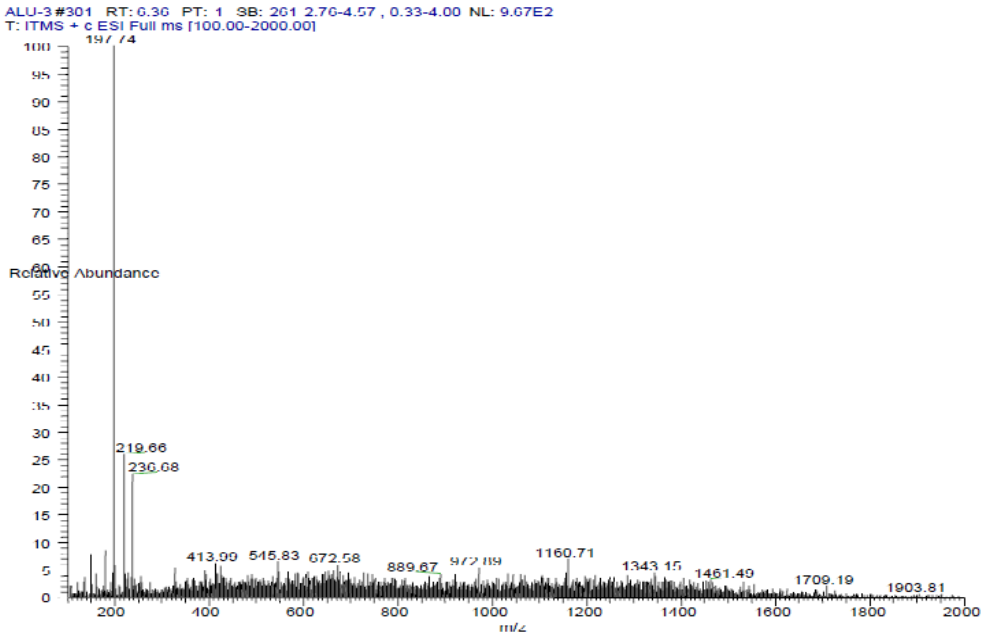


Fig: 3 LC-MS Analysis of *Padina tetrastomatica*



CONCLUSION

In humans, *Helicobacter pylori* colonizes the stomachs of about half of the world's population and highly associated with the number of most important disease of the upper gastrointestinal tract particularly prevalence in developing countries. Eradication of the *H. pylori* primarily dependent on continued application of antibiotics. Although effective, repeated use of these chemical drugs have sometimes resulted in the development of resistance and had undesirable effects. These problems have highlighted the need for the development of new strategies for selective *H. pylori* eradication. Marine plants may be an alternative source of materials for the *H. pylori* eradication because they constitute a rich source of bioactive chemicals.

The present study also found that the seaweeds *Gracilaria edulis*, *Padina tetrastomatica* showed antihelicobacterial activity. The importance of seaweed for human consumption is well known in many countries, they offer a good source of recovery of various useful chemicals particularly polysaccharide present in most of the seaweeds may used for the treatment of intestinal and stomach disorders. In this present investigation *Padina tetrastomatica* has proven antihelicobacterial activity based on the outcome of this investigation the active metabolites derived from seaweeds may be an alternative therapeutic agent for the treatment of Helicobacter infection but still further evaluation and complete structural characterization of active metabolites is warranted.

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DIVERSITY AND DISTRIBUTION OF SEAWEEDS IN THE SOUTHERN COAST OF TAMIL NADU, INDIA

SARAVANAVEL. R[1] , MATHAVAN PILLAI.M[2] and ANANTHARAMAN.P[3]

[1]Department of Botany, Lekshmipuram college of arts and science, Neyyoor-629 802 Tamil Nadu, India

[2]PG and Research Department of Botany, ST. Hindu college, Nagercoil, Tamil Nadu, India

[3]CAS in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India

INTRODUCTION

India has vast coastline of more than 7000 Km, and a number of Islands, which harbor a large number of species of marine algae. More than 20,000 species of seaweeds have been reported from all over the world. In India, 624 species of seaweeds have been reported with a potential of 77,000 tons (Wet weight/ Annum)Seaweeds are a group of large marine non flowering plants attached to the bottom in relatively shallow coastal waters. Seaweeds refer to any large marine benthic algae that are multicellular, macrothallic and thus differentiated from most algae that are of microscopic size (Smith, 1944). These plants form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial. The seaweed flora of India is highly diversified and comprises mostly of tropical species, but temperate and subtropical elements have also been reported. About 6000 species of red seaweeds (Rhodophyceae), 2000 species of brown seaweeds (Phaeophyceae) and 1200 species of green seaweeds (Chlorophyceae) occurred globally and theworld production of seaweeds was estimated as 21,65,675 million tons per year (Kaliaperumal, 2007). Several species of green, brown and red seaweeds were found with luxuriant growth along the south coast of Tamilnadu from Rameswaram to Kanyakumari.

Many types of seaweeds live in rocky intertidal communities, because they cannot get up and follow the water when the tide goes out. Such intertidal seaweeds are subjected to the stresses, associated with exposure to air and weather conditions. To survive in the intertidal regions, seaweeds must be able to tolerate or minimize the effects of evaporate water loss, temperature and salinity changes (Sahu and Athikary, 1999). The distribution of marine macro algae is controlled by many factors. Growth and biomass production have been determined in some seaweeds (Christiaen *et al.,* 1987). There are only some studies on the distribution, frequency, density and abundance of seaweeds. Boergesen (1934) was the first researcher to have worked on the geographical distribution of some marine algae. Anand (1940, 1943) and Nizermuddin and Gessner (1970) also worked on the distribution, dominance and abundance of some seaweeds. Saifullah (1973) listed out the abundance, biomasses variation and tidal variation of seaweeds. Qari (1985) worked on ecology and biochemical composition of seaweeds. The ecology of seaweeds is dominated by two specific environmental requirements. These are the presence of sea water and the presence of light sufficient to drive photosynthesis and another important common requirement is to have a firm point of attachment. Therefore, seaweeds are most commonly found in the littoral zone and within that zone more frequently on rock shores than on sand or shingle shore (Smith, 1944).

The region was having more than 200 species of seaweeds. Now the biodiversity and density of seaweeds of southern coastal region of Tamilnadu have come down gradually over a period of years (Krishnamurthy, 2006; Kannan and Thangaradjou, 2006). Seaweeds constitute a major part of the food chain of the aquatic life. Whatever alters in the marine water, the seaweed growth and the composition also affects all other organisms. The southern coastal region of Tamil Nadu has rich seaweed diversity because it has a lot of rocky coastal regions which face several problems due to various environmental changes and human activities.

Although a number of systematic surveys have been made on seaweed resources of the southern coastal region of Tamil Nadu, there is no proper documentation for the seaweed resources availability with reference to the seasons. There are only a few scattered reports about seaweed resources in the selected area of the southern coastal region of Tamil Nadu. All the previous studies carried out during the last 50 years are only from scattered localities with small area as follows: Kanyakumari region (Nair *et al.,* 1993; Stella *et al.,* 1997; Edwin, 2004), Tirunelveli region (Kaliaperumal and Pandian, 1984; Kaliaperumal *et al.,* 1995; Selvaraj and Selvaraj, 1997), Thoothukudi region (Mahadevan and Nagappan, 1967; Krishnamurthy, 1980; Kaliaperumal and Pandian, 1984; Kaliaperumal *et al.,* 1998) and Ramanathapuram region (Chacko *et al.,* 1955; Varma and Krishna, 1962; Umamaheswara Rao, 1969; Krishnamurthy and Joshi, 1970; Subbaramiah *et al.,* 1977; Kaliaperumal and Kalimuthu, 1994; Kaliaperumal *et al.,*
1998). From such scattered and sporadic reports, the actual number of seaweeds is obscure from the southern coastal region of Tamil Nadu, because each and every researcher has given different number of seaweeds from the same locality. To fulfill this lacuna, a comprehensive study was aimed to understand the seaweed resource along with its seasonal variation in the entire southern coastal region of Tamil Nadu. Seaweeds are mostly affected by various ecological factors. Seaweeds are highly climate specific. Some of the seaweeds are present throughout the year but most of the seaweeds are found at specific climate.

MATERIALS AND METHODS

The investigation was carried out at southern coast of Tamilnadu India. The rocky shore of southern coast inhabits an astonishing biodiversity, representing nearly almost all the invertebrate phyla and urochordates. Hard rocky bottom of this area greatly supports the algal diversity and provide suitable shelter and feeding ground for grazers. The entire study area was divided in to four stations namely kudankulam (S1), Panchal (S2), Arrokiyapuram (S3) and Manavalakurichi (S4). The survey of green seaweed species from the intertidal area was carried out during low tide Seasons at southern coast may be classified into pre-monsoon (June-September), monsoon (October-January), and post-monsoon (February-May). Field surveys were undertaken to the selected sampling stations of the southern region over a period of one years from Aug 2013 to Sep 2014. The algal samples were collected in every month during the study period by detaching a portion from the seaweed bed, kept in polythene bags with fresh seawater, transported to the laboratory and fixed in 4% formaldeyde for further studies. The seaweeds were identified using the taxonomic keys provided by Umamaheswara Rao (1987), Desikachary et al. (1990, 1998) and Krishnamurthy (1999), and the nomenclature was updated using Appeltans et al. (2012).

The seasonal distribution of seaweeds was studied by submerging test panels for a period of one year from Aug 2013 to Sep 2014. Test panels made from teak wood with a size of 10 x 10 x 2 cm were vertically placed in a suitably designed wooden raft with grooves in such a way so as to keep a 10 cm distance between panels. The raft with panels (in replicate, n = 6) was suspendedat 2 m depth in the coastal waters using floats and sinkers. Each panel was studied for the seaweed species composition and biomass. The total and the differential biomass (wet weight) of the seaweeds were estimated after carefully scrapping them from the panels and weighing them.

RESULT

A maximum of 96 seaweed taxa were collected from the southern coast (Table 1).Out of the 96 species recorded 32 species occurred in all the month of the study period. Among the Chlorophyta prevailed with 30 taxa followed by Rhodophyta (49 taxa) and Phaeophyta (16 taxa). *Ulva fasciata* Delile, *Sargassum wightii* Greville, *Chaetomorpha linum* (O.F.Müller) Kützing, *Gracilaria edulis* (Gmelin) Gurgel et Fredericq, *Dictyota dichotoma* (Hudson) Lamouroux, *Caulerpa sertulariodes* (Gmelin) Howe, *Acanthophora muscoides* (Linnaeus) Bory de Saint-Vincent and *Ulva compressa* Linnaeus were the commonly found seaweeds in the rocky shores and other submerged hard surfaces. *Ulva fasciata* is the common green alga inhabiting the rocky shores of this region. During the monsoon season (October-January), *Ulva fasciata* forms thick mats covering the entire rocky substratum *Chaetomorpha linum*, and *Caulerpa sertulariodes* are the other dominant green seaweeds taxa observed during this period of study.

Table 1: List of seaweed occurring at four stations of southern coast region of Tamilnadu

S.No.	Name of the Seaweeds	1	2	3	S4
1	*Enteromorpha compressa (L.) Nees*				+
2	*Enteromorpha flexuosa (Wulfen) J. Ag.*				+
3	*Enteromorpha linza (L.) J. Ag.*				+
4	*Enteromorpha prolifera(O.F.Muller)*				+
5	*Ulva fasciata Delile*				+
6	*Ulva lactuca L*				+
7	*Ulva reticulata Forssk*				-
8	*Chaetomorpha antennina (Bony) Kutzing*				+
9	*Chaetomorpha litorea Harvey*				+
10	*Chaetomorpha glamerata L*				+
11	*Chaetomorpha area (Dillwyn) kutzing*				+
12	*Halimeda macroloba Decaisne*				-
13	*Halimeda tuna (Ell. et. Sol.) Lamour*				-
14	*Halimeda opuntia L*				-
15	*Halimeda gracilis J.Agardh*				_
16	*Caulerpa chemnitzia (Esper) Web v Bosse*				
17	*Caulerpa recemosa(Forsskal) J.Agardh*				+
18	*Caulerpa peltata (J.V.Lamouroux)*				+
19	*Caulerpa sertulariodes (S.G.Gmelin) M.A.Howe*				+
20	*Caulerpa taxifolia(Vahl) G.Agardh*				+
21	*Caulerpa serrulata(Forsskal) J.Agardh*				+
22	*Caulerpa fergusoni G.Murry*				+
23	*Caulerpa `scalpeliformis(R.Brown) C.Agardh*				_
24	*Caulerpa lativerance Montagne*				+
25	*Caulerpa crassifolia (C. Ag.) J. Ag*				+
26	*Bryopsis plumosa (Huds.) C. Ag.*				+
27	*Cladophorapora facicularis*				-
28	*Cladophorapora glomarata*				

29	*Codium decorticatum Howe*				-
30	*Valoniopsis pachynema (Martens) Boergs.*				+
31	*Dictyota bartayresiana Lamouroux*				+
32	*Dictyota dichotoma (Huds.) Lamouroux*				+
33	*Dictyota divaricata Lamouroux*				+
34	*Stoechospermum marginatum (C. Ag.) Kutzing*				+
35	*Padina pavonica (L.) Thivy ex Taylor*				+
35	*Padina boergesenii Allender & Kraft*				+
36	*Padina austrilis*				+
37	*Padina boryana*				+
38	*Spatoglossum asperum J. Ag.*				-
39	*Chnoospora implexa J. Ag.*				-
40	*Chnoospora minima pappenfuss.*				+
41	*Colpomenia sinuosa (Mertens ex*				+
42	*Rosenvingea intricata (J. Ag.) Boergesen*				+
43	*Sargassum duplicatum C.Ag.*				+
44	*Sargassum duplicatum C.Ag.*				+
45	*Sargassum linearifolium (Turner) C.Ag.*				+
46	*Sargassum tenerrimum J.Ag.*				+
47	*Sargassum wightii Greville*				+
49	*Ecotocarpus simplicursculas*				-
50	*Hydroclathrus clathratus*				+
51	*Cystoseria indica*				+
52	*Hormophysa cuniformis*				-
53	*Cryptonemia coriacea Schmitz*				+
54	*Amphiroa anceps (Lamarck) Decaisne*				-
55	*Amphiroa fragilliissma*				+
56	*Dermonema frappieri (Mont. & Millard. ex Maill.) Boerg.*				–

57	. *Liagora ceranoides Lamouroux*				-
58	*Gelidium micropterum Kuetz. A.*				+
59	*Gelidium pusillum (Stackhouse) Le Jolis*				+
60	*Gelidiella acerosa (Forssk.) Feldm. & Hamel*				
61	*Hamel Corynomorpha prismatica (J. Ag.) J. Ag.*				+
62	*Grateloupia filicina (Wulf.) J. Ag.*				+
63	*Halymenia floresia (Clem.) C. Ag.*				+
64	*Jania rubens (L.) Lamouroux*				-
65	*Portieria hornemannii (Lyngbye) Silva*				-
66	*Gracilaria corticata J. Ag.*				+
67	*Gracilaria crassa (Harvey) J. Ag.*				+
68	*Gracilaria cylindrica Boergesen*				+
69	*Gracilaria edulis (Gmelin) Silva*				+
70	*Gracilaria fergusonii J. Ag.*				+
71	*Gracilaria foliifera (Forsskal) Boergesen*				
72	*Gracilaria kanyakumariensis Umamaheswara Rao*				+
73	*Gracilaria verrucosa (Hudson) Papenfus*				+
74	*Ahnfeltiopsis densa (J. Ag.) Silva et Decew*				+
75	*Sarconema scinaioides Boergesen*				+
76	*Solieria robusta (Grev.) Kylin*				+
77	*Hypnea musciformis (Wulf.) Lamouroux*				+
78	*Hypnea valentiae (Turner) Montagne*				
79	*Rhodeminia sonderi Silva*				+
80	*Botrycladia leptopoda (J.Agardh) Kylin*				-
81`	*Gelidiopsis variabilis (Grev.) Schmitz*				-
82	*Champia indica Boergesen*				-
83	*Champia parvula (Agardh) Harvey*				-
84	*Ceramium procumbens Setchell et Gardner*				+
85	*Centroceras clavulatum (C. Ag.) Montagne*				+
86	*Spyridia hypnoides (Bory) Papenfuss*				-
87	*Griffithsia corallinoides (Linnaeus) Trevisan*				-

88	*Dictyurus purpurascens Bory*				+
89	*Enantiocladia prolifera (Greville) Falkenberg*				+
90	*Neurymenia fraxinifolia (Mertens) J. Ag.*				+
91	*Acanthophora muscoides (L.) Bory*				+
92	*Acanthophora spicifera (Vahl.) Boergesen*				+
93	*Laurencia flagellifera J. Ag.*				+
94	*Laurencia obtusa (Hudson) Lamouroux*				-
95	*Laurencia papillosa (Forsskal) Greville*				-
96	*Laurencia poiteaui (Lamouroux) Howe*				+

The brown seaweeds (Phaeophyta) are represented by 16 taxa and *Sargassum wightii* is the dominant one. *Dictyota dichotoma* and *Padina antillarum* (Kützing) Picone are also abundantly observed on the intertidal rocky reefs. A rich growth of *Sargassum* sp.pl. was observed during pre-monsoon and monsoon months. *Sargassum* sp. pl. was harvested during October-December period by the local people. *Colpomenia sinuosa* (Mertens ex Roth) Derbès et Solier was commonly observed on the artificial substrata submerged in the seawater. Rhodophyta of the southern coastal waters consisted of 69 taxa. *Gracilaria corticata* (Ag.) Agardh, *Hydropuntia edulis* (Gmelin) Gurgel et Fredericq, and *Acanthophora muscoides* (Linnaeus) Bory de Saint-Vincent were the dominant red seaweeds observed during this study period. *Amphiroa* sp. and *Hypnea valentiae* (Turner) Montagne were also commonly observed on the rocks. *Gracilaria* sp.pl. were abundantly observed during May-October period. *Acanthophora muscoides* and *Hypnea valentiae* were abundant during November-January period on the rocky shores.

Table 2. Biomass of seaweeds settled on the wooden test panels submerged in pre-monsoon, monsoon and post-monsoon season period at southern coast. The wet biomass values are expressed as g/dm2.

S. No		**Pre-monsoon**	**Monsoon**	**Post-monsoon**
1	*Ulva fasciata*	5.66±1.17	2.92±0.80	1.70±0.04
2	*Ulva compressa*	3.18±0.90	4.70±0.90	1.60±0.20
3	*Caulerpa sertulariodes*	2.50± 0.76	4.70±0.90	1.87±0.09
4	*Padina pavonica*	3.67±1.17	3.80±0.71	1.71±0.48
5	*Sargassum wightii*	4.56±1.14	0.81±0.12	2.2±30.69
6	*Hypnea musciformis*	4.30±1.70	4.54±1.79	1.32±0.40
7	*Acanthophora muscoides*	3.34±1.00	2.21±0.85	2.32±0.19
8	*Laurencia papillosa*	2.43±0.75	3.24±0.10	1.43±0.43
	Total algal biomass	27.84±8.90	32.13±6.16	11.83± 2.99

The test panels immersed during Aug 2013 and examined at the end of September 2014 (pre-monsoon) showed a total algal biomass value of 32.13±6.16 g/dm2 (Table 2). The macro-algal community of the panels submerged during this period was dominated by *Ulva fasciata* (5.66±1.17 g/dm2) and *Ulva compressa* (4.70±0.90 g/dm2) (Table 2). (2.5± 0.78 g/dm2) was also observed as one of the dominant groups in this panel series. *Sargassum wightii*, *Padina* and *Hypnea valentiae* were also observed. The panels exposed during the monsoon season showed a biomass value of *Ulva compressa* (4.70±0.9 g/dm2) and *Acanthophora muscoides* (3.34±1.00 g/dm2). The biomass of *Ulva fasciata* Delil on this panel series was 1.92±0.72 g/dm2, while *Hypnea valentiae* recorded a very low biomass value of 1.32.6±0.40 g/dm2. *Sargassum wightii* was also observed on the panels with a biomass of 2.2±0.69 g/dm2.

DISCUSSION

The Tamil Nadu coast was surveyed during 1971-1976, covering a distance of 320km from Rameswaram and adjoining islands to Melmidalam (Colachal) by CSMCRI, in collaboration with Central Marine Fisheries Research Institute (CMFRI), Cochin and Department of Fisheries, Government of Tamil Nadu. The survey was conducted in five sectors. The total standing crop of seaweeds in the intertidal region of Tamil Nadu was estimated at 22,044 tons (FW) in a potential area of 9891.35ha of the 20,000ha total area surveyed (Anon, 1978). In India, about 850 species of seaweeds were reported (Oza and Zaidi, 2001) and their commercial exploitation has been commenced from 1966 onwards. At present industries were annually utilizing 1518 tons (DW) of red seaweed and 2285 tons (DW) of brown seaweed for the manufacture of agar, algin and liquid fertilizer (Kaliaperumal *et al.*, 2004).

Seaweeds play a pivotal role as one of the main groups of primary producers in marine ecosystems. Diversity, distribution and abundance of seaweeds are known to be influenced by both physical and biological factors (Lobban and Harrison, 1994; Nybakken, 2001). Grazing pressure, a biological factor, has been regarded as the major factor controlling the structure of macro algal communities (Anderson and Underwood, 1997; Underwood, 1998). There have been various studies on inter and intra specific competition for nutrients and space of macroalgae during the last fifteen years (McCook, 1997, 1999, 2001; Miller and Hay, 1998; McCook *et al.*, 2001; Lirman, 2001) and they are known to determine patterns of macro algal dominance or exclusion in coral reefecosystems.

Studies made on Indian seaweeds have been reviewed from time to time by many workers (Agharkar, 1923; Biswas, 1932, 1934; Joshi, 1949; Iyengar, 1957; Randhawa, 1960; Srinivasan, 1965). Boergesen published a series of papers on the green, brown and red algae of the northern parts of the west coast (Boergesen, 1930, 1931, 1932a, b; 1933a, b; 1934 a, b; 1935) and red algae of South India (Boergesen, 1937 a, b; 1938). A general review of the marine algae of the western coast was published by Biswas (1945). Srinivasan (1946) studied the marine algal flora of Mahabalipuram. Varma (1960) studied the seaweeds growing on the pearl and chank beds off Tuticorin. Srinivasan (1960) gave a detailed account of marine algae of the east and west coasts of India and reported on the occurence of 162 genera and 413 species of marine algae from the Indian seas. Misra (1966) prepared a monograph on brown algae occuring along the Indian seas. The algal flora of Tiruchendur wasmarine algae and their distribution along the maritime states of India. Ecology and zonation of seaweeds in the Gulf of Mannar along the southeast coast of Bay of Bengal have been studied by Umamaheswara Rao (1972), Subbaramaiah *et al.* (1977), Krishnamurthy (1980), Krishnamurthy and Balasundaram (1990), Ganesan and Kannan (1995), Roslin *et al.* (1997) and Edwin James *et al.* (2004). The marine green algal flora of Kollam coast, Kerala, South India was reported by Sulekha and Panikkar (2006). The present scenario on algal flora of Krusadai Island has been depicted by Krishnamurthy (2008). Arulmurugan *et al.* (2008) have studied the seasonal variation in hydrography and diversity of seaweeds at Kodiakkarai coast and reported by Krishnamurthy (1980). Untawale *et al.* (1983) enumerated 624 species ofMuthupet lagoon. Although all seaweeds are beneficial to man in one way or the other, only 49 species are presently found to be useful either as directly edible materials or as industrial raw materials (Chennubhotla *et al.*, 1987a).

Studies on the diversity and distribution of seaweeds in Indian waters were carried out by several authors (Untawale et al., 1989; Kalimuthu et al., 1995; Jayachandran & Ramaswamy 1997; Kaliaperumal & Kalimuthu, 1997; Stella Roslin et al., 1997; Selvaraj & Selvaraj, 1997; Mohammed et al., 1999; James et al., 2004; Krekar, 2004; Rath & Adhikary, 2006). Southeast coast of India is a unique marine habitat characterized by a high biodiversity. Results of the present study indicate the occurrence of 32 seaweed taxa in the Kudankulam coastal waters; most of the seaweeds such as *Sargassum wightii*, *Ulva fasciata*, *Gracilaria corticata* and *Chaetomorpha linum*, are abundantly observed on the rocks during the pre-monsoon (June-September) and monsoon months (October- January). The richness of seaweed resources is due to the intertidal rocky reefs available in the Kudankulam region. The seaweed flora observed in the present study is similar to that reported from the nearby Tiruchendur coast (Chennubhotla et al., 1991). Marine ecologists have a long history of using artificial substrate and habitats to test hypothesis about sessile plants and animals (Osman, 1977; Sutherland & Karlson, 1977). In this study, settlement panels were used to analyse the seasonal distribution of macroalgal communities. The seaweed biomass on test panels was high during pre-monsoon and monsoon seasons. In an earlier study (Satheesh & Wesley,

2007), we have reported that *Gracilaria* sp., *Enteromorpha* sp., and *Ulva* sp., showed dense settlement during pre-monsoon and post monsoon months on test panels.

The observed pattern of seasonal distribution is likely to be related to the life history of the alga, particularly the dispersal abilities of its spores. The supply from macroalgal propagule may influence the abundance of algae in littoral habitats (Worm et al., 2001). As the test panels provide limited space for the settlement of marine organisms including seaweeds, the seasonal biomass of only a few species could be observed in this study. Gradual rise in the anthropogenic influence, impact of the possible thermal discharge from the emerging nuclear power station and the indiscriminate collection of algae (mostly *Sargassum* sp.) may be the cause of concern for the biodiversity of algal species at Kudankulam coast. Both frond bleaching and cell plasmolysis of algae were observed in thermal effluent discharge areas (North, 1969; Lobban et al., 1985). These negative effects may reduce the survival and growth of seaweeds, resulting in extensive reductions in the number of species of marine algae (Wood & Zieman, 1969). The present study could be useful as new baseline record for future biomonitoring studies in this coast. Further systematic studies on the seaweed resources may provide useful data for the conservation of marine algal resources in this region.

An account of 46 species of seaweed occurring at Trichendur was given by Krishnamurthy (1980). Seaweeds were collected from six localities in Gulf of Mannar namely Tuticorin, Manapad, Trichendur, Idinthakarai, Kovalam and Muttam (Kaliaperumal and Pandian, 1984). The number of seaweed species recorded from these places was 56, 43, 34, 41, 38 and 25 respectively. A total number of 155 species have been reported in the seaweed resources survey conducted from Rameswaram to Athankarai and from Thonithurai to Melamidalam covering 21 islands in Gulf of Mannar at 0 to 4m depth during 1971-1976. In this survey, 102 species of seaweeds were recorded (Anon, 1978). Varma (1960) reported 51 seaweed species from pearl beds from Tuticorin. Mahadevan and Nagappan (1967) recorded 12 numbers of in deep waters ofTuticorin.

A total number of 99 seaweed species (20 species of green algae, 18 species of brown algae and 61 species of red algae were encounted in the seaweed resources survey conducted at deep waters from Dhanushkodi to Kanyakumari (Kaliaperumal *et al.,* 1998). The total number of genera and species of seaweed belonging to three divisions, occurring at Kanyakumari, Vattakottai, Kootapuzhi, Kudankulam and Idinthakarai were 18 listed. Maximum number of 98 seaweed species from Kudankulam and minimum number of 62 species from Kootapuzhi were recorded. A total number of 96 species from Idinthakarai, 94 from Kanyakumari and 67 species from Vattakottai were collected. The red algae dominated in all these places than the green and brown algae. Totally 121 species were recorded in all the five places of which 31 species belong to Chlorophyta, 25 specie s to Phaeophyta and 65 species to Rhodophyta (Edwin, 2004).

Water motion, a physical factor, has been proven to be a key determinant of macro algal production (Lobban and Harrison, 1994), influencing a number of abiotic and biotic factors that control macro algal zonation and community structure, including nutrient availability, temperature (Costa *et al.*, 2002) and rates of herbivory (Lubchenco, 1978; Kim, 1997; Lotze *et al.*, 2000; Belliveau and Paul, 2002). Water motion can also influence the community structure via wave action (Lobban and Harrison, 1994), which influences propagule dispersal, fertilization, settlement and recruitment (Vadas *et al.*, 1990; Serrao *et al.*, 1996; Costa *et al.*, 2001).

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ROLE OF *SPIRULINA MAXIMUS* AND VITAMIN C ADDED DIETS ON GROWTH RESPONSES OF *CYPRINUS CARPIO*

M.Manohar,[1] M.Mohan,[2] P.Ganesh[3]

[1]E.R.K.College of Arts &Science, Erumiyampatti, Dharmapuri Dt

[2]COE., Mahindra Engineering College, Salem

[3]Asst.Prof., Department of Microbiology, Annamalai University, Chidambaram.

Introduction

Aquaculture is one among the fastest growing food producing systems, which has been emerged recently as an industry and now it is made possible to supply protein rich food throughout the world. *Spirulina* is often used as the converse of antibiotics. *Spirulina* can be viewed as promoter of life.

Spirulina

Spirulina, a genus of blue green algae (BGA), is a ubiquitous component of phytoplankton. *Spirulina* is a nutrient dense material containing over 60% protein, a rich source of all vitamins (Venkataraman, 1998). Even though application of *Spirulina* in aquaculture has been practically followed for long period less is known particularly to Indian-aqua farmers. This may be attributed to the special feeds, particularly larval feeds being imported from Taiwan, USA and Japan (Avigad Vonshak, 1997).

Materials and Methods

Growth experiments were carried out in the Center for Aquaculture Research Extension (CARE-aqua-farm), St. Xavier's College, Palayamkottai, Tamil Nadu. The experimental fish *Cyprinus carpio* (Common name: Common carp, Tamil: Satha Kendai) were procured from the Government Fisheries Farm, Manimuthar. The reared juveniles had an average length of 4.7±0.02 cm and body weight of 12.4±0.25 g. The ten juvenile carps

were introduced into each trough of 40-liter capacity filled with 30 liter of water. Triplicates were maintained for each experimental diet. The fresh water was well aerated and the water quality parameters (pH, dissolved oxygen, temperature, total alkalinity, ammonia, and electrical conductivity) were measured periodically following standard methods (APHA, 1998).

Feed Preparation

For our present study, seven diets were prepared by incorporating *Spirulina maximus*, and Vitamin C viz.

- SD1 – Algae, *Spirulina maximus* incorporated diet at 10%
- SD2 – Algae, *Spirulina maximus* incorporated diet at 20%
- SD3 – Algae, *Spirulina maximus* incorporated diet at 40%
- VD1 – Vitamin C incorporated diet at 0.05%
- VD2 - Vitamin C incorporated diet at 0.1%
- VD3 - Vitamin C incorporated diet at 0.2% and Control diet.

The composition and formulation of all the diets are also given in Table 1.

Incorporation of *Spirulina maximus* in feed

The *Spirulina maximus* incorporated feeds were prepared with various levels of *Spirulina* viz., 10%, 20% & 40% and were designated as SD1, SD2 and SD3 respectively. The algal strain *S.maximus* were collected from Elvess Biotech Consultancy Pvt. Ltd. Mysore and mass cultured in the Laboratory using simplified CFTRI medium. Wet algal biomass was washed using sterile water in 1:10 ratio and re-filtered. The washed algal culture was dried in trays and spreaded on a plastic sheet kept in aluminium tray of 1.2mm thick. They were dried in sun/ cross flow drier for 4-6 hours, and stored in polyethylene bags, at room temperature and kept away from sunlight (Venkataraman, 1998).

Vitamin C. Incorporated Fish Diet

A stable and bio available form of vitamin C (Rovimix stay C-95%) was obtained from Roche chemicals, Mumbai. Diets incorporated with vitamin C at 0, 0.05%, 0.1% and 0.2% were fed to common carp.

Chemical Analysis of ingredients and test diets.

The ingredients of feeds were analyzed following the standard procedures (AOAC, 1990).

Protein: The protein present in the feed sample was estimated following Lowery method (Lowry *et al*., 1951). Lipid: The Lipid present in the feed sample was estimated by following chloroform methanol method (Floch *et al.,* 1957). Carbohydrate: The carbohydrate present in the feed sample was estimated by following Anthrone method (Carral *et al*., 1956).

Growth Parameters of *C.carpio*

To study the growth of fish fed with different diets, various growth parameters and survival rate were determined at every 15 days of intervals for the total duration of 60 days (Shivananda Murthy and Ramachandra Naik 2001).

RESULTS

Percentage composition of experimental diets

The percentage composition of ingredients used in the formulated diets of *Cyprinus carpio* is given in the Table 1. The control feed has fish meal (32%), groundnut oil cake (30%), rice bran (13%), wheat flour (12%), tapioca flour (10%), cod liver oil (2 ml) along with vitamin mineral mix (1%). The experimental diets were grouped into two types. The first type of feed had *Spirulina maximus* in three different concentrations (*viz.,* 10%, 20% and 40%) by partial replacing the fish meal and groundnut oil cake and second group of feed was incorporated with vitamin 'C' in three different concentrations (*viz*., 0.05%, 0.1% and 0.2%) by partial replacing the tapioca flour.

Proximate estimation of ingredients

The proximate composition of ingredients used in formulated diets is given in Table 2. Among the ingredients *S. maximus* has the highest protein content (61.7%) followed by fish meal (52.05%) and then groundnut oil cake (47.3%). The carbohydrate level was found to be higher in tapioca flour (44.9%) followed by wheat flour (29.5%) and rice bran (21.7%). The carbohydrate content was found to be least in fish meal (1.7%). The crude fat content was found maximum in fish meal (12.3%) and it was found minimum in tapioca flour (0.2%). The crude fiber content was found to be maximum (19.9 %) in rice bran and it was followed by wheat flour (12.4 %) and *S.maximus* (11.8 %).

Proximate composition of formulated diets

The proximate composition of test diets is given in Table 3. Among the 7 formulated feeds, the highest protein content was noticed in SD3 diet (40.79 %), followed by the diet SD2 (39.63%).The ash content was noticed as 10.57% (SD3).

Growth parameters of *Cyprinus carpio*

The growth responses of *C. carpio* fed with *S. maximus* and vitamin C is given in the Table 4.The experiments showed that the all the diets were readily accepted by *C.carpio.* During the feeding trial, the observed survival rate ranged from 73 % (control diet) to 97 % (SD3). The growth parameters like weight gain, specific growth rate (SGR), food conversion ratio (FCR), feed efficiency ratio (FER), and protein efficiency ratio (PER), of the experimental fish are found to be highly significant ($P \leq 0.001$).

The results pointed out that the growth response of *C.carpio* is found to be positively influenced by the higher concentration of *S. maximus* incorporated diet SD3. It is evident from table 4 that the fish fed with SD3 diet showed maximum SGR (0.41±0.009%), followed by those fed with SD2 (0.37±0.008 %).

The average weight gain of the fish ranged between 16.43% and 75.56%. The lowest value was recorded in fishes fed with control diets. The maximum weight gain (75.56±0.23) was recorded in fish fed with SD3 diet. The best food conversion ratio (FCR) of 1.78±0.231 was observed in the experimental fish fed with SD3 diet, whereas control diet fed fishes showed the poor FCR (6.79±1.27). The maximum feed efficiency ratio (FER) was observed in SD3 (56.20±4.73) and minimum FER in control diet (14.72±2.14). The better protein efficiency ratio (PER) of 1.40±0.23 was also found in SD3 followed by SD2 diet (1.24±0.13) fed fishes. The very low PER value (0.40±0.04) was observed in control diet.

It is evident that a gradual increase in specific growth rate was observed in fish fed with SD3 diet and there after the values showed a decreasing trend in the rest of the fishes.

Discussion

Growth parameters of *Cyprinus carpio*

In our present study, an addition of algae (*Spirulina maximus* 10%- SD1; 20%- SD2; 40% - SD3/g) and vitamin 'C' (50mg – VD1; 100 mg VD2; 200mg – VD3/g) in different levels resulted better performance. Growth and feed utilization responses of fishes mainly depend on the food quality and quantity as well as the body size (Pandian, 1967). Of the different types of experimental diets tested, encouraging results were observed when the test fish were fed with SD3, SD2, and SD1 diets, whereas the rest of the fishes showed comparatively lesser growth. Among all the experimental diets, the SD3 (75.56 ±0.23%) gave maximum weight gain, followed by SD2 (67.04±0.24%), and SD1 (59.69±0.43%) (Table 4). The percentage of increase in weight gain of the experimental fishes over the control fish fed fishes. The maximum percentage weight gain (75.56 ±0.23%) was observed in SD3 fed fishes over the control diet. Our findings correlated well with those of Uma and Subramanian (2001). They found that *Synechococcus elongates* and *Spirulina rebselse* were promoted the growth of ruminants. The results of our present study is also supported by Nandeesha et al., (1993). They have demonstrated the suitability of *Spirulina* algal biomass as a valuable feed supplement in *C.carpio*. De Silva *et al*., (1990) conducted field trials with *Spirulina* based diets in Indian major carps and recorded encouraging results. Mohanty *et.al.,* (1996) also observed better specific growth rate(2.33%) and weight gain (52.18%) in *Labeo rohita* fingerlings fed with higher concentration of algal diet. The above all findings recommended *Spirulina* as promoter of nutrition such as vitamins, minerals etc that promote growth.

The specific growth rate (SGR) was maximum (0.41±0.009%) in SD3 fed *C.carpio* followed by SD2 (0.37±0.009%). The maximum percentage of increase of SGR (0.41±0.009%) observed in SD3 over the control (0.11±0.009%), which is followed by SD2 (0.37±0.009%). There is a significant difference in SGR ($P \leq 0.001$) observed between the treatments (Table 4). Similar results were also reported by Mbahinzireki (2001) who found the highest average weight gain in *C.carpio* (79.29±1.96%) fed with 40% *Spirulina* substitution with fishmeal in the diet. He also observed that the growth rate and FCR for common carp varied depending on the experimental diets including 10%, 20% and 40% of *Spirulina* which showed better significant daily weight gain, feed efficiency ratio and SGR.

The growth studies (food conversion ratio – FCR; feed efficiency ratio – FER; protein efficiency ratio – PER) of *C.carpio* have clearly indicated that *S.maximus* incorporated diets (SD3 and SD2) induced growth and digestibility due to the high protein and fiber content of *Spirulina*. There is a significant difference ($P \leq 0.001$) between the treatments in the observed FCR, FER and PER (Table 4). The percentage increase in FER and the percentage decrease in FCR observed in experimental fishes over the control diet fed fishes. It was revealed that the maximum increase in FER (56.20±4.73%) and maximum decrease (1.78±0.231%) in FCR were observed as a function of diet SD3 followed by SD2,. Umesh *et al.,* (1999) too observed similar evaluation of *Spirulina platensis* (10-90%) in *C.carpio* at different levels. The maximum digestibility was observed in 50% *S.platensis* incorporated diet. The results of the present study were supported by Mohanty *et.al.,* (1996) who found the best FCR(1.69), and higher PER(1.77%) in catla (*Catla catla*. Ham) fed with *Spirulina* incorporated diet.

C.carpio with the vitamin C incorporated diets was found to have maximum growth rate (0.20±0.009%) in VD3 over the control fed (0.11±0.009%) ones. Our findings well

coincided with the results of Aguirre (1999) who obtained better results with vitamin C incorporated diets fed to red drum (*Sciaenops ocellatus*).

The overall performance of different diets with algal, and vitamin 'C' in the present investigation was found to be better, when compared to the control diets (Table 4). Among these diets the maximum weight gain, specific growth rate, feed efficiency ratio, protein efficiency ratio and minimum food conversion ratio were attained in the case SD3 (40% *Spirulina maximus* incorporated diet) followed by SD2 (20 % *Spirulina* incorporated diet),

Table 1 Percentage composition of experimental diets

Ingredients	**Control**	**D 1**	**SD 2**	**D 3**	**VD 1**	**VD 2**	**VD 3**
Fish meal	32		13		32	32	32
Groundnut oil cake	30	1	29	1	30	30	30
Rice bran	13	3	13	3	13	13	13
Wheat flour	12	2	12	2	12	12	12
Tapioca flour	10	0	10	0	9.5	9	8
Cod liver oil	2		2		2	2	2
Vitamin & Mineral mix	1		1		1	1	1
Spirulina maximus	-	0%	20%	0%	-	-	-
Vitamin 'C'	-		-		500 mg	1000mg	2000 mg

*Values represent means of triplicate

Table 2 Proximate composition of feed ingredients

Ingredients	**Crude Protein (%)**	**Crude Carbohydrate (%)**	**Crude fat (%)**	**Crude fibre (%)**	***Ash** (%)*
Fish meal	54.05	1.7	12.3	4.5	28.9
Groundnut oil cake	47.3	7.1	7.7	6.5	7.9

Rice bran	11.2	21.7	3.9	19.9	1.9
Wheat flour	19.7	29.5	11.91	12.4	4.3
Tapioca flour	13.9	44.9	0.2	9.6	11.4
Spirulina maximus	61.7	8.5	4.9	11.8	2.1

*Values represent means of triplicate

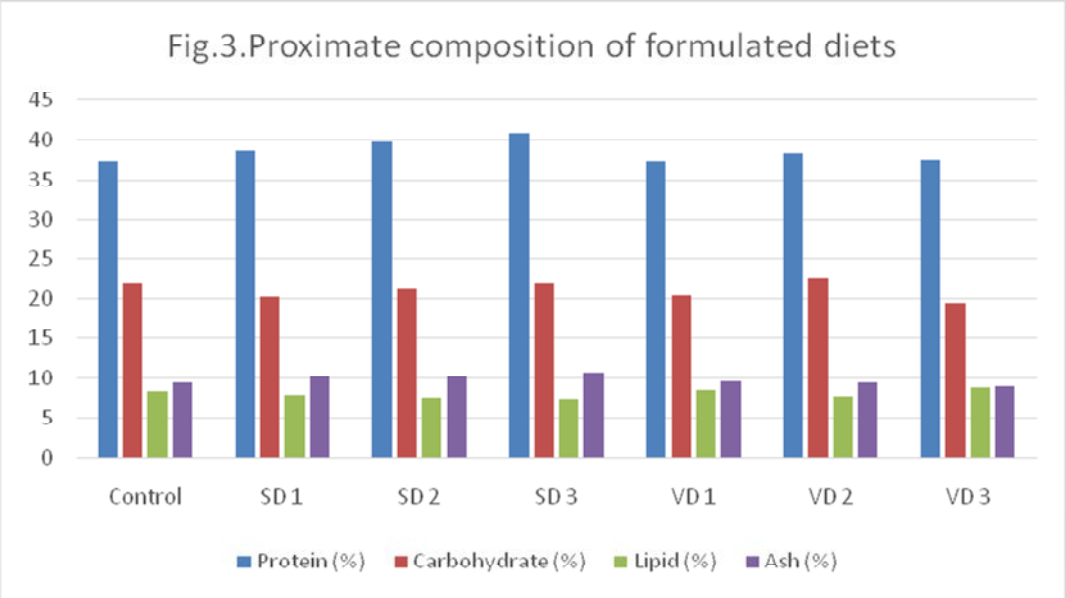
Table 3 Proximate composition of formulated diets

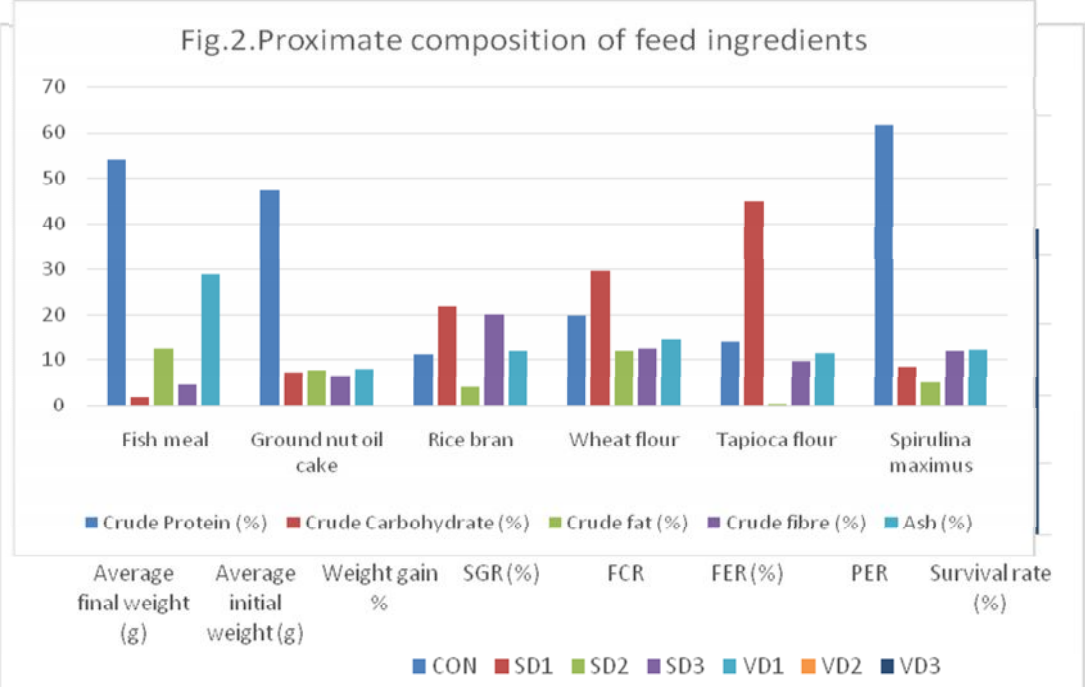
Diet	Protein (%)	Carbohydrate (%)	Lipid (%)	Ash (%)
Control	37.32	21.72	8.19	9.41
SD 1	38.49	20.17	7.75	10.17
SD 2	39.63	21.09	7.43	10.23
SD 3	40.79	21.84	7.21	10.57
VD 1	37.18	20.33	8.41	9.52
VD 2	38.23	22.58	7.63	9.28
VD 3	37.48	19.39	8.68	8.91

*Values represent means of triplicate

Table 4 Growth responses of *Cyprinus carpio* fed with *Spirulina* and vitamin 'C'

Growth parameters	CON	SD1	SD2	SD3	VD1	VD2	VD3
Average final weight (g)	14.03 ±1.34	16.48 ±0.49	16.57 ±1.25	16.59 ±1.33	15.84 ±1.18	16.07 ±1.06	16.13 ±1.17
Average initial weight (g)	12.05± 0.93	10.32 ±1.19	9.92 ±1.32	9.45 ±1.57	12.82 ±0.7	12.64 ±0.3	12.17 ±0.21
Weight gain %	6.43^{h} ±1.27	59.69^{bc} ±0.43	67.04^{b} ±0.24	75.56^{a} ±0.23	23.56^{gh} ±0.98	27.14^{fg} ±1.01	32.54^{f} ±0.97
SGR (%)	0.11^{i} ±0.009	0.34^{c} ±0.012	0.37^{b} ±0.009	0.41^{a} ±0.009	0.15^{h} ±0.006	0.17^{h} ±0.006	0.20^{g} ±0.009
FCR	6.79^{d} ±1.27	2.26^{ab} ±0.331	2.07^{ab} ±0.241	1.78^{a} ±0.231	4.83^{c} ±0.984	4.49^{c} ±1.015	3.98^{bc} ±0.964
FER (%)	14.72^{g} ±2.14	44.25^{b} ±3.74	48.40^{b} ±4.19	56.20^{a} ±4.73	20.71^{fg} ±2.93	22.27^{ef} ±2.07	25.10^{def} ±2.13
ER	0.40^{e} ±0.04	1.16^{ab} ±0.19	1.24^{a} ±0.13	1.40^{a} ±0.23	0.56^{de} ±0.10	0.59^{cde} ±0.12	0.68^{cde} ±0.11





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IN VITRO INHIBITION OF CLINICAL PATHOGENS BY THE EXTRACTS OF *CERAMIUM* Sp: A PRIMARY STUDY

F. Arockiya Aarthi Rajathi, V. Thanappan, S. Saravanan and P. Anantharaman*

1. Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamilnadu, India.

1. Introduction

The microorganisms have developed resistance to many antibiotics because of indiscriminate use of antimicrobial drugs that create a big problem in the treatment of infectious diseases (Davis, 1994). Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur (Parekh *et al.,* 2007).

Marine algae represent a great source of a vast variety of complex natural products and could be a promising source of a novel bioactive compound that can help plant survival by offering protection against stress imposed by pathogens. Marine algae may have several

applications in agriculture (Chandia *et al*., 2008). It has been reported that seaweeds possess compounds exhibiting antimicrobial potential against the pathogenic microbes of medical, agricultural, and environmental importance. Thus, antiviral, anthelmintic, antifungal, and antibacterial activities have been detected in green, brown, and red algae (Damonte *et al*., 2004, Mayer *et al*., 2009). There are numerous reports on the biological activities of macroalgae against human pathogens, fungi, and yeast (Newman *et al.,* 2003).

Seaweeds might use targeted antimicrobial chemical defense strategies by eliciting secondary metabolites, which are important in ecological interactions between marine macro organisms and micro organisms (Kubanek *et al.,* 2003). Therefore, seaweeds could be a promising source of novel bio-active compounds that can help plant survival by offering protection against stress imposed by the environment. The present study was, therefore carried out to assess the antibacterial activity of six different solvents of *Ceramium Sp.* against human bacterial (Gram negative) pathogens.

2. Materials and Methods

2.1. Chemicals

All the Chemicals procured were analytical grade. Muller Hinton broth, Agar and sterile disc were purchased from Hi-Media, Mumbai, India.

2.2. Collection and Processing of Algal sample

The seaweed samples were collected during the low tidal conditions from Muttom (8º07''N 77º18''E) India. The seaweed was handpicked or collected with the help of scalpel and immediately cleaned with seawater to remove foreign particles, sand and epiphytes. Then the seaweed was kept in an ice box and immediately transported to the laboratory and cleaned thoroughly using tap water to remove the salt on the surface of the sample. Then it was spread on blotting paper to remove excess amount of water. The dried sample was powdered in a mixer grinder and stored.

2.3. Phytochemical screening

Phytochemical screening of aqueous extract of *Ceramium* sp. was made to check the possible biomolecules involved in the *Ceramium* sp. The extract was then centrifuged at 5,000 rpm for 20 min at 4°C and filtered. Phytochemical characterization was carried out qualitatively to detect the presence of alkaloids, phenols, saponins, proteins, carbohydrates, steroids, tannins, flavonoids and anthocyanins by standard methods [Harbone, 1998; Sofawara, 1993].

2.4. Preparation of algal extract

The finely ground algal samples were weighed and 10 g were mixed with 100 ml of various solvents (1:10, w/v); 100% Ethanol, Methanol, Acetone, Hexane, Diethyl ether and Ethyl acetate respectively. The crude preparation was left overnight in the shaker at room temperature and then centrifuged at 4000rpm for 24 hrs. Then the samples were filtered using Whatman filter paper No. 1 to separate the filtrate and the extracts were freed from solvent by evaporation.

2.5. Growth and Maintenance of Test Microorganisms

Bacterial culture of *Escherichia coli, Salmonella typhi, Vibrio cholerae* and *Proteus mirabilis* were obtained from R.M.M.C.H, Annamalai University, Annamalai nagar,

Chidambaram. The bacterial culture were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C and centrifuged at 10,000 RPM for 5 min.

2.6. *In vitro* antibacterial assay

Antimicrobial activity of various extracts of *Ceramium* sp. was evaluated by the well diffusion method on Muller Hinton agar medium. The sterile nutrient agar medium (20 ml) in petri dishes was uniformly smeared using sterile cotton swab with tested pure culture of *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Vibrio cholera* and *Salmonella typhi*. The wells of 5 mm diameter were made in each petri plates and various extracts of *Ceramium sp.* was added. The plates were incubated at 37ºC for 24 h during which activity was evidenced by the presence of a zone of inhibition surrounding the well. The antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the crude extract when compared to the controls. Streptomycin was used as positive control (Cappuccino *et al*., 1996).

3. Result and Discussion

The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites, which are not found in terrestrial environments. Thus, marine algae are among the richest sources of known and novel bioactive compounds (Faulkner, 2002; Blunt *et al*., 2006).

The red alga *Ceramium sp. w*as extracted to produce 6 kinds of condensed extract, each of which is the fraction of Ethanol, Methanol (polar), Diethyl ether, Ethyl acetate (semipolar), and n-hexane, Acetone (nonpolar) solvents.

Phytochemical screening on *Ceramium* sp. by conventional methods confirmed the presence of Phenolics and tannins whereas the remaining phytoconstituents were absent. This observation suggests that the ingredients present in the extract are responsible for antibacterial activity (Mulvaney, 1996). From the previous phytochemical studies it is evident that *Ceramium virgatum* is rich in phytochemical constituents like Phenolics, tannis and lacking alkaloids, flavonoids, saponins, proteins, quinines, steroids, xanthoproteins, carboxylic acid, coumarins, carbohydrates anthrocyanins and coumarins, [Domettila *et al*., 2013]

The results indicated various degrees of growth inhibition on the test microorganisms. Ethanolic extract of *Ceramium* sp. exhibited inhibitory effects against almost the tested strains.

The five types of bacteria representing Gram-negative bacteria and Gram positive bacteria were used in this study. Antibacterial activity of the extracts against the five test bacteria *E. coli, K. pneumoniae, V. cholerae, P. vulgaris* and *S. typhi* characterized by a clear zone, indicating the involvement of phytoconstituent in algae. Ethyl acetate extract of *Ceramium* sp. has antibacterial activity compared to most large fraction of ethanol and n-hexane.

Among the six different solvent extracts of *Ceramium* sp. the inhibitory activity was found in the series of Ethanol, methanol, Hexane, Ethylacetate, Diethyl ether and Acetone against the gram negative bacterium.

From this study, the ethanolic extract shows promising antibacterial activity against almost all bacterial strains. The maximum activity was recorded against *E. coli* (8 mm), *K. pneumoniae* (6 mm), *V. cholerae* (6 mm), *P. vulgaris* (4 mm) and the minimum activity was

observed against *S. typhi* (3.8 mm). The extracts using methanol showed maximum activity against *P. vulgaris* (5 mm), *V. cholerae* (4 mm), *K. pneumoniae* (4 mm), *E. coli* (3 mm) and the minimum activity was observed against *S. typhi* (1 mm). Acetone extract of *Ceramium sp.* exhibited maximum activity against *P. vulgaris* (3 mm) and the minimum activity was observed) in *E. coli* (1 mm), *K. pneumoniae* (1 mm), *V. cholerae* (1 mm) and *S. typhi* (1 mm). Ethylacetate extract produced maximum zone of inhibition against *E. coli* (5 mm), *V. cholerae* (3 mm), *K. pneumoniae* (2 mm) and minimum was observed in *P. vulgaris* and *S. typhi* (1 mm). The diethylether extract observed the maximum activity against pathogen *S. typhi* (5 mm) and the minimum activity was observed in *E. coli* (1 mm), *K. pneumoniae* (1 mm), *V. cholerae* (1 mm) and *P. vulgaris* (1 mm). The low polar solvent as Hexane exhibited maximum inhibitory activity against *E. coli* (6 mm), *V. cholerae* (3 mm), *S. typhi* (3 mm), *P. vulgaris* (2 mm) and minimum activity was recorded in *K. pneumoniae* (1 mm).

In general, phenolic compounds possess specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-feedant, anti-viral, anticancer and vasodilatory actions [Aliyu *et al*., 2009]. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent [Kolodziej and Kiderlen, 2005]. In general, gram-positive bacteria appeared to be more tolerant than gram-negative cells. The cell wall of gram-positive bacteria contains multiple layers of peptidoglycon compared to the cell wall of gram-negative bacteria. Thus, gram-positive bacteria may allow less phytochemicals to reach the cytoplasmic membrane than gram-negative bacteria and may therefore be less susceptible. The *Ceramium* sp. are also reported to be most effective against bacteria, very low concentrations.

Conclusion

In conclusion, the antibacterial activity of the extracts was found to be almost high against all gram negative strains. The activity varied according to type of solvents used for the extraction. Further studies are needed for isolation of bioactive compounds from seaweed extract.

Acknowledgement

The authors are sincerely thankful to higher authorities of Annamalai University, for providing research facilities and Centre for Marine Living Resource and Ecology, Kochi, through Harmful Algal Bloom for their financial support throughout my study period.

AN ASSESSMENT OF ANTIOXIDANT ACTIVITY OF PHYCOERYTHRIN FROM A RED ALGA *CERAMIUM*SP.

D. Babitha and Vasuki Subramanian*

CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India
**Corresponding author: vasukipackiaraj04@yahoo.com*

INTRODUCTION

Seaweeds are macroalge found abundantly in coastal areas. Seaweeds produce many biologically active phytochemicals, which includes carotenoids, terpenoids, xanthophylls,

chlorophylls, phycobilins, polyunsaturated fatty acids, polysaccharides, vitamins, sterols, tocopherol and pigments. Usually red Seaweeds contain red pigments known as Phycoerythrin (PE), Phycocyanin (PC) and Allophycocyanin (APC). In Particular, Phycoerythrin (PE) a red protein-pigment complex from the light-harvesting phycobiliprotein family, present in red algae and cryptophytes, accessory to the main chlorophyll pigments responsible for photosynthesis.Amoung them *Ceramium*sp. are associated with other seaweeds which contains R-phycocyanin, Allophycocyanin and R-phycoerythrin, a phycobiliproteins, can be exploited for pigment extraction and utilization as natural colorant. The phycobiliproteins are water soluble pigments, pinkish in colour and unstable in light, pH and temperature (Sudhakar *et al.*,2014). Extraction of Phycoerythrin from this seaweed is a difficult process due to its morphological appearance of small thallus. Due to the protein nature, unique colour and fluorescence characteristics of PBPs, they have wide range of promising applications viz. nutritional ingredients in food, colorants in cosmetics and markers in different fluorescence techniques (Raja *et al.*,2008).Antioxidant activities have been identified in various marine algae, including red, green, and brown algae species.(Kelman*et al.*, 2012).It is well known that reactive oxygen species (ROS) are involved in a diversity of important pathological processes in medicine including among others: inflammatory and neurodegenerative diseases, atherosclerosis, cancer and reperfusion injury (Shon*et al.*,2003). In addition to antioxidant compounds from plant sources, numerous studies are also being carried out to identify antioxidant substances from macro and micro algae because of their abundance and easy availability in nature.

Many studies have been devoted towards the recognition of antioxidant potential of natural proteins originated from microalgae because of their broad spectrum antioxidant activity. Therefore in present study, an attempthas been made to evaluate the antioxidant potential of crude Phycoerythrin pigment from *Ceramium*sp.

MATERIALS AND METHODS

Seaweed sampling

The red alga *Ceramium*sp. was collected during August 2015, from the hard substratum in the Pondicherry coast (Lat. $11^0$56' 27.22" N; Long. $79^0$50' 14.67" E). The alga was collected at the intertidal rocky shore on the coast, where they are usually abundant in the month of May, August and January.The collected samples were put in buffered solution to prevent protein denaturation. Then, the samples were brought to laboratory in ice containing box for the extraction of R-Phycoerythrin.

Extraction of crude R-Phycoerythrin (Bennett and Bogorad, 1973)

One gram of *Ceramium*sp.was taken in a sterile container and grind with acid-washed sand using mortar and pestle in phosphate buffer (pH 7.2; 1M)by centrifugation at 8000 rpm at 4^0C for 15 minutes. The proteins in the supernatant were precipitated with 60% ammonium sulphate saturation and the mixture was stirred overnight. Then the precipitate was centrifuged to get the crude pigment.ThePE, PC and APC pigments were assayed by reading the supernatant at 562, 615 and 652 nm using a DU-40 Spectrophotometer (Beckman, USA). All the subsequent purification steps were carried out at 4or repeated freezing and thawing. Later the biomass was separated 4^0C.the calculation of phycobilins was calculated using the following equations:

Phycocyanin (mg/ml) = [A620-0.474(A652)]/5.34

Allophycocyanin (mg/ml) = [A652-0.208(A620)]/5.09

Phycoerythrin (mg/ml) = [A562-2.41(PC)-0.849 (APC)]/9.62

Table: 1. Extraction of crude R-Phycoerythrin.

Pigment type	R-phycocyanin mg/ml	Allophycocyanin mg/ml	R-phycoerythrin mg/ml
Crude	**0.38**	**0.48**	**0.77**

Antioxidant activity

The antioxidant activity was measured by the total antioxidant method and the free radical DPPHmethod.

DPPH Assay

DPPH radical-scavenging activity was measured according to the method of Kang and Saltveit (2002). Two millilitres of tested sample were mixed with 1 ml of 200 lM DPPH in ethanol solution and incubated for 30 min at 25^0C. The absorbance of the mixture was measured at 517 nm.

Results and Discussion

Pigment Quantification

The Crude phycobiliprotein contains a mixture of PC, APC and PE pigments. The content of PC, APC and PE in crude samples was observed to be 0.38, 0.48, 0.77mg/ml respectively (Table 1). The present results are in the better side than the previous report of Beer and Esel, 1985 andTello-Ireland *et al.*, 2011, had reported PE (0.46 mg/g and 0.54 mg/g) and PC (0.28 mg/g and 0.24 mg/g) from the red alga *Gracilaria chilensis*. In fresh samples of *Gracilaria* sp. Gomez *et al* reported higher amount of PE (1.25 mg/g) and a lesser amount of PC (0.11 mg/g) as compared to the present study (PE=0.78 mg/g and 0.49 mg/g FW). Senthilkumar*et al* (2013) reported 0.781 mg/g of R-phycoerythrin from *G. corticata*as compared to the result of present study. Sudhakar *et al* 2014reported 0.78 mg/g of R-phycoerythrin from *G.corticata*J. Agardh var. *corticata* which is found to be similar to the result of present study. Through this study the yield of R-phycoerythrin obtained was 0.77 mg/ml in crude extract.

Antioxidant activity is depends on the method adopted and the lipid system used as substrate. Hence, the following method has been adopted in order to assess the antioxidative potential of crude Phycoerythrin pigment from red seaweed.The results inferred that, the crude PE exhibited highest DPPH-scavenging activity with 75.23±0.67% at the dose of 150mg. Ravi RaghavSonani*et al* (2014) reported that phycoerythrin found promising antioxidant activity compared to phycocyanin and allophycocyanin.The ascorbic acid showed 85.22 % inhibition at 150 mg.

Conclusion

The present study reveals that R-phycoerythrin in crude form extracted from red algae *Ceramium*sp. *rubuns* was analysed for phycobiliproteins R-Phycoerythrin and its antioxidant activity was studied. The study revealed that crude R-phycoerythrin pigment from the red alga showed potential activity against free radicals that's taking as a baseline to check the antioxidant activity. The findings of this work are useful tofurther research to identify, isolate

and characterize thespecific pigment which is responsible for higher antioxidant activity. Bioactive pigment found in seaweedawaits a major breakthrough for a variety of food/medicalapplications as they have the potential for application ofsuch compounds as natural antioxidants in differentfood/pharmaceutical products.

Acknowledgement

The authors are sincerely thankful to higher authorities ofAnnamalai University for providing research facilities and Ministry of Human Resources and Development for financial support throughout my study.

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EFFECT OF COLCHICINE AND GIBBERELLIC ACID ON THE GROWTH OF COMMERCIAL SEAWEEDS UNDER *IN VITRO* CONDITION

Dr. C.P. BALAKRISHNAN[1] and Dr. VENKATARAMAN KUMAR[2]
[1]Assistant Professor and Head, Department of Botany, Aditanar College of Arts and Science, Virapandianpatnam, Tiruchendur, Tamil Nadu
[2]Former Head, Dept. of P.G. and Research Centre in Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu

INTRODUCTION

Seaweeds are macroscopic algae, which form an important component of the marine living resource. They are available largely in shallow coastal waters wherever there is a substratum on which they can grow and flourish. Based on their pigmentation, the seaweeds are broadly grouped into green, brown and red. They are harvested by man for centuries, particularly in Japan and China, where they form a part of the staple diet. The uses of seaweeds as food, fodder and manure are well known in many countries. The three major commercial phycocolliods extracted from seaweeds are agar agar and carrageenan derived from red seaweeds and algin from brown seaweeds. These products are difficult to synthesize chemically because of formidable chemical barriers and hence for these commercially important products we have to depend on seaweed resources (Dhargalkar and Pereira, 2005). The demand for phycocolliods in the Indian market is much higher than what is produced and so it imports these phycocolliods from countries like Norway, Scotland, Chile, France, Spain, China, Japan and the Philippines. The industrial uses of seaweed phycocolliods are immense. Some of the well recognized uses are as emulsifiers in dairy, leather, textile and pharmaceutical industries.

MATRIALS AND METHODS

Survey

Seaweeds were collected from the intertidal to the subtidal region during low tide of Gulf of Mannar region. Samples were taken from randomly selected spot at each site. Herbarium specimens were prepared and identified based on the keys given by Umamaheswara Rao (1970 and 1987). The collected plants were authenticated and the voucher specimens (Voucher No. VOCB4098 to VOCB4128) were lodged in the herbarium of Ethno pharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu, India.

Laboratory culture experiments

The agarophyte *Gracilaria edulis* and the carrageenophyte *Hypnea musciformis* were cultured in the Botany Research Laboratory for studying the effect of colchicine and gibberellic acid on the yield of agar and carrageenan respectively

Colchicine

The alkaloid colchicine ($C_{22}H_{25}NO_6$, Himedia) was used in algal culture at three different concentrations (10, 25 and 50 µg) and maintained in triplicate.

Phytohormone

Three different dosages ((10, 25 and 50 µg) of the phytohormone, Gibberellic acid ($C_{19}H_{22}O_6$, Loba Chemie P. Ltd.) were employed in algal culture maintained in triplicate.

RESULTS AND DISCUSSION

Maximum amount of agar was obtained from *Gracilaria edulis* culture having 25µg of colchicin whereas that of carrageenan from *Hypnea musciformis* culture containing 50 µg of colchicin (Fig. 1 and 3). The phytohormone Gibberellic acid triggered maximum amount of agar and carrageenan at 50 µg concentration in the culture of *Gracilaria edulis* and *Hypnea musciformis* respectively (Fig. 2 and 4). Colchicine is a narcotic alkaloid, related chemically to morphine and codeine. It is a very potent poisonous substance induces chromosome doubling in plant at very small concentration that may observe in *Datura* palnt (Blakeslee and Avery, 1937). Plant growth regulators or the so called plant growth hormones accelerated agar yield at low concentrations (Hemalatha and Rengasamy, 1999; Kaliaperumal *et al.*, 2003). These earlier observations support the present finding of promotion of agar and carrageenan yield by colchicines and gibberellic acid treatment at varying concentrations.

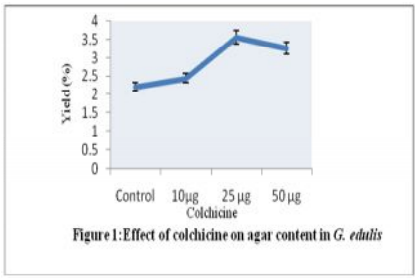

Figure 1:Effect of colchicine on agar content in *G. edulis*

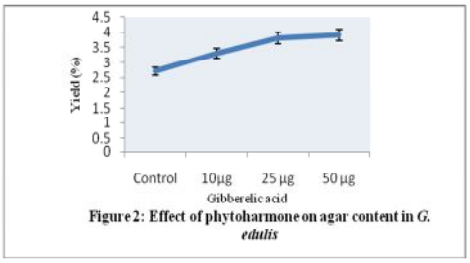

Figure 2: Effect of phytoharmone on agar content in *G. edulis*

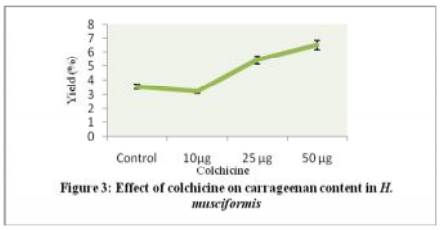

Figure 3: Effect of colchicine on carrageenan content in *H. musciformis*

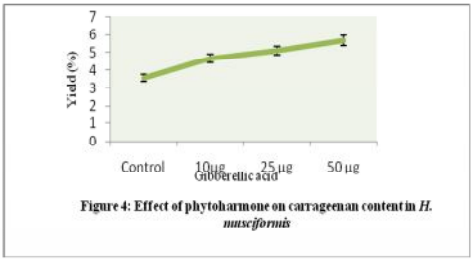

Figure 4: Effect of phytoharmone on carrageenan content in *H. musciformis*

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ANATOMICAL STUDY OF AGAROPHYTIC SEAWEED *GRACILARIA CORTICATA* OF MANAPAD COAST, TAMIL NADU

Jenifer. P[1]., C.P. Balakrishnan[2] and S. Chidambaram Pillai[3]

[1]UGC Project Fellow, Department of Botany, Aditanar College of Arts and Science, Virapandianpatnam, Tiruchendur - 628 216, Tamil Nadu, India
[2]Assistant Professor & Head, Department of Botany, Aditanar College of Arts and Science, Virapandianpatnam, Tiruchendur - 628 216, Tamil Nadu, India
[3]Associate Professor, Dept. of P.G. and Research Centre in Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu

INTRODUCTION

Marine macro algae are abundantly occurs in the shallow coastal area of Manapad coast, Tamil Nadu. Marine algae are the raw material for the production of many bioactive secondary metabolites. More number of bioactive substances may extract from macro algae using different organic solvents. Normally the insoluble polysaccharide may found in the cell wall of certain seaweeds are having certain bioactivity. In the present study the basic dye toluidin blue was used to identify the polysaccharide of *G. corticata*.

MATERIAL AND METHODS

Collection and Identification

The test specimen *Gracilaria corticata* was collected from Manapad during the low tide. Collected plant was thoroughly washed with seawater to remove the epiphytes and sand particles and transported to the laboratory for further use. Herbarium specimens were prepared and identified based on the keys given by "Economical Important Seaweeds – CMFRI Bulletin 41" by Umamaheswara Rao 1970 and 1987. The collected plants were authenticated and the voucher specimens (Voucher No. ACBH28 to ACBH33) were lodged in the Botany Research laboratory, Department of Botany, Adtianar College of Arts & Science, Tiruchendur, Tamil Nadu.

ANATOMICAL ANALYSIS

The fresh sample of *Gracilaria corticata* was cut into small pieces and fixed immediately in FAA (Formalin: Acetic Acid: Alcohol) for 24 hours. After fixation the specimen was washed thoroughly in distilled water simultaneously dehydrated using alcohol and xylol for remove excess of water. Dehydrated specimens was embedded in paraffin wax (Merck Product, 55^0C Melting Point) after infiltration (at 55^0C in Hot air Oven) and sectioned using Rotary Microtome to the thickness of 5 to 10 µm (Sass, 1940). Sectioned materials were stained by Toluidine Blue (O' Brien et al 1964) method. Stained specimen was mounted using DPX (Distrene Plasticizer Xylene); is the most commonly used non fluorescent mountant that preserve most routine stains and dries rapidly (Culling, 1963). The images were taken by using Olympus Ch20i built with analogue camera.

RESULTS AND DISCUSSION

The seacoast of the study area Manapad coast is rocky and deep sea area placed in India state of Tamil Nadu located at the longitude of 77.5^o to 78.30^oE and the latitude of 8^o to 8.88^oN. The study area is highly dynamic with many cyclic and random processes owing to a variety of lithophytic algal resources like the members of Chlorophyta (Green), Phaeophyta

(Brown) and Rhodophyta (Red) are in rich. Agar yield red agarophytic coralline seaweed *Gracilaria* are rich in the near shore of the study area.

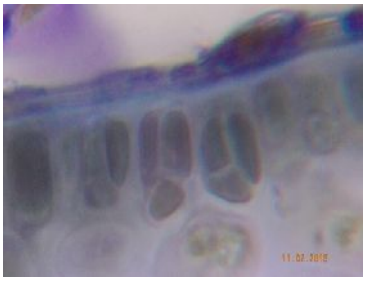
Identification and Preservation

Collected seaweeds were preserved in the form of herbarium specimens. The specimens were identified based on the keys given by "Economical Important Seaweeds – CMFRI Bulletin 41" by Umamaheswara Rao 1970 and 1987. The macroscopic observations are as follows

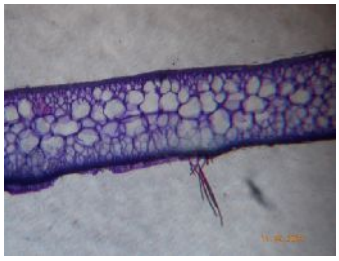
Gracilaria corticata - Thallus is flat, rough and leathery, minute hairs are present, dark green with light red and the apex are dichotomously and entire.

Microscopic Observation

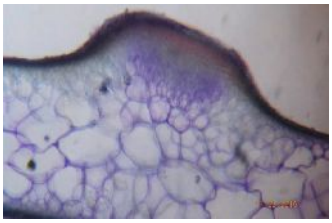
The photomicrograph at varying magnification showed distinct outer covering epidermis, cortex and broad medulla. The outer thickest mucilaginous matrix appeared purple in colour and the cytoplasm and nucleic acids showed blue in colour under 2.5 µm and 1µm of Toluidine blue, crystal violet and safranin staining. Cortex consists 1-3 layered followed by parenchymatous broad medulla were present (Plate).



View of cell wall (100X)



View of thallus (4X)



View of Medulla (10X)

View of cell wall polysaccharide (40X)

Agar is a phycocolloids derived from galactan polysaccharides, the major polysaccharides constituents' present in the cell walls of most marine seaweeds. Marine red algae are the main source of polysaccharides is used for pharmaceutical applications including anticoagulant, antiviral and immune – inflammatory activities that might find relevance in neutraceutical, cosmeceutical and food. These sulfated galactans are primarily classified as agar and carrageen. Specifically galactans with 4-linked α - galactans residues of the L-series are termed as agar. The present microtechnique study revealed that extra cellular

colloidal mucilaginous substance appeared dark purple colour that may indicate the presence of insoluble polysaccharides are abundant in *Gracilaria corticata*.

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IMMUNOSTIMULATORY EFFECT OF SARGASSUM WHITTI EXTRACTS ON CYPRINUS CARPIO (FISH)

Immanuel Suresh*. J, Revathy[1] P and Kumerasan[2]
*Assistant Professor,
[1,2]Department of Immunology and Microbiology,
The American College, Madurai. Email : immanuelsuresh1978@gmail.com

Introduction:

Costal environment play a vital role in nation's economy by virtue of the resources, productive habitat and rich biodiversity. India has a coastline of about 7,500 kms. The coastline of Tamil Nadu a length of about 1076 kms constitutes of about 15% Arabian Sea. The Tamil Nadu coast is straight along the Bay of Bengal, Indian Ocean and except the Vedaranyam. Fringing and patch reefs are present near Rameswaram and Gulf of Mannar, Pitchavaram, Vedaranyam and Point calimere have well developed mangrove systms Tamil Nadu is endowed with one of the largest and richest fisheries in India.(Kannan and Thangaraj, et al 2006).

The State has 1.9 lakh sq. m of EEZ covering the three coastal zones already described, besides 21 coral islands in the Gulf of Munnar, with rich habitats of corals, Coastal Lagoons (Pulicat Lake and Muthupet Swamp) and estuaries. Mandapam camp was built in the early 1900s by the British Government to house migrant plantation workers coming to India from Sri Lanka. The Camp is located in South India, 700 km South of Chennai (formerly known as Madras), the capital of the state of Tamil Nadu (Ramachandran and Asir Ramesh et al., 2000).

A number of species such as Sargassum have adapted to a fully planktonic niche and are free-floating, depending on gas-filled sacs to maintain an acceptable depth to live in tidal rock pools. In this habitat seaweeds must withstand rapidly changing temperature and salinity and even occasional drying. Seaweed compounds in different sectors,such as food

supplements, cosmetics, biomedicine and biotechnology, are constantly under development. Recent trends in life – style towards natural, healthy products are favourable for advancing seaweed aquaculture in Ireland. The most suitable seaweed species for cultivation in Ireland for the near future are those, which are already used in trials or commercial cultivation operations in Ireland and other western countries, or species that have high levels of desired molecules. For these specific species, a real market demand exists. These include seaweeds for human consumption, functional food ingredients, nutraceuticals and cosmetics.

IMMUNOSTIMULANT'S:

Immunostimulants can be applied either orally (or) intravenously. The effect generated depends on the dosage, time and mode of administration as well as on the immune status of the organisms (Wagner et al., 1999)

Use of immunostimulants is a unique approach for fish culturalists as they under take methods of controlling disease losses in their facilities. Immunostimulants may give by themselves to active non-specific defense mechanisms or they may be administered with a vaccine to activate non-specific defense mechanism as well as specific immune response.

COMMON NAME:

Blourkurper (Africans – South Africa), Common Kendai

(English:Greas) carpioCyprinidae (English, India), carpio mouth breeder and gills injection(English), carpioGrase (English), GraseCarpio (Dominician Republic).

Although tolerant of most conditions, common carp prefer large bodies of slow or standing water and soft, vegetative sediments. As schooling fish, they prefer to be in groups of five or more. They naturally live in temperate climates in fresh or slightly brackish water with a pH of 6.5-9.0 and salinity up to about 0.5% and temperatures of 3 to 35c (Sterba, 1962). The ideal temperature is 23 to 30^0c, with spawning beginning at 17-18^0c; they easily survive winter in a frozen-over pond, as long as some free water remains below the ice. Carp are able to tolerate water with very low oxygen levels, by gulping air at the surface.

Various seaweed which have the potential of immunostimulatory or immunomodulatory actively are identified and found to be more effective than the use of synthetic substance as immunostimulant (Agarwal and Singh et al., 1999).

The specific objective of the present study is to investigate the effect of both acetone and ethanol crude extract of *sargassumwhitti*for their specific immunostimulatory properties with reference to antibody response in *Cyprinuscarpio.*

Material and methods:

SEAWEED SELECTED:

Seaweeds (*Sargassumwhitti)* was collected by scuba diving and handpicking from the rocky substratum at depths of 1-2 m along the subtidal areas of Sta. Ana, Cagayan ($18^0$25' 25.50" N, 122^0 07'28.20" E), and Indian Ocean. The collected seaweeds were cleaned of epiphytes, extraneous matter, and necrotic parts, washed withclean fresh water, air-dried at room temperature for two weeks, cut into small portions, and powdered using a hammer mill and a mechanical food blender. Extracts (Methanol, ethyl acetate) of the powdered seaweed were obtained with organic solvents to increase polarity.

COLLECTION OF SAMPLE:

Based on pilot study, seaweeds were obtained from the Gulf of Munnar Coastal region.

PREPARATION OF SEAWEED:

Fresh seaweeds of *Sargassumwhitti*was washed thoroughly in tap water followed by distilled water and were then shade dried until the water content lost completely. Dried seaweed was powdered using mixer grinder. Fine powder was obtained after sieving.

PERCOLATION:

Percolation – liquid passing through holes or porous substances. It can be used informally to mean to become lively and to make seaweeds in a percolation known as percolate (Mark and Ziff, 2000).

COLLECTION OF EXTRACTION:

The powdered plant material was soaked initially in a solvent in a percolator, Additional solvent is then poured on top of the plant material and allowed to percolate slowly (Dropwise) out of the bottom of the percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator (Newman et al.,2000). Percolation is adequate for both initial and large-scale extraction. Then seaweed extraction was collected from conical flak, after collection of sample and using evaporation of water bath and small: amount of sample was taken in the eppendrof tube.

ADMINISTRATION OF EXTRACT TO THE FISH – ORAL AND GILL INJECTION:

Oral seaweed injection can be done with virtually all fish sizes. This methods stress-free and inexpensive way to boosts of fish in any culture environment. The gill area injection of the extract of seaweed in dosage 1.0 ml to five number of fishes and maintaining the one as control fish. The incubation time 8-10 days.

BLEEDING TECHNIQUE:

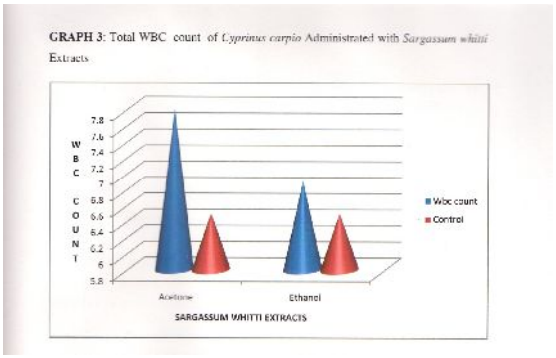
GILLS:

Gills are very efficient at collecting free oxygen from water; they have to be because water typically contains only a fraction of free oxygen of that found in air. Air typically contains 280 mg/l of oxygen while fresh water typically only contains 5 – 10 mg/l. So the gills area injection of seaweed in dosage 1.0 gm and maintaining the 1 control fish.The incubation time 8-10 day. Standard gill bleeding technique was followed to bleed the fishes.

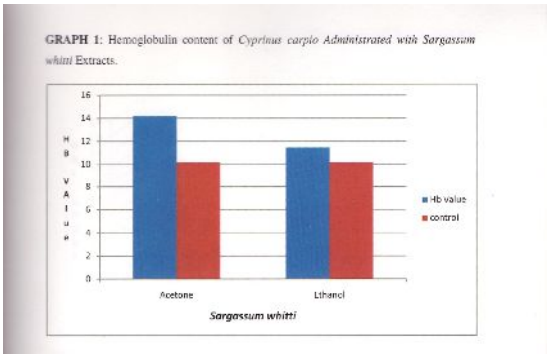
ESTIMATION OF BLOOD PARAMETERS (Haemoglobin and WBC):

From the collected blood the Haemoglobin and WBC count was estimated by the following procedures and Haemoglobin content estimation work was done at Bose Clinical Laboratory, Madurai. Haemoglobin was estimated by cyanmethemoglobin method.

Total white blood cells (WBC) were counted using an improved Neubaurhaemocytometer (Shah and Altindag 2005; Mgbenka et al., 2003). Blood was diluted 1:20 with WBC diluting fluid and placed in haemocytometer. 4 large (1 sq mm) corner squared of the haemocytometer were counted under the microscope (Olympus) at 40 X. The total number of WBC was calculated in $mm^{3.}$ X $10^{3.}$



RESULT AND DISCUSSION:



The two solvents Acetone and Ethanol of *Sargassumwhitti* extracts showed significant immunostimulatory effect on the secondary antibody response in *Cyprinuscarpio* to comparative study of seaweed.

Hemoglobulin content in fish showed (Graph) when compared with the control one *Sargassumwhitti*Acetone (14.2g/dl) extracts increased the content of hemoglobulin content compared with the *sargassumwhitti*with ethanol (11.5g/dl) extracts.

The total WBC count of *Cyprinuscarpio*indiacated in graph (2) shows the increase in total WBC count in *Sargassumwhitti*Acetone extract (7.8mm x 10^3) compared with *Sargassumwhitti*Ethanol extract (6.9mm x 10^3)

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PHYTAL FAUNA ASSOCIATED WITH THE MARINE MACRO ALGA *CHAETOMORPHA AEREA* (DILLWYN) KUTZING, (CHLOROPHYCEAE) IN PULICAT ESTUARY, TAMILNADU

Azhagu Raj R[1*]., J.Ganesh[1] ., M.C. John Milton[1] and Palavesam. A[*]

[1]School of Biodiversity and Environmental Monitoring, PG & Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai 600 034, Tamil Nadu, India.

[1*]Department of Animal Science, M. S. University, Tirunelveli, Tamilnadu ,India

*Email: drazhaguraj@gmail.com

1.Introduction

Marine macroalgae, popularly known as seaweeds are potential renewable resources in the marine environment. Seaweeds are primary producers and they play a significant role in the benthic food web. Many macroalgae build biogenic habitats which give shelter and provide a suitable physical environment for a great variety of organisms Day *et al.* (1989).

They offer a considerable variety of habitats, food resources and nursery areas for many species (Day *et al.,* 1989; Herman *et al*., 1999; McLusky, 1999; Little, 2000 and Thiel and Potter, 2001). Macro algal beds are one of the most productive habitats in the marine environment and frequently support high densities of mobile invertebrates including small crustaceans, gastropods, copepods and polychaetes (Edgar, 2001). The fauna associated with algal beds forms an important link to higher trophic level organisms such as juvenile fish (Andrew and Jones, 1990 and Fletcher and Day, 1983).

Marine seaweed regions harbor rich fauna of many diverse kinds of animals, and such animals lifelong or temporarily living in, on or among seaweeds are generally called "phytal animals" (Kenji, 1975). Numerous important studies on seaweed communities including these phytal animals have been so far carried out mainly in European seas from various biological view-points by many authors (Colman, 1940; Chapman, 1955; Ohm, 1964; Hagerman, 1966 and Nagle, 1968). Marine macrophytes such as algae and seagrasses often occur as dense aggregations, forming habitat patches to the associated fauna (Gunnill, 1982; Edgar and Moore, 1986). Vagile organisms may select patches with higher availability of food and refuges from both predators and unfavorable physical conditions (Brawley, 1992).

Fauna associated with seaweeds have attracted the attention of many scientists from various parts of the world. Mukai (1971) from Mukaishima Island, Japan and Zaleha *et al*.,(2010) studied the seaweed assemblage from Pulau Besar, Melaka, Malaysia . In India, Sarma and Ganapathy (1972; 1975), Sarma (1974), studied the phytal fauna from Visakhapatnam coast. Joseph (1978[a,b,]) studied the phytal fauna from Mandapam; Yogamoorthi (1982) studied the phytal fauna from Vellar estuary; Muralikrishnamurthy, (1983) studied the phytal fauna from Visakhapatnam coast ; James, *et al*.,(1986) studied the phytal fauna from Palk Bay and Gulf of Mannar ; Selva Ranjitham *et al*. (2008) studied the phytal fauna from Vellar estuary and Jansi, *et al*.(2009) studied the phytal fauna from Manakkudy estuary. Hitherto report on seaweed associated fauna from Pulicat Lake. Hence the present study is aimed at to study the phytal faunal association in Pulicat Lake.

2.0. Materials and Methods

2.1. Study area –Pulicat Lake

Pulicat Lake is the second largest brackish water body in India and is located between 13°26'and 13°43' N latitude and 80°03' and 80°18' E longitudes, with an average water spread area of about 461 sq. km on the Coromandel Coast. The study area is restricted to the lake waters of Pulicat. The sampling stations Station I -13°25'57.60 N 80°18°40.28"E, Station II-13°26'15.76N 80°19°02 .31"E, Station III-13°26'02.11'N 80°19°17.78"E, Station IV-13°25'41 N80°18°54.86"E.

2.2. Collection and Identification

The macroalgae *Chaetomorpha aerea* were collected from Pulicat estuary (stations I-IV, Quadrate, 25 X 25 cm^2; each10 replicates). The collected macroalga were kept in separate polythene pack containing filtered seawater brought to the laboratory. Then the *C. aerea* were submerged in 5-7 % buffered formalin solution. Vigorous shaking in formalin solution dislodges most of the clinging animals. After sieving, (mesh size 1.0 mm), 100 gram of the alga were taken into a Petri dish and carefully examined for every frond under a binocular microscope with strong incident illumination. The animal groups were sorted, counted and preserved in 4 % formalin for specific determination. The collection of phytal fauna associated with the seaweeds was carried out following the procedure advocated by Sharma

and Ganapati (1972). The *C. aerea* associated fauna were calculated by the quantitative data were expressed in terms of number of animals per unit of weight of seaweed (100g).

The structure and composition of seaweed associated faunal data were approached to various statistical methods namely univariate, graphical/distributional and multivariate methods. The computer programme PRIMER-E (ver. 6.1.7) (Plymouth Routines in Multivariate Ecological Research), was used for univariate and multivariate analyses of data (Clarke and Warwick, 1994).

3.0. Results and Discussion

3.1.Seaweed *Chaetomorpha aerea* associated fauna

In the present study, seaweed *C.aerea* associated fauna of the following six groups were recorded. 1. Mollusca, 2.Amphipods, 3.Fishes, 4.Prawns, 5. Crab and 6. Polychaete.Twenty one species of macrofauna were recorded from four stations of Pulicat Lake. Among the 21 species recorded, Crustaceans (Amphipods, Crab and Prawns) were found to be the largest component in the collection with ten species. Mollusca and fishes emerged as next dominant group in the order of abundance with five species. The polychaetes came last in the order with one species (Table1).

Table1. Seaweed *Chaetomorpha aerea* and its associated fauna in the Pulicat Lake

Crustacea			**Mollusca**	**Polychaetes**	**Fishes**
Amphipods	**Crab**	**Prawn**			
Grandidierella gravipes	*Clibanarius longitarsus*	*Penaeus semisulcatus*	*Clithon oualaniensis*	*Nereis chilkensis*	*Ambassis ambassis*
Ampelisca scabripes	*Clibanarius clibanarius*	*Fenneropenaeus indicus*	*Nassarius coronatus*		*Lutjanus johnii*
Parorchestia morini	*Portunus hastatoides*	*Penaeus monodon*	*Odostomia babylonica*		*Mugil cephalous*
Eriopisa chilkensis			*Cerithidea cingulata*		*Terapon puta*
			Cerithium scabridum		*Atule mate*

In stations I to IV, the population density of seaweed associated macrofauna were 2004 nos/m^2; 1304 nos/m^2; 1500 nos/m^2 and 1156 nos/m^2 respectively. Sharma and Ganapthi (1972) observed 13 algae associated fauna in Visakhapatnam coast. Amongst the algae the fine, bushy, tufted, *Spongomorpha indica* supported the maximum numbers of animals (78807.9/100g and1134833.0/m^2 of rock surface) and *Chaetomorpha antennina* associated fauna showed the minimum number of animals 550.4/100g and 11117.6/m^2 of algal coverage. The faunal composition of *C.aerea* ranged between 1.41 and 36.63%. It is well known that the phytal faunal density is also dependent on the morphology of the algae (structure, texture, colour and contour) and its sediment retaining capacities (Sarma and Ganapathi 1972).

Penaeid prawns constitute a major fishery in Pulicat Lake. There are about 12 species of penaeid prawns in the Pulicat Lake, of which seven species belong to the genus *Penaeus* and five belong to the genus *Metapenaeus* (Paulpandian and Ramasamy, 1991). Amphipods support the growth, and production of estuarine fishes and prawns (Aravind, *et al.,* 2005). Seaweed, Seagrass and emergent marshes, mangrove substrates may support higher densities of amphipods (Lyla, *et al*., 1998; Vainola, *et al*., 2008 and Walkar, l904). Hermit crabs occupy empty gastropod shells, and these shells act as shelters from biotic factors including predation and abiotic factors such as desiccation and osmotic stress (Ajmalkhan, 1992; Rajagopal, *et al*., 1998 and Ramesh, *et al*., 2009).

The distribution of polychaetes in Pulicat Lake is determined chiefly by the salinity of water and the nature of substratum. *Nereis chilkensis,* are widespread, in diverse habitats in the Pulicat lake. Polychaetes constitute important links in the food-chains of Pulicat Lake and they are the common food items for several species of top carnivores like fishes and birds in the lake (Srikrishnadhas *et al.*(1998); Sharma and Ganapthi (1972); Yogamoorthi, (1982); James, *et al*.(1986); Selva Ranjitham *et al*. (2008); Jansi and Ramadhas, (2009) and Zaleha *et al*.(2010)**.**

The density of phytal fauna in *C.aerea* is ranged from 1304 to 2004 and is good agreement with earlier report by Saravanakumar *et al.* (2007).The lower density of phytal fauna might be due to the higher saline nature of the estuary. The assemblage of polychaetes, amphipods and gastropods in *C.aerea* it may be due to providing more area of substratum. The faunal register were showed varies feeding habit such as filter feeders, detritus feeders, scavengers or carnivores and algaivores. (Selvaranjitham *et al*., 2008).

In the present study, a marked variation in diversity indices was observed between the stations. In station I to IV, the species diversity (Shannon Weinner index) varied from 2.254 to 2.091. The species richness between the stations I to IV ranged from 2.598 to 3.217. The species evenness, varied from each stations the values were 0.7235 to 0.7789. The present values observed in all the stations are comparable with the results of other earlier studies in India. (Sunilkumar, *et al*.,1996).

The faunal diversity was higher at station I situated near bar mouth than the interior three stations. Among the regions, the maximum diversity value was found in station I. The species richness, it's generally recognized that the muddy or clay sediments of mangrove forest act as home for a variety of epifaunal and infaunal invertebrates (Kumar, 1995). True to this, in the present study, the organic carbon and nitrogen percentage was more in station I which might be the plausible reason for the higher diversity and richness in station I.

The observations of the present study are in general agreement with the earlier observation made by Sharma and Ganapthi (1972); Yogamoorthi,(1982); James, *et al*.(1986); Ajmal Khan *et al*. (2005); Selvaranjitham *et al*. (2008); Jansi and Ramadhas, (2009) and Zaleha *et al*.(2010). However, knowledge of seasonal fluctuations of seaweeds and associated macrofauna is essential for future monitoring, conservation and for making reliable management decisions, especially in protected areas such as Pulicat Lake, Tamil nadu, India.

Acknowledgements:

We extremely thank late Dr. V. Krishna Moorthy, Director, Krishna Moorthy Institute of Algalogy, Chennai, for the identification of Algae; Dr. R. Venkitesan, Scientist- C, Southern Regional Station, ZSI, Chennai for Identification of Mollusca;Dr. S. Raffi, and Dr. C. Viswanathann, CAS, Marine Biology, Annamalai University, Parangipettai, for the

identification of Crabs. Dr. José Manuel Guerra García, Professor, Departamento de Zoología, Universidad de Sevilla, Spain, for the identification of Amphipods.

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ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF BROWN SEAWEED *Padina gymnospora*

Stella Mary J[*], John Marshal[1]and Christy Joshi[2]
[*]Assistant Professor,
[1,2]Department of Immunology and Microbiology, The American College, Madurai,
email.id: stella.mim@gmail.com

INTRODUCTION:

The marine world offers an extremely rich resource for important compounds of structurally novel and biologically active metabolites. Over the past several decades seaweeds have generated an enormous amount of interest in the pharmacological industry with enormous medicinal properties [1]. It produces variety of primary and secondary metabolites. Over 2,400 secondary metabolites have been isolated and many of which have been reported to have excellent biological activities [2] such as antibacterial, anti-cancer, anti-diabetic, anti-tumor, anti-coagulant and antioxidant [3].

Reactive oxygen species attack the biological molecules of the human body and leadingto cell or tissue injury associated with aging, atherosclerosis carcinogenesis and may lead to the development of chronic diseases related to the cardio and cerebrovascular systems [4]. Free-radical scavengers are antioxidants which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species. The most commonly used synthetic antioxidants like butylatedhydoxyanisole (BHA), butylatedhydoxytoluene (BHT) Propylgallate (PG) have side effects such as liver have side effects such as liver damage and carcinogenesis. Seaweeds have received special attention as a source of natural antioxidants [5].Recently, the potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolic acid, flavonoid, tannins e.g.). Those compounds are widely distributed in plants or seaweeds and are known to exhibit higher antioxidant activities [6].

Bacterial diseases are also the challenging threat to human population. Nowadays the use of antibiotics increases significantly due to heavy bacterial infections. The indiscriminate use of antibiotics has resulted in the accumulation of more resistant pathogenic bacterial strains and also causes some side effects to human [7] The ultimate solution to combat antimicrobial resistance is to development drugs from natural sources. The present study was aimed to evaluate the invitro antioxidant and antibacterial activities of brown seaweed *Padinagymnospora.*

MATERIALS AND METHODS

Collection and processing of algae species:

*Padinagymnospora*macro brown algae were collected from Mandapamcoastal region, in Gulf of Mannar, Tamilnadu, Rameshwaram South India on low tide during December 2014 and brought to the laboratory in polythene bags and washed several times with fresh water to remove sand, mud and attached fauna. The sample was cleaned using brush for the removal of the epiphytes with distilled water. The washed sample was shade dried in shade for one week and homogenized to fine powder.

Preparation of extracts:

Powdered algal sample (500g) was taken and extracted successively with different solvents such as ethanol and water using soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to get their corresponding residues.

Phytochemical analysis

Phytochemical analysis aims to reveal the presence of active compounds in the sea weed sample using several chemical protocols.

Antimicrobial Activity

Estimation of the antimicrobial potential is most important in finding the therapeutic value of the sea weed. Using the Agar well diffusion method the antimicrobial activity was carried out against the gram positive organisms such as *Staphylococcus sp.* and gram negative organisms such as *Escherichia sp. , Pseudomonas sp. , Vibrio sp. and Klebsiella sp.* Muller Hinton agar was prepared, sterilized and wells of 5mm diameter were cut on the agar. The extracts of *Padinagymnospora*was then dispensed into the wells in various concentration of 1.0 mg/ml and 0.05 mg/ml.. The plates were then incubated at 37°C for 24 hrs in an incubator.

Hydrogen Peroxide Inhibition Assay:-

Hydrogen peroxide radical inhibition assay could be used to estimate the anti-oxidant potential of the desired sample. This assay was measured according to the method of Govindarajan*et al*. Extract was rapidly mixed with 2ml of 10mM phosphate buffer (0.1M, pH 7.4) and hydrogen peroxide solution. The absorbance was measured at 550 nm in the UV spectrophotometer incubation for 10 min at 37^{o} C against a blank without Hydrogen peroxide. The antioxidant activity could be expressed as Gallic acid equivalents (GAE/g).

Percentage (%) of Antioxidant Activity = (A0-A1)/A0 * 100

A0 = Absorbance of the Blank Solution

A1 = Absorbance of the Solution added with the extract

FT-IR Analysis:-

FT-IR - 8400 S, SHIMADZU model Fourier Transmission Infra- Red Spectroscopy was used for the analysis of the solvent extracted potential seaweed sample. The spectrum was taken in the mid IR region of 400 – 4000 per centimeter. The spectrum was recorded using ATR (Attenuated Total Reflectance) technique. The sample was directly placed in the Potassium bromide crystal and the spectrum was recorded in the transmittance mode.

Statistical Analysis:-

Student t test was performed for the variables obtained from the aqueous and ethanol seaweed extracts of *Padinagymnospora* in the Hydrogen Peroxide Inhibition Assay.

RESULT AND DISCUSSION

Marine organisms are a rich source of structurally novel and biologically active metabolites like laminarin, fucoxanthin, fucoidan etc. These bioactive compounds from the sea are very attractive. Secondary or primary metabolites produced by these organisms may be of importance in pharmaceutical industry. Phytochemical analysis of *Padinagymnospora* reveals the presence of phytoactive components such as glycosides, phenolics, flavanoids, terpenoids, steroids and coumarins in their ethanol extracts, while the phenolic compound were not observed on the aqueous extracts of the seaweeds, but it contains the compounds such as resins, steroids etc.

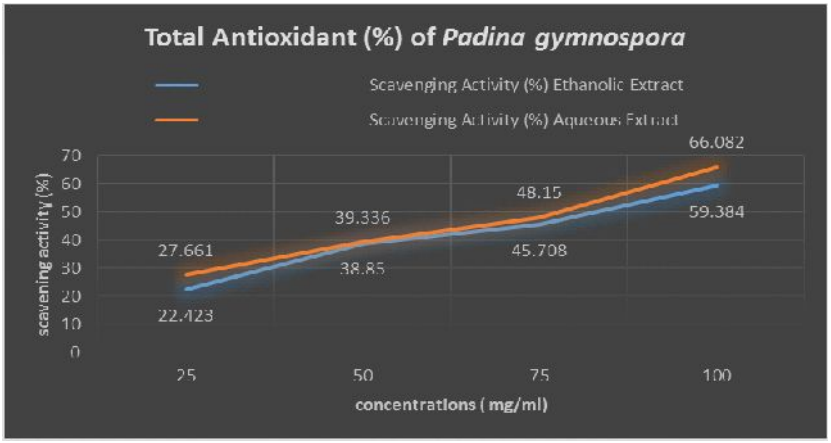
Ethanol extract of *Padinagymnospora* are shown to have potential inhibitory action against the bacterial culture isolates such as *Pseudomonas spp, Salmonella spp, Staphylococcus spp, Escherichia spp*and *Vibrio spp.* The zone of inhibition was found to be in uniform among the clinical isolates. The aqueous extract of*Padinagymnospora* doesn't show any inhibitory activity against the clinical isolates. The active components such as

flavanoids, terpenoids, steroids and phenolics present in the ethanol extract of *Padinagymnospora* rendered an effective inhibitory action on the Gram positive and Gram negative cell wall of the bacterial culture isolates.

Table 1: Antimicrobial Activity of Aqueous and Ethanol Extracts of *Padinagymnospora* against Clinical isolates.

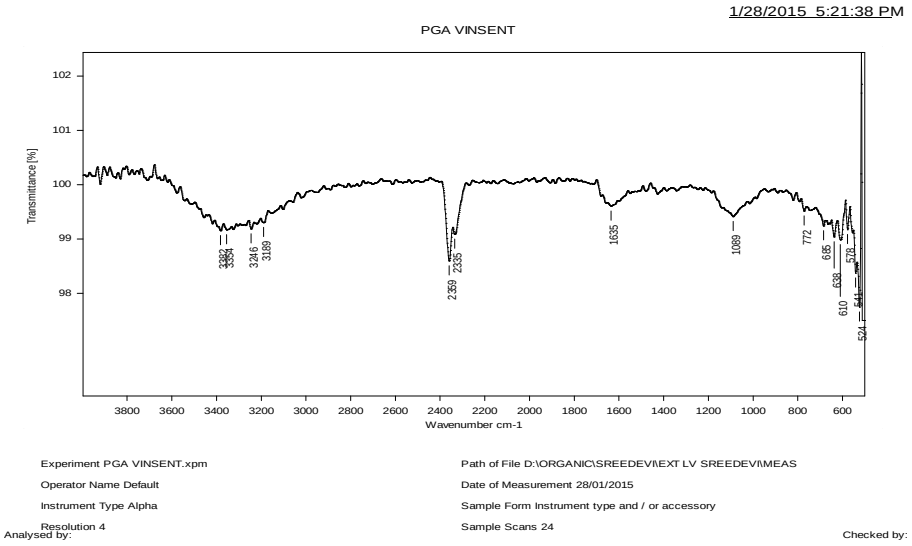
.No	Name of the Organism	Aqueous (0.1mg/ml)	Ethanol (0.1mg/ml)	Aqueous (0.05mg/ml)	Ethanol (0.05mg/ml)
	Pseudomonas sp.	-	10 mm	-	09 mm
	Salmonella sp.	-	09 mm	-	10 mm
	Staphylococcus sp.	-	10 mm	-	-
	Escherichia sp.	-	11 mm	-	10 mm
	Vibrio sp.	-	10 mm	-	10 mm

Fig 1: Total Antioxidant Activity (%) of *Padinagymnospora*



Hydrogen peroxide, a reactive non free radical compound, is very important as it can penetrate biological membranes. Although hydrogen peroxide itself isn't very reactive, it may convert into more reactive species such singlet oxygen and HO radicals. The phytochemical study has revealed the presence of polyphenolic compounds, flavanoids and terpenoids which are shown to be effective antioxidants. The total antioxidant activity of the aqueous and ethanol extracts of *Padinagymnospora*was found to be between 20-70%, with the maximum of 66.082% in aqueous extract of *Padinagymnospora* and with the minimum of 22.423% in ethanol extract of *Padinagymnospora.* It was observed that as the concentration of the sea weed extracts increases, the antioxidant activity also got increased.

Fig 3: FT-IR Profile for the Aqueous Extract of *Padina gymnospora*.



The FTIR spectrum of the ethanol and aqueous extract of *P.gymnospora* shows the band at the range of 3346cm-1 and 3348cm-1 which is assigned to the stretching vibration of primary and secondary amines and alcohols. The FTIR spectrum supports the presence of primary and secondary aromatic compounds and thus the presence of phenolic compounds.

Conclusion

The present study suggests that the seaweed*Padinagymnospora* might be used as potential antimicrobial and antioxidant agents due to the presence of various bioactive components. Selective isolation, characterization and in-depth research on the bioactive components of this unexplored sea weed may use as an effective therapeutic tool in future.

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ANTI INFLAMMATORY ACTIVITY OF *AVICENNIA MARINA* IN DEXTRAN SULFATE SODIUM INDUCED ULCERATIVE COLITIS IN WISTAR RAT

A.Mohamed Hanifa[1], M.Syed Ali[1], V.Anuradha[2], N.Yogananth[1], V.Saravanan[3]
[1]PG and Research Department of Biotechnology,
Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai – 119.
[2]PG and Research Department of Biochemistry,
Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai – 119.
[3]Department of Advanced Zoology and Biotechnology ,
The M.D.T.Hindu College, Pettai, Tiruneveli
* Corresponding author: syedmicro555@gmail.com.

Introduction:

The word "*Inflammation*" is derived from the Latin word "*inflammare*" (to burn).It is defined as the biological response of the immune system to an injury. It is characterized by redness (*rubor*), heat (*calor*), swelling (*tumour*), pain (*dolor*) and sometimes dysfunction of the organs are also involved (*functiolaesa*). The first four characteristics have been known since ancient times; *functiolaesa* was added to the definition of inflammation by Rudolf Virchow in 1858 and it became evident that if the injury persists, for longer period, it may leads to chronic inflammatory diseases.

Inflammatory processes are orchestrated by inflammatory cells through a complex set of chemical signals and can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic, allergic and/or autoimmune injury (Haraoui *et al*., 1991). It is one of the most important processes involved in the defence of an organism against local injury and infections. However, it often progresses to painful or chronically harmful diseases requiring pharmacological treatment, when the injury is not eliminated and the recovery of the inflammatory processes is hampered. Inflammation may be classified as acute or chronic. Acute inflammation is the immediate or early response to injury and is of short duration lasting for minutes, hours, or at most a few days. Chronic inflammation is of longer duration and may last from weeks to years (Kumar *et al.*1999).

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD), characterized by remitting and relapsing inflammation of the large intestine, resulting in symptoms such as weight loss, chronic diarrhea and bloody stool. UC and Crohn's disease (CD) are the two major subtypes of IBD. UC affects only the colon, and the inflammatory response is confined to the mucosa. CD may affect any part of the gastrointestinal (GI) tract, and it is not confined to the mucosa but affects the entire bowel wall. Although IBD is rarely lethal on its own, the disease is associated with increased morbidity and decreased quality of life among such patients.

Mangrove forest is economically and ecologically important. Unlike common terrestrial plants, they can withstand high salt concentration, can remain submerged in water, and maintain an efficient nutrient retention mechanism. Mangroves offer numerous benefits and are greatly important in maintaining the ecology of the coastal zones and the diversity of species. Mangroves and related species, are known to have many metabolites possessing antibacterial and antifungal, [Ravikumar *etal.,* 2010] antiviral, antidiarrhoeal, hepatoprotective, antifeedant , larvicidal [Syed Ali *et al.,* 2012] , cytotoxicity and antiplasmodial [Jacob *et al.,* 2010] properties. It is also reported that, mangrove is a folk

remedy for angina, diabetes, diarrhea, dysentery, hematuria and haemorrhage. Mangrove plants are well-known for their flavonoids, alkaloids, coumarins, polyphenols and are a rich source of tannins[Lin *et al.*,2005].

Considering the large number of mangrove plants, *Avicennia marina is the* most important of all and plays a vital role in the overall mangrove ecosystem.[Duke and Allen, 2006]. *Avicennia marina* commonly known as Asiatic mangrove, is widely distributed along the coastal tropical and subtropical region. This plant is well known for its medicinal properties. For centuries it has been used as a traditional medicine in the treatment of diarrhea, dysentery, blood in urine, fever, angina, diabetes, hematuria, and haemorrhage. Of the various plant parts of *Avicennia marina*, stilt root is well known for its free radical scavenging property. [Ravikumar *et al.*,2012]. It donates hydrogen atoms to the free radicals and convert them into stable products thus inhibits tissue damage. The present study made an attempt to investigate anti-inflammatory effect of *Avicennia marina* extract against Dextran Sulfate Sodium Induced Ulcerative Colitis in Wistar Rat

MATERIALS AND METHODS:

Collection of plant materials

Fresh stilt root samples of *Avicennia marina* was collected from Kovalam canal, Chennai the East coast of India.

2.Extraction

Stilt root samples of *Avicennia marina* were preferred for percolation by soaking in ethanol/water (3:1 v/v). This extract was further concentrated by using rotary flash evaporator (Superfit, INDIA) to get the fine residues and further lyophilised (BENCHTOP 2K) to remove the excess organic residues. DSS (mol wt 40 000; ICN) at a final concentration of 2 % (wt/vol) were prepared using drinking water. Dextran Sulfate Sodium fraction was prepared by cold maceration extract was dried in vacuum desiccators to obtained constant weight.

Animals

All the studies were conducted in compliance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA no. 971/bc/06/CPCSEA),Government of India and approved by the Institute of Animal Ethics commission All the studies were conducted as per the norms of the committee for the purpose of supervision of experiments on animals. Female wistar rats weighing 140-170g were purchased from Biogen Bangalore, India. All animals were housed 2/cage and kept in the animal house for one week for proper acclimatization before starting the experiment under controlled conditions of illumination (12 h light/12 h darkness) and temperature ranging 20-25°C. They were housed under the above laboratory conditions, maintained on standard pellet diet and water.

Colitis was induced by the method previously reported by Kitajima *et al*., (1999) with some modifications. After one-week quarantine, the rats were divided into 5 groups composed of 5 rats each. In the control group (Group 1), mice were given fresh water and micro fluidized pellets *ad libitum*. In the DSS group (Group 2), DSS (2%) in feeding water was given one week to induce colitis. In other three groups viz 3, 4, 5 E. *littorale*350 and 500 mg/kg was administered p.o and Group 6 was dose with Dexamethasone (1 mg/kg), which remained as standard control. In this experiment 2% DSS drinking water was given to all groups, except the control group. The body weight of each rat was recorded from day 1 to 7.

The myeloperoxidase activity in the colon was assessed by (Bradley *et al*., 1982). The colon tissue (50 mg) was minced Homogenized in 1 ml of 50mM phosphate-buffered saline (PBS) pH 6.0, containing 0.5% hexadecyltrimethyl ammonium bromide (HETAB). The homogenate was subjected to three cycles of freezing (-30^{o}C) and thawing (37^{o}C) with intermittent brief periods (15 s) of sonication and centrifuged at 12,000 X g for 15 min at 4^{o}C. Supernatant (0.1 ml) was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml O-dianisidinedihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 470nm was then measured within 5 minutes using a Beckman spectrophotometer (Beckman DU 640B). The MPO activity was calculated as...

$$\text{MPO} = \frac{X}{\text{wt of the piece of tissue (50mg) taken}}$$

$$\text{Whereas } X = \frac{10 \times \text{change in absorbance}}{\text{wt of the Volume of supernatant taken in the final reaction piece of tissue taken}}$$

Statistical analysis

The results are expressed as mean ± S.D from 5 rats per group. For statistical analysis, ANOVA followed by Dunnett test was used. A $P<0.05$ was considered statistically significant using graph prism version 5.0.

RESULTS:

DSS Induced Colitis Model in rats:

DSS treated rats displayed signs of moderate colitis. This was indicated by a decreased food intake results in body weight reduction (Table.1), shortening of the colon (Figure.2), increased colon weight (Table.2), and increased colon weight (Figure.1). MPO activity (Figure 3) compared to normal rats. *Avicennia marina* (500mg/kg) treated rats displayed a 40% increase in body weight gain compared to control group. *Avicennia marina* 350mg/kg and 500mg/kg treated rats also had an increase in body weight gain compared to control group were 35.43% and 41.64% respectively). Comparative therapy with Dexamethasone (1mg/kg) doesn't showed much increase in body weight significantly.

Table.1 Mean Body Weight of DSS Induced colitis in rats

ays	Normal		Vehicle		*A.marina* 350mg/kg		*A.marina* 500mg/kg		Dexa1mg/kg	
	Mean	SD	Mean	SD	Mean	SEM	Mean	SEM	Mean	SEM
	123.31	1.51	121.73	0.69	101.63	0.48	111.68	0.59	91.65	0.53
	121.33	1.51	131.28	0.68	102.36	0.51	120.11	0.70	95.44	0.46
	125.45	0.96	132.56	0.65	103.26	0.50	119.99	0.73	92.94	0.47
	128.32	0.92	129.10	0.74	106.32	0.56	123.12	0.83	94.15	0.72

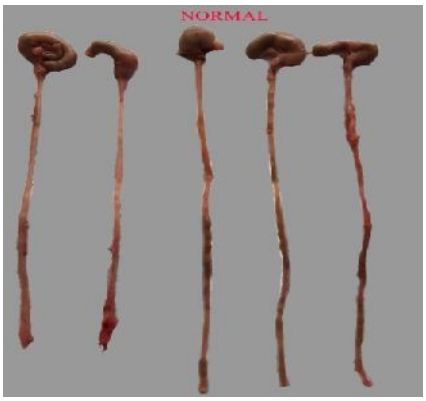
	132.01	0.98	127.15	0.77	107.99	0.39	124.85	0.80	95.96	0.59
	133.96	.96	124.52	0.42	110.21	0.47	125.99	0.88	99.33	0.87
	131.11	1.55	123.59	0.42	120.02	0.47	127.89	0.88	101.19	0.87

Table.2 Colon Weight (mg)

Groups	Mean	SD
Normal control	7.9	1.1
DSS control	132	2.4
Dexamethasone 1mg/kg	82	2.3
Avicennia marina 350mg/kg	111	1.5
Avicennia marina 500 mg/kg	99	0.9

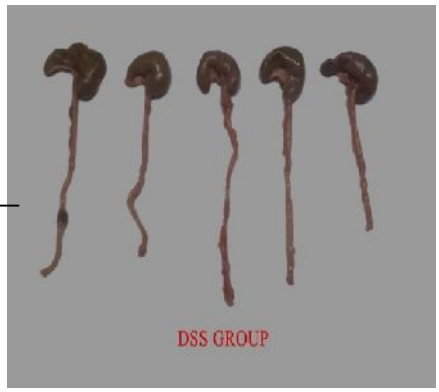
PLATE-1

MORPHOLOGY OF RAT COLON



Normal colon

DSS



Dexamethasone

The DSS-induced model of colitis is associated with a significant decrease in colon length (Rumi *et al*., 2004). Increase in colon length in *Avicennia marina* group dosed at 350mg/kg and 500mg/kg was 10.73, 27.85 % and dexamethasone at 1mg/kg was 46.49% when compared to control group. *Avicennia marina* at 350 and 500mg/kg showed 33% and 49% reduction in fractional colon weight, whereas dexamethasone showed 54.46% compared to control (Table.3). When colon weight was expressed as a proportion of colon length, *Avicennia marina* administration resulted in a significantly lower colon weight compared to dexamethasone administration in DSS treated rat.

The MPO levels (Table.4) in normal animals were 0.2µg units/gm of tissue. Tissue MPO activity in the DSS treated animals was 706.74µg units/gm of wet colon. *Avicennia marina* at 350 and 500mg/kg significantly decreased the MPO levels to 328.91and 364.41 µg units/gm, which shows 46.90%& 56.09%. Dexamethasone significantly inhibited the MPO activity of 85.75%. Which was significantly lower than DSS treated animals.

Table.4 Effect of embelin in Myeloperoxidase assay (µg/mL)

Groups	Mean	SD	% Inhibition
Normal	0	0	0.00
Vehicle	706.74	32.06	0.00
Avicenni marina 350mg/kg	328.41	28.28	46.90
Avicenni marina 500mg/kg	364.26	25.48	56.09
Dexamethasone1mg/kg	118.95	21.53	85.75

DISCUSSION:

The aim of the study was to establish an inexpensive model of experimental colitis resembling UC in wistar rats. Although several models of experimental colitis have been reported previously, none of these showed the optimum characteristics. In recent years, some kinds of knockout (KO) mice have been reported (Hibi *et al*., 2002). Unfortunately, these strains of mice are not widely available, thus limiting their usefulness. The most widely used models are induced by administering toxic chemical such as TNBS or DSS.

The DSS-induced colitis results in inflammation mainly in the distal colonic mucosa and the histopathology of this model showed some characteristics resembling UC. But this colitis can only be induced by administration of DSS at a high concentration for several days (Faure et al., 2003; Gaudio et al., 1999). Thus the amount of DSS used for rats will be very large, which limited its wide use in many regions because DSS is very expensive.

In this study we showed that intracolonic administration of the “barrier breaker” after 5% DSS treatment resulted in a rapid development of severe ulceration and inflammation of the distal part of rat colon. These features are similar to what happen in human UC where the major symptoms include diarrhoea, rectal bleeding and weight loss. Macroscopic finding showed the damage was characterized by marked ulceration and haemorrhage and that the diseased site was limited to the distal colon. Moreover, dysplasia was commonly found in DSS group. The most important clinical issue in the management of patients with IBD is an increased risk for development of dysplasia and neoplasia. Taken together, these features

indicate that DSS induced colitis and human UC shares many similar clinical and morphological aspects.

DSS can induce reproducible acute colitis in rodents when given at a concentration of 5% for more than 6 d (Faure *et al*., 2003; (Gaudio *et al*., 1999; Okayasu *et al*., 1990). The exact mechanism by which DSS causes inflammation is not fully elucidated. It appears that the potential roles of DSS in induction of colitis may be: (a) direct cytotoxicity; (b) interference with the normal interaction between intestinal lymphocytes and epithelial cells (Ni *et al*.,1996); (c)

DSS causes a change in the intestinal microflora, and particularly an increase in the number of Gram-negative anaerobes (Okayasu *et al*., 1990). Administration of DSS also activates the immune response (Ni *et al*., 1996; Vicario *et al*., 2005) and stimulates chemokine production by epithelial cells (Ohtsuka and Sanderson, 2003). DSS at a lower concentration upregulates cytokines, although it does not cause bloody diarrhea and ulceration in colon (Egger *et al*., 2000; Vicario *et al*.,2005). We also found aggregated lymphocytes in the colonic mucosa after the administration of DSS for 3 d. This fact indicates the administration of lower concentration of DSS may also activate the immune response.

In reviews, it was found that 30% ethanol cannot cause any change of stool in SD rats. However, it does destroy the intestinal epithelium, which is considered to be a part of the innate immune system. The intestinal epithelium forms a tight, highly selective barrier between the body and the intra luminal microenvironment. It plays an active role in the maintenance of mucosal homeostasis (Yu et al., 2004). Failure of this barrier may result in intestinal inflammation, most likely through exposuretofecal antigens (Bamias et al., 2005).

The administration of 5% DSS for 3 d could only cause loose stool in rats and no obvious macroscopic damage was observed at any time. Thirty percent ethanol treatment induces acute injury that could be observed at 24 h and 3 d, but with no obvious change at 1 week. For rats treated with DSS, the mucosal injury was still present at 1 week. Our data confirmed that the DSS produced a more severely acute injury in the distal colon than that induced by methanol. Therefore, it seems the activated immune response after administration of DSS and the disruption of the superficial epithelium are essential to enable the consequent induction of a more severe inflammatory reaction. Colitis may be a result from a dysregulated response of the mucosal immune system toward intra luminal antigens of bacterial origin (Duchmann *et al*.,1995; Lu *et al*., 2003). Several advantages of this model make it a useful one for the study of the pathophysiology and therapy of UC, especially in developing countries. First, the animal used is the wistar rat, which is inexpensive and widely available. Second, the concentration of DSS is 5% and the duration of administration is 2 d, thus the model is relatively inexpensive. Third, this model is relevant in that it has several features of human UC. Finally, the inflammation is easy to be induced and very reproducible.

Avicennia marina extract was found to be anti-inflammatory compound, but an novel approach was made to test this extract using this DSS induced colitis model. The plant extract found to be active against colitis and showed similar activity to dexamethasone. This indicates that this plant can be used against anti-ulcerative model. It is inferred from the present study *Avicennia marina* extract was proved to work against colitis. Even though, it proved similar activity to dexamethasone but not equivalent. The activity of this plant extract can be enhanced to encapsulating with nanoparticles and also in chronic studies this plant extract may show more activity without side effects in comparison to dexamethasone.

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COASTAL VEGETATION OF POINT CALIMERE WILDLIFE AND BIRD SANCTUARY, TAMIL NADU

M. Padma Sorna Subramanian[1] A. Saravana Ganthi[2] & K. Subramonian
[1] Survey of Medicinal Plants Unit (S), CCRAS, Salam, Tamil Nadu, India
[2] Department of Botany, Rani Anna Govt. College for Women,
Tirunelveli, Tamil Nadu. India
[3] Department of Plant Biology and Biotechnology,
The MDT Hindu College, Tirunelveli, Tamil Nadu. India
E-Mail: saran_gan@rediffmail.com

Introduction

Maintenance, management and sustainable utilization of plant coverage have required scientific recognition. The information resulted from vegetation can be useful to solve the ecological problems such as biological conservation and natural resources management and the future of an ecosystem trends can be forecasted using those information. In other words, vegetation can be useful to exhibit some ecological factors, which might be hard to measure directly (Daubenmire, 1976). The indigenous vegetation of an area is the direct expression of the physical environment that has been influenced by geological history. The vegetation is adapted to the long-term rainfall patterns, to the soils, to the temperature regime. The constituent species represent the families and genera that have had access to the area over the geological epochs. Heydari and Mahdavi (2009) studied biodiversity of plant species in related to physiographic factors (aspect, elevation above sea level and slope percentage) in Melah Gavan area in Ilam province of Iran and inferred that seasons have great influence on soil characteristics and species diversity. An increase in species diversity was observed during. India has 7,500 km of coastline under 53 coastal districts of 10 maritime states and six union territories. Point Calimere (10 18" N, 79 51"E) the Calligicum of Ptolemy is a low promontory on the Coromandal coast of Tamil Nadu jutting out into the Bay of Bengal (Map: 1). Perusal of literature revealed that no intensive and systematic floristic investigation with all its climatic characteristics has been carried out in Point Calimere, which has the unique vegetation of coastal, halophyte and tropical dry evergreen forest. Thus the entire vegetation has remained with very little enumerative studies and hence in this study an attempt has been made to highlight the distribution of vegetation types in Point Calimere.

Area of Study

The area of study Point Calimere falls under the Nagapattinam district and it was under the erstwhile district of Tanjore of Tamil Nadu before bifurcation. Point Calimere (Kalli-medu in Tamil) is also called as Cape Calimere and Kodikkarai. It is the apex of the Cauvery river delta, and marks a nearly a right-angle turn in the coastline. In 1988, the sanctuary was enlarged to include the Great Vedaranyam Swamp and the Talaignayar Reserve Forest, and renamed the Point Calimere Wildlife and Bird Sanctuary, with a total area of 377 km². Point Calimere, at a sea level above MSL in the eastern side ending with the sea gradually raises in the west up to 25 mts in Ramarpadam located in the high sand dunes.

Methodology

The data were gathered from October 2005 – September 2008 with follow up visits to all sites to record seasonal changes in the communities. The vegetation of different parts of the area of study was thoroughly explored by repeated visits during different seasons of

the year, covering all ecological habitats represented in the area. During the visits, different forest types and microhabitats were identified.

Results and discussion

Vegetation in contrast to flora identifies the distinct assemblage of plant species in a given area.Vegetation of the area of study can be classified into sea-shore vegetation, aquatic vegetation,dry evergreen vegetation and mangrove vegetation (Plate:

1). **Seashore and saline vegetation**

As the Point Calimere forest is surrounded on the East and South by the sea and on the north and west by the extensive salt swamps the littoral vegetation is marked. *Spinifix* along with *Ipomoea, Launaea, Pandanus* etc., serve as sand binders or soil binders at several places. *Pandanus* serves as sand binder and wind breaker. *Spinifix littoreus* is predominant on sandy seashores. Sand dunes observed adjoining the sea and inhabited by *Pandanus fasicularis, Prosopis chilensis, Tribulus terrestris, Solanum surattense, Spinifix littoreus* and *Ipomoea pes-caprae.*

The next zone consists of *Phyla nudiflora, Enicostema axillare* and *Gisekia pharnaceoides* etc. Interior to this zone, where sand mat expands, is covered by a thin layer of brine during high tide. The vegetation comprises *Aleuropus lagopoides, Suaeda, Salicornia, Arthrocneum* etc. During the maximum rainfall season of October – December *Peplidium* occurs over extensive areas indicating the lowering of salt content in the water. *Astercantha* occurs alongside as a common plant during this period. Untawale and Nair reported that the sand dune flora of India comprised of 63 species and *Ipomea pescaprae, Spinifix squarrosus, S. littoreus, Vitex negundo, Launea pinnatifida, Anacardium occidentale, Pandanus* and *Opuntia* spp. dominate the vegetation.

Nearer to the two villages, *Prosopis* an introduced plant is progressively encircling patches of natural vegetation and strangles them extending inwards. This acts very well as soil binder. Further inside the vegetation comprises of *Manilkara hexandra, Canthium diccocum, Carissa carandus, Canavalia virosa, Cyphostema setosum, Cassis sps, lablab purpureus, Ixora parviflora, Memecylon umbellatum, Sapindus emarginata* etc. *Ochna obtusata, Capparis oppositifolia, Salacia chinensis, Mucuna pruriens* and *Walsura piscidia* are the typical flora of this area. The following herbaceous plants are found very common in this area; *Tinospora cordifolia, Asystasia gangetic*a, *Rivea hypocrateriformis, Asparagus racemosus, Crotalaria striata, Indigofera aspalathoides* and others. *Viscum* is a parasite on *Excoecaria agallocha* and *Loranthus falcatus* on tree species like *Azhadirachta, Sapindus* etc.

On alluvial halomorphic soil, the forest is not continuous but intersected by numerous tidal inlets and creeks. *Salvadora persica* is the predominant tree. *Manilkara hexandra* is the principal tree on the dune is slowly disappearing on poorly drained, salty terrain. The thicket then presents a more regular appearance, less high, but the natural openings are many wherever there is saline efflorescence.

Aquatic vegetation

In the fresh water pools of the rainy season, aquatic ephemerals such as *Ludwigia Lindernia, Bacopa, Limnophila, Marsilea* etc., come up. The ridges are predominant with *Salacia chinensis* and *Mucuna pruriens* which are unique to this area. The usually low-lying salt marshy habitat is generally inhabited by *Salicornia, Sporobolus, Suaeda* and *Arthrocnemon*. Emergent species include *Limnophilla* and *Typha*. Floating-leaved

hydrophytes are *Aponogeton natans, Ipomoea aquatica, Nymphaea pubescens* and *Nelumbo nucifera*. Submerged species are *Vallisneria natans* and *Ottleia alismoides*.

The Tropical Dry Evergreen Vegetation

The area consists of stunted, almost exclusively formed of bushed much branched plant species measuring 2 to 4 m in height. Here and there emerge some evergreen arborescent shrubs under 10 m in height with dark green voluminous crowns. High thorny evergreen thickets are found on the sand dunes. The continuous and principal stratum is thorny with a slight numerical dominance of *Zizyphus, Catuneragam, Maytenus.* Other non-thorny ligneous species are also common but their density varies from place to place. This is in the case with *Memecylon, Sapindus, Premna, Ixora, Canthium.* Therefore, in the thicket, the majority of the species are non-thorny though the number of the individual thorny plants appears to be higher than the others. They make the entry into the thicket particularly difficult especially in the presence of the invading spinescent climbing shrubs like *Toddalia asiatica.* Generally, the lianas grow well even in the interior of the thickets but relatively few species. The herbaceous and ground cover is exclusively poor.

Being accessible, this vegetation is often traversed by man and openings are made in places. Also, some anthropic stages appear here and there with anthropochore trees like *Syzygium cumini, Borassus flabellifer, Lannea coromandelica* and *Manilkara hexandra*.

This is the most productive vegetation of the forest in terms of biomass as well as utility. Dendroid forms of *Manilkara, Calophyllum, Walsura, Syzygium, Dryptes* and climbers or stragglers like *Mucuna, Canavalia, Lablab, Salacia, Cissus, Toddalia, Tinospora* occur here are valuable as food and medicine.

At the borders, roads and pathways, some unarmed species like *Vitex negundo, Clerodendron inerme, Cassia auriculata* and others are common. *Cassytha, Loranthus* and *Viscum* are the parasites. *Vanda* is the only orchid, fairly common in these forests as it could thrive in the warm humid atmospheres.

Mangrove vegetation

Mangroves are salt-tolerant species of divergent groups. They play vital role in land-stabilizing and building, preventing soil erosion in coastal zones and are a source of nutrients for the inhabitants therein besides being a supplier of a variety of forest produce for human use. The presence of luxurious mangrove vegetation in the locality called Muniappan Lake on the western side of the road and vertises of such patches in several other places suggests that the once exuberant mangrove formations are progressively dwindling and disappearing due to human interference in the form of salt pars.

Dynamism of the vegetation

The thickets are often invaded by the human population to get their requirement of fuel wood. On the dunes, the equilibrium of the woody species is precarious. There are hardly any seedlings of these species observed colonizing the clear areas. As their growth is apparently very low, special attention should be paid to the protection of the vegetation.

Practically all the thorny shrubs of the thickets may be considered as the pioneers. Under their light shade and protection and due to the thin superficial humus layer some seedlings have developed. These shrubs, if not cut, may give rise to the discontinuous dominant stratum of the formation.

On the halomorphic soils of the tidal inlets and creeks, almost a continuous herbaceous cover precedes the woody species. There appears quick growing species like *Prosopis chilensis, Clerodendrum inerme, Salvadora persica, Excoecaria agallocha* are capable of forming a thick bush within 5 – 6 years. Between the clumps of shrubs develop a low dense grassy carpet of *Poaceae, Cyperaceae* and *Scrophulariaceae* on the halomorphic soils. It is grazed by the cattle and represents a sort of equilibrium between dynamism of shrubs on the one hand and the biotic factors on the other.

This pasture is likely to be immersed under a thin layer of brackish water during the strong tides of October – November. *Fimbristylis* and *Kyllinga* are the dominant species. Another *Cyperaceae* member *Cyperus arenarius* is also locally very abundant. *Poaceae* species like *Chloris barbata, Chrysopogon fulvus, Eragrostis japonica* and *Oplismenus composites* also observed along with the *Cyperaceae.*

The remaining flora is essentially comprised of *Phyla nodiflora, Portulaca oleracea, Leucas aspera, Oldenlandia umbellata, Evolvulus alsinoides, Hybanthus enneaspermum, Tribulus terrestris, Boerhaavia diffusa, Phyllanthus amarus* and *Spermacoce hispida*.

Although the bushy species are evergreen, some may be partially defoliated for some days per year. The arborescent species are evergreen (*Manilkara)* or deciduous for a very short time (*Diospyros ferrea)* or yet distinctly deciduous (*Albizia, Vitex*). The number of individuals of deciduous species is quite low compared to that of the evergreens. Tobacco is cultivated at several places. Leaves of *Manilkara hexandra* used as manure for tobacco fields.

Across the study site, the highest value of plant diversity and richness. The reason can be the arid environment which results in the lower tree cover diversity and thus increase the light level in the forest floor. The plant diversity is related to the edaphically heterogeneity in the semi-arid environment in USA (Moustafa and Zayed, 1996). So that, the more rugged area would have more biodiversity that the other areas. The effects of environmental factors on vegetation established on alluvial plains of Sina desret stated that the species richness is different toward humidity difference (Sabestia, 2004). Also, humidity change is a mixture of changes related to altitude, slope, climatic drought and texture and nature of top soil, so that plant species diversity is higher in drier aspects than another aspect. The soil fertility is the main environmental factor in vegetation establishment (Shmida and Wilson, 1985). This paper concludes that a proper management from human disturbance and scientific management of medicinal plants of the forest area may lead a rich biodiversity site in India.

Map 1: Land cover map showing various sites in Point Calimere

Source: District Forest Office, Nagapattinam

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STUDIES ON PHYTORESOURCES OF SAND DUNE VEGETATION IN COASTAL ZONES OF KANYAKUMARI DISTRICT TAMIL NADU

Padma Sorna Subramanian M[*], Ramarajan Sekar, Kanakarajan A. and Saravana Gandhi A[**]
*Siddha Medicinal Plants Garden, CCRS, Mettur Dam, Tamil Nadu, India.
SCRU, Central Council for Research in Siddha, Palayamkottai, Tamil Nadu, India.
**Department of Botany, Rani Anna Govt. College for Women, Tirunelveli – 627 008
E-mail: sramabio@gmail.com

Introduction

Coastal sand dunes are the natural ecosystem which protect the coastal environment by absorbing energy from wind, tide and wave action (Corre Jean-Jacqes, 1991). These species are playing a vital role in protecting soil erosion and flooding (Desai, 2000). Dune restoration is primarily required when natural dunes have been significantly modified or damaged by human activities. In the rare absence of human damage, most natural fore dunes in Kanyakumari are self-maintaining. Human modification of coastal dunes is common worldwide (Nordstrom, 1994). A variety of exotic species are now widely established in dune systems (Partridge, 1992 and Johnson, 1992). In rural India around 200 million people are dependent on forest plant resources (Khare et al., 2000)

The coastal length of India is 7500km with many lagoons, beaches, estuaries and mangrove swamps, supporting rich biotic and abiotic organisms (Anonymous 1987). Tamil Nadu has an extensive coastline of nearby 1,076 Kms and 3 marine protected areas covering more then 1300 sq. kms are present on the Coramandel coast of Tamil Nadu. With respect to geographic location and physical distinctiveness, the coast of Kanyakumari District is part of the Gulf of Mannar Biosphere Reserve (8°21'N and long. between 77° 26' and 77° 30' E).

There are different types of vegetation on the coast of Kanyakumari, this includes mangroves and their associates scrub jungles, aquatic vegetation and coastal sand dune. The loss of biodiversity is a worldwide concern in today's perspective and more often it is accompanied with ecosystem destruction and degradation. Although much effort has been put into restoring and preserving tropical ecosystem such as rainforests, mangrove swamps and coral reefs, preservation of tropical, costal sandy plant communities has been largely ignored (Condit, 1995, Mulkey et al., 1996, Sheil and May, 1996, Kennish, 2001).

The most noticeable threat to the Kanyakumari coastal dune open area is a huge invasion of trees like *Prosobis juliflora, Parthemium sp.* and *Lantana camera*. In order to mitigate the current decline of medicinal plant biodiversity, it is necessary to understand the ecological processes in coastal dunes and a critical step in such an understanding is the establishment of a detailed description of medicinal species diversity at different coastal dune habitats. The current deforestation scenario which threatens the existence of medicinal plants encourages for conservation of plants in coastal area. Therefore, an attempt has been taken to document the medicinal plants and their indigenous knowledge prior to its extinction.

Methodology

Study Area

Coastal length of Kanyakumari district along Arabian Sea is 59 km and along Bay of Bengal is 11 km. Kanyakumari District, having a coastline of about 67.59 km, is situated on the southern extremity of the Indian peninsula (lat.between 8°21'N and long. between 77° 26' and 77° 30' E) from Leepuram in the east coast to Neerodi in the west (Fig. 1).

There are three important river line ecosystems, which confluence with Arabian Sea in Kanyakumari

- Thengapattinam estuary, formed by the confluence of river Tampirabarani in between Thengapattinam and Eraiummanthurai.
- Valliyar estuary formed by the river Valloiyar near Kadiapattinam.
- Manakudy estuary formed by the confluence of river Pazhayar in between East and West Manakudy villages.

Another two minor estuaries also: they are

- Pambar estuary near Colachel and
- Pantri estuary near Rajakkamangalam

Pattern of Survey

The plant samples were randomly collected 10×10m Quadrate method were followed.

Medicinal Plants Survey

Medicinal plants were collected during June 2015 to December 2016 through field survey in different remote villages of coastal districts. During the period of study, door to door visits were made to identify local people with specialized knowledge on use of medicinal plants. Plants were collected with noting their local names, parts used and ethno medicinal uses.

Identification and Preservation of Specimen

The plant species Flora of Gulf on Mannar and Presidency of Madras

Results and Discussion

A total of 34 species belonging to 21 families were recorded. Plants of Fabaceae (4 species) was largely represented followed by Euphorbiaceae, Verbanaceae, Malvaceae, Ceasalpineaceae (3 sp). The remaining families had only one species each. The present study provides information about some beneficial uses of 34 plant species. The plants are either used individually or in mixture with some other plants or plant parts. Some plant species are claimed to be quite effective remedies for diarrhea, febrifuge, malaria, cough, cold, leukemia and stomach troubles etc. The dominant index of plant species showed herbs, followed by shrubs and trees, climber. Various plant parts namely flowers, bark, roots, leaves and root stem, whole plants and leaves were used for the treatments of diseases such as skin, bones,

bronchitis, ears, eyes, headache, kidney, intestine, liver, lungs, muscles, teeth, throat, etc. Maximum species were used for cold & cough, followed by stomach ache, skin diseases. The same pattern of trees, herb, shrub and climber are used for various ailments (Sharma and Samant, 2014)

Floristic Study

The floristic composition of these tropical communities was dominated by some of the larger plant families including Poaceae was the most common and dominant family followed by Leguminosae, Cyperaceae, Euphorbiaceae, Caesalpiniaceae, Amaranthaceae, Compositae, Lamiaceae, Malvaceae, Molluginaceae, Mimosaceae, Nyctaginaceae, Sollanaceae, Asclepiadeaceae, Cucurbitaceae and Liliaceae etc.. Although species composition at various sites on the coastal zone of Kanyakumari, there were similarities among species encountered at sites within this regions.

Perennial herbaceous plants dominated growth forms in these sandy communities. Low lying herbs such as *Leucas aspera* and *Andrographis paniculata* were less frequently encountered. Two growth forms commonly encountered among the sandy plant communities were succulent perennials (*Ipomoea pes-caprae, Sesuvium portulacastrum*) and sclerophylous grasses (*Sporobolus sp.*). Annual plants, *Boerhavia diffusa* and *Hemidesmus indicas*, encountered at sites examined where not as numerous, with the communities dominated by perennial species such as *Panicum repens* and *Spinifex littoreus* which are in the table 2.

Indian coastal area consist of 154 species belonging to 108 genera and 41families

(Rao and Sherieff, 2002). Musila et al (2001). Several authors have reported that various parts of the world, many dune ecosystems support high plant richness and diversity values.

Medicinal Floristic Study

This information was checked with available literatures (Chopra *et al*., 1982; Jain, 1995; Kirtikar and Basu 1980 and Pal, 1980).

The total of 402 species of medicinal plants belonging to 98 families and 266 genera were reported and used by the inhabitants of the area for curing various ailments. These medicinal plants comprise of 34 trees, 301 herbs, 61 shrubs, and climbers and ferns, 03 species (Sharma and Samant, 2014). These domestic substances are probably to increase the efficacy of the drug. Since the uses are based on empirical knowledge, the scientific study of all these herbal drugs is highly desirable to establish their efficacy for safe use. Similar ethanobotanical studies have been reported in surrounding are (Ramarajan and Murugesan, 2012, Ramarajan and Muthukumarasamy, 2012 and Ramarajan *et al*, 2012). A total of 157 species of plants belonging to 58 families were recorded (Patel, 2012).

Various plants parts used as a traditional namely bark and flowers 5%, root 10%, leaves and root 11%, whole plant 16%, stem 21% and leaves 32% in figure 3. Among the parts used lesf105 species, followed by root 87 species, flowers36 species and seed 33species (Man and Samant, 2011).

Management

Coastal areas represent a fragile ecosystem; its stability is an important pre-condition for the environmental sustainability of the marine and inland life. Coastal ecosystem in the degradation and pollution have both local and distant inland source. Proper management of natural resource is necessary for its best utilization. As such, grazing should be limited by

marking areas left for grazing and other should be kept strict surveillance or a barbed wire used for demarcation of the areas. Census of wild life is also important since it is dependent on the vegetation of the area. Before any number of livestock is allowed to grazing in a particular area the possible number of wild life should also to be taken into consideration. Monitoring of the economic plants of the area is needed. This plant should be allowed to be cut in such a manner that they do not jeopardize the existing bad situation.

Ecological research

Research on stabilization and protection, dynamics of sand dune including other perspectives as well as documentation of flora and fauna urgently needed.

Implementation of law and legislation properly

The coastal regulation zone notified in 1991 issued under the Environment Protection Act, 1986. But unfortunately the implementation is very poor by the pressure of industrial and development lobbies. Development is also required to enhance the concept of eco-tourism but the dune vegetation should be undisturbed as it is the natural guard wall at sea shore.

Conclusion

The sand dune species of costal Kanyakumari are extremely important resources, which play a vital role in the economic and social life of nearby people. Conservation and judicious utilization of the costal plant wealth is important because they have become threatened by over exploitation, clearing of forest for industrialization, rapid urbanization, human settlements, etc.

The inventory of 34 medicinal plant species as used by the costal people throws some light on the economic and medicinal importance of these species, Hence, there is a need for detailed investigations of ethno- botanical knowledge held by these local villagers before such valuable knowledge is lost forever. A rational and sustainable method of utilization can help improving the life of the local people while maintaining ecological balance of costal habitats.

Table 1: List of medicinal plants in coastal zones of Kanyakumari district

Sl. No	Plant name	Family	Tamil Name	Habit
.	*Acalypha indica* L.	Euphorbiaceae	Kuppaimeni	Herb
.	*Achyranthus aspera* L.	Amaranthaceae	Nayuruvi	Herb
.	*Alternanthera sessilis* R.Br.	Amaranthaceae	Ponnanganni	Herb
.	*Argemone mexicana* L.	Papaveraceae	Prematha nd	Herb
.	*Asparagus racemosus* Willd.	Liliaceae	Tannirvitta n	Climber
.	*Borassus flabellifer* L.	Palmaceae	Panaimaram	Tree

.	*Calophyllum inophyllum* L.	Casurinaceae	Punnai		Tree
.	*Calotropis gigantea*(L.)R.Br. ex.Ait.	Asclepiadaceae	Erukku		Shrub
.	*Cassia occidentalis* L.	Caealpiniaceae	Peravirai		Herb
0.	*Cassia tora* L.	Caealpiniaceae	Usittahara i		Herb
1.	*Cissus quadraangularis* L.	Vitaceae	Pirandai	er	climb
2.	*Clerodendrum inerme* (L.) Gaertn.	Verbena ceae	Peechalat hi		Shrub
3.	*Crotalaria retusa* L.	Fabacea e	Sannappu		Shrub
4.	*Crotalaria verrucoasa* L.	Fabacea e	Kilukilupp ai		Herb
5.	*Cyanodon dactylon* (L) Pers.	Poaceae	Arugampu l		Herb
6.	*Euphorbia hirta* L.	Euphorb iaceae	Amampatc haiarisi		Herb
7.	*Evolvulus alsinoides* L.,	Convolv ulaceae	Vishnukra nthi		Herb
8.	*Hemidesmus indicus* L. R.Br.	Periploc aceae	Nannari	er	Climb
19.	*Hibiscus tiliaceus* L.	Malvace ae	Neerparut hi		Shrub
0.	*Ipomoea pes-caprae*(L.) *Sweet*	Convolvulaceae	Atappan kolai	er	Climb
1.	*Jatropha gossypifolia* L.	Euphorbiaceae	Kattamanakku		Shrub
2.	*Launaea sermentosa* (Willd.)	Asteraceae	Eluthanippondu		Herb
3.	*Leucas aspera* (Willd.) Link.	Lamiaceae	umbai		Herb
4.	*Mimosa pudica* L.	Mimosaccae	Thottal sinungi		Herb
5.	*Phyla nodiflora*(L) Greene.	Verbenaceae	Koduppai		Herb
	Physalis minima L.	Solanaceae	Tottakkali		Herb

6.				
7.	*Salvadora persica* L.	Salvadoraceae	Vasamaram	Tree
8.	*Sesbania bispinosa* (Jacq.)W.F Wight.	Fabaceae	mutcempai, uravi	Shrub
9.	*Sida cordifolia* L.	Malvaceae	Palampasi	Herb
0.	*Tamarindus indica* L.	Caesalpiniaceae	Puli	Tree
1.	*Tephrosia purpurea* (L.) Pers.	Fabaceae	Kolinchi	Herb
2.	*Thespesia populnea*(L.) Soland ex Corr.	Malvaceae	Poovarasu	Tree
3.	*Vitex negundo* L.	Verbenaceae	Nochi	Shrub
4.	*Ziziphus mauritiana* Lam	Rhamnaceae	Ilanthai	Shrub

Table 2: List of potential sand binder plant species.

Plant Name	Family
Bulbostylis barbata Roth.	Cyperaceae
Ipomoea pes-caprae (L.) R.Br.	Convolvulaceae
Launaea sermentosa (Willd.) Schult-Bip.ex O.Kuntze	Asteraceae
Panicum repens L.	Poaceae
Spinifex littoreus (Burm.f.) Merr.	Poaceae

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STUDIES ON GROWTH RESPONSE AND BIOCHEMICAL CHANGES IN ROCK LOBSTER *PANULIRUS HOMARUS* FED WITH DIFFERENT DIETARY SOURCES

Suganya A.M[1]., NavinChandran M[2]., Lingeswari S[1]., Palavesam A[2]. and Immanuel G.[1]

1. MNP laboratory, Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam-629502, Tamilnadu, India.

2. Department of Animal Science, M.S.University, Tirunelveli-627012.

Corresponding author email: gimmas@gmail.com

1. Introduction

Lobster is an edible, strong clawed marine crustacean. In India, lobsters are called as spiny lobsters also known as langouste or rock lobsters; they are placed next to shrimp in export value. There is a good demand for Indian lobsters throughout the world particularly in USA (Radhakrishnan, 1995). *Panulirus homarus* is a species of spiny lobster that lives along the coasts of the Indian and Pacific Oceans. It lives in shallow water, and feeds on mussels *Perna* sp.. They are exported to Southeast Asian countries due to its excellent market demand and price. They are flavored food in many countries because of their fine flavor. Lobsters are rich sources of protein, vitamins, minerals etc. They provide a protective food containing good source of animal protein, which are vital for human life. Commonly live lobsters achieve a highest price compared to frozen lobsters (James and Marian, 2003) due to its expensive delicacies (PCARRD, 1981). To meet out the demand of lobster export, lobster farmers adopted a new technique called "Lobster fattening". It is by rearing of under sized lobsters in captive condition. The success of lobster farming largely depends on the fattening of wild caught under sized lobsters. These lobsters are stocked at high densities in fattening ponds. Although farming trials in indoor systems have shown culture potential for this species, the high stocking density and poor water quality management can lead to disturbing consequences on the successful maintenance of these lobsters. Therefore, in the present study an indoor experimental trial was performed on fattening of juvenile lobsters for a duration of 30 days to access the growth responses and biochemical constituents in *P. homarus* fed with different dietary sources.

2.Materials and Methods

2.1Collection and maintenance of experimental animals

The juvenile *P. homarus* was collected from the fish landing centre at Chinnamuttom, Kanyakumari district. They were collected in live condition and transported carefully to the laboratory under moist condition without much disturbances to the animals. The collected lobsters were gradually released into 2000 litre capacity FRP tank containing fresh and well aerated seawater. During the period of acclimatization, the lobsters were fed with anchovies to saitation. The unfed remains were siphoned out during the next day prior to water exchange and 50% fresh seawater was added to maintain the initial level of water. Lobsters were maintained in this stock tank for a duration of 10 days.

2.2Collection of chicken intestine

The dried chicken intestine was placed in a hot air oven at a temperature of 50^0C in order to facilitate grinding. Then it was ground well and used as an ingredient for the preparation of lobster diet.

2.3Collection of Anchovies and Sardines

Anchovies and Sardines were obtained from the local fish market at Rajakkamangalam. The collected fishes were washed properly and chopped in order to obtain the flesh and head separately. The chopped head and flesh were stored separately in plastic containers in a deep freezer until they were used as diet for lobsters.

2.4Experimental set up

In order to evaluate the growth responses of lobsters on different diets (i.e., pellet diet, anchovy head, anchovy flesh, sardine head and sardine flesh) lobsters weighing about 72.43 – 88.70 were collected from the stock tank and segregated into respective experimental tanks (500 litre capacity FRP tanks) provided with proper aeration. In each tank 10 nos of lobsters were maintained. The entire experiment was conducted in an indoor culture system for a duration of 30 days.

2.5Feeding and experimentation

Lobsters reared on experimental tanks were maintained in duplicates and they were fed on respective experimental diets twice daily at *ad libitum*. The unfed remains were collected next day by siphoning prior to water exchange and 50% fresh seawater was added to maintain the original level. Lobsters were provided with hides (PVC pipes) in order to prevent cannabalism during moulting. To begin with the experiment, the initial wet weight of lobsters, carapace length (CL), and total length (TL) were recorded during the initial day of the experiment and also during the end of the experiment.

2.6Preparation of pellet diet

In order to prepare a pellet diet supplemented with chicken intestine waste, the appropriate quantity of ingredients such as fish meal, casein, chicken intestine, rice bran, tapioca powder, groundnut oil cake and soya meal (Table 1) were mixed thoroughly by adding sufficient quantity of distilled water and made into a dough. The dough was then cooked in a pressure cooker for a duration of 15 minutes. The cooked dough was taken and then other additives such as vitamin and mineral, egg white, BHT, NaCl and finally 2 ml of gelatin was added and mixed well. Then the dough was allowed to pass through a pelletizer having pore size of 1.15 cm in diameter. The pellets were sun dried and stored in a air tight plastic container and it was used for the experimentation.

Table 1. Feed ingredients used for the preparation of pellet diets supplemented with chicken intestine waste

Ingredients	Weight (g)
Casein (g)	10
Fish meal (g)	25.0
Chicken intestine (g)	40.0
Rice bran (g)	5.0
Tapioca powder (g)	6.5
Groundnut oil cake (g)	5.0

Soya meal (g)	5.0
Gelatin (g)	2.0
BHT (g)	0.5
NaCl (g)	0.5
Vitamin and Mineral	0.5
Egg white (ml)	2.0

2.7Water quality parameters

Water quality parameters such as temperature, pH, dissolved oxygen, salinity, ammonia and alkalinity were recorded once in 10 days time interval by following the standard methodology described in APHA (1995). Water quality parameters were maintained at an optimum level by providing proper aeration and regular water exchange.

2.8Growth parameters

The growth parameters such as Carapace and abdomen length, Body weight, Production (Growth), Food consumption, Food conversion efficiency (FCE), Growth percentage, Absolute Growth Rate (AGR), Specific Growth Rate (SGR) and Food conversion ratio (FCR) were analyzed.

2.9Biochemical estimation

Biochemical elements such as protein (Lowry *et al.,* 1951), carbohydrate (Roe *et al.,* 1955) and lipid (Folch *et al.,* 1957) were analyzed in the tissue samples such as muscle, gill and hepatopancreas once in 10 days interval.

3.Results

Water quality parameters recorded during fattening of lobsters are shown in Tables 2. The result on the growth performances such as length, weight and SGR of *P. homarus* received various diets are presented in Table 3. The results indicated that, the total length (TL), carapace length (CL), total weight (TW) and growth responses showed variation with the progress of experimentation. For instance, among the tested dietary sources, the growth and production, displayed maximum by lobster received sardine flesh as the diet against to that of lobsters those received pellet diet (1.66 ± 0.03g) which displayed a minimum value. The biochemical constituents of muscle, gill and hepatopancreas of *P. homarus,* those received various diets are presented in Fig.1 - 3. The results indicated the variation among *P. homarus* those received different diets and also among tissues.

4.Discussion

Farming of spiny lobsters as a commercial scale and for experimental purpose has gained importance worldwide, due to the limited availability, high consumer demand and high market value. In India, three species of lobsters are most suitable for farming which include *Panulirus homarus* of southwest and south east coast, *P. polyphagus* of northwest coast and *P. ornatus* of southeast coast. In the present study, *P. homarus* was taken for fattening studies in an indoor culture system. Mussels have been shown to be a good diet for a broad range of spiny lobsters. But there are problems associated with collection, seasonal variation in quality; storage and handling (Booth and Kittaka, 1994) may take that unfeasible and uneconomical. So an alternate method of diet for lobsters should be adopted. Very little research has been devoted to the development of formulated diets for spiny lobsters, even though their availability is seen on a important component of development of spiny lobster culture (Booth and Kittaka, 1994). In the present study, artificial pellet diet supplemented with chicken intestine waste was formulated for spiny lobster *P. homarus*, however the

results inferred a poor growth rate and a minimum production of 1.655 g in lobsters those received pellet diet as against to that of maximum production observed in lobsters those fed on natural sardine flesh as a diet (10.14 ± 0.24 g). The above mentioned results were in consistence with the previous study of Crear *et al.* (2000), they observed that formulated diets were readily ingested, but they did not support growth rates comparable to that of natural diet mussels. Similar results have been seen with formulated diets in studies on other spiny lobsters (Lellis, 1992) and with clawed lobsters (Bordner *et al.*, 1986). Lobsters feeding strategies rely on chemical cues to locate food (Kanazawa, 1994). So the characters of diets such as high attractiveness, palatability and water stability should suit the feeding behavior of lobsters, however, in the present study, lobsters those provided with pellet diet (supplemented with chicken intestine waste) refused to take it efficiently. Spiny lobsters can survive in a low dissolved oxygen content, but exposure to a level below 3 ppt may result in mortality especially of moulting lobsters (Radhakrishnan, 1996). The above mentioned facts was realized in our present study in which *P. homarus* were reared in an optimum salinity of 30ppt and good survival rates were obtained even in a dissolved oxygen content of 4 mg/l. Reduction of oxygen in the culture system is likely during early morning, where the unfed and excreta of lobsters might consume oxygen quickly. Vigorous aeration and continuous flow of fresh seawater is essential for the culture system during this period to maintain the water quality.

Available commercial pelleted feeds for marine shrimp have not been particularly successful as a sole diet for feeding spiny lobsters. Several reasons have been advanced to explain why pelleted feeds have not been as effective in promoting growth of spiny lobsters as natural foods. The inadequate knowledge of the nutritional requirements of particular species of spiny lobsters is clearly a fundamental reason (Williams, 2007). Our present findings support to the above said view that lobsters those fed on pellet diet which displayed a low SGR value of 0.066 ± 0.001% and a less growth percentage of 5.516 ± 0.011%, when compared to that of lobsters which displayed a better specific growth rate of 0.436 ± 0.003% and growth percentage of 33.8 ± 0.74% by the lobsters those received sardine flesh as the diet. The better production and growth responses of *P. homarus* those received sardine flesh as the diet may be due to the fact that spiny lobsters are opportunistic foragers and the natural diet of spiny lobsters are thought to be dominated by molluscs (mussels and barnacles), coralline algae and crustaceans with minor dietary contributions from echinoids, worms and seagrasses (Frusher *et al.*, 1999).

From the above obtained results, it may be concluded that lobsters those received natural diets particularly sardine flesh and anchovy head displayed a better growth and further experiment may be carried out to ascertain the specific nutrient required to the particular lobster species and also to formulate the efficient, cost effective and more stable artificial feed.

Table 2. Water quality parameters recorded in the lobster fattening system with respect to change in dietary source during different days of culture period

Dietary Sources	Experimental Duration	Parameters					
		Tem (°C)	pH	DO (mg/l)	Salinity (ppt)	Alkalinity (mg/l)	Ammonia (mg/l)
Pellet	10	26.20±0.40	7.80±0.12	4.12±0.02	30±0.00	95.00±1.48	0.020±0.00
	20	28.50±0.42	7.50±0.10	4.00±0.04	30±0.00	94.00±2.42	0.060±0.00

Feed	30	28.2±0.46	7.62±0.20	5.40±0.02	30±0.00	120.00±1.80	0.065±0.00
Anchovy head	10	26.25±0.40	7.21±0.10	4.00±0.04	30±0.00	90.00±0.86	0.013±0.00
	20	28.20±0.34	7.80±0.14	4.15±0.02	30±0.00	100.00±0.89	0.028±0.00
	30	27.80±0.26	7.30±0.12	4.83±0.03	30±0.00	110.00±1.10	0.016±0.00
Anchovy flesh	10	26.20±0.31	7.10±0.10	4.00±0.01	30±0.00	80.00±1.10	0.024±0.00
	20	28.60±0.40	7.69±0.11	4.12±0.02	30±0.00	100.00±1.40	0.016±0.00
	30	28.70±0.32	7.65±0.10	4.53±0.02	30±0.00	105.00±2.10	0.029±0.00
Sardine head	10	26.34±0.34	7.85±0.12	4.10±0.04	30±0.00	112.00±0.98	0.020±0.00
	20	28.60±0.26	7.77±0.10	4.21±0.02	30±0.00	114.00±1.14	0.019±0.00
	30	28.42±0.24	7.60±0.08	4.54±0.03	30±0.00	110.00±1.50	0.137±0.00
Sardine flesh	10	26.40±0.24	8.04±0.10	4.20±0.04	30±0.00	105.00±0.80	0.015±0.00
	20	28.50±0.22	7.77±0.12	4.18±0.02	30±0.00	110.00±1.20	0.018±0.00
	30	28.40±0.21	7.90±0.08	5.40±0.03	30±0.00	105.00±1.10	0.017±0.00

Each value is the mean ± S.D of triplicate analysis

Table 3. Growth responses of spiny rock lobster *P. homarus* fed on different dietary sources during 30 days of culture period

Parameters		Experimental diets				
		Pellet diet	Anchovy head	Anchovy flesh	Sardine head	Sardine flesh
Carapace length (cm)	Initial	5.18±0.11	4.75±0.10	4.93±0.13	5.05±0.14	4.75±0.10
	Final	6.10±0.14	5.95±0.12	6.00±0.15	5.97±0.14	5.77±0.13
Total length (cm)	Initial	12.22±0.24	12.20±0.24	12.02±0.26	12.40±0.31	11.65±0.34
	Final	13.57±0.26	13.15±0.28	13.13±0.32	13.70±0.41	12.87±0.22
Total weight (g)	Initial	82.26±1.40	88.70±1.24	76.21±1.16	80.85±0.02	72.43±1.21
	Final	83.91±1.42	91.63±1.14	78.47±1.32	82.61±2.20	82.57±1.40
Initial weight (g)		82.26±1.40	88.70±1.24	76.21±1.16	80.85±2.04	72.43±1.21
Final weight (g)		83.91±1.42	91.63±1.14	78.47±1.32	82.67±2.20	82.57±1.40
Mid body weight		83.08±2.10	90.16±1.40	77.34±2.20	81.73±1.15	77.50±2.40
Feed consumption (g)		35.90±1.20	57.02±0.85	59.08±0.82	61.91±1.10	60.8±0.62
Production (g)		1.66±0.03	3.00±0.04	2.26±0.04	1.77±0.03	10.14±0.24
GPE (%)		4.61±0.10	5.26±0.12	3.82±0.13	2.85±0.11	16.65±0.40
SGR (%)		0.066±0.001	0.108±0.001	0.097±0.002	0.072±0.001	0.436±0.003
FCR		21.69±0.65	19.00±0.42	26.14±0.72	35.03±0.84	6.00±0.21
Growth (%)		5.51±0.11	10.00±0.24	7.53±0.32	5.89±0.21	33.80±0.74

Each value is the mean ± S.D of triplicate analysis

Fig. 1. Protein content (%wet weight) recorded in various tissue samples of *P. homarus* fed on different dietary sources at the end of culture period

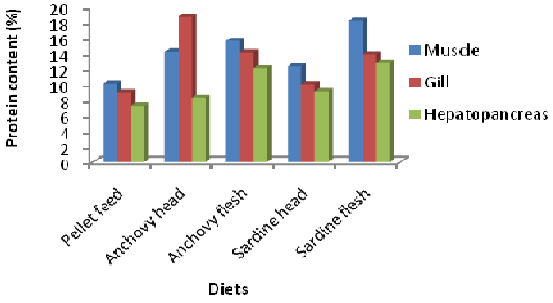


Fig. 2. Carbohydrate content (%wet weight) recorded in various tissue samples of *P. homarus* fed on different dietary sources at the end of culture period

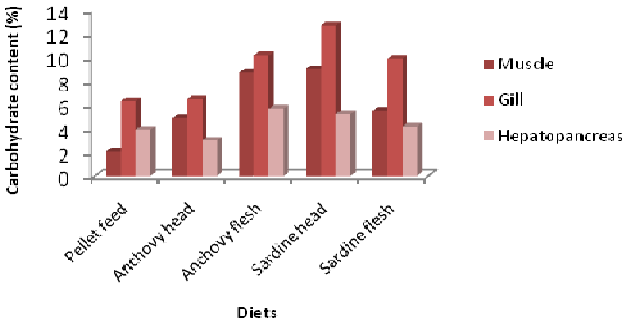
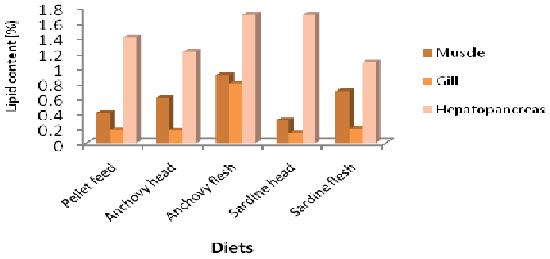


Fig. 3. Lipid content (%wet weight) recorded in various tissue samples of *P. homarus* fed on different dietary sources at the end of culture period



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BIOPRESERVATIVE EFFICACY OF BACTERIOCIN FROM LACTOBACILLUS SP., IN WHITE LEG SHRIMP (*LITOPENAEUS VANNAMEI*)

Nagarajan, S[1],Yogananth, N[1]. Syed Ali, M[1]. Anuradha, V., [2]and Muthezhilan, R[3].

[1]PG and Research Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai – 119.

[2]PG and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai – 119.

[3]Department of Biotechnology, D.G.Vaisanav College, Arumbakkam, Chennai.

* Corresponding author: bioyogaa@gmail.com

Introduction

Prawn and shrimp are the most important products from aquaculture: more than 3.7 million tons were produced in 2010 with a value of more than 16 billion US dollars. The white leg shrimp *Litopenaeus vannamei* is the most important penaeid shrimp species farmed worldwide (Alcivar – Warren *et al*., 2007). Because of the high demand for shrimps in Japan, the United States and Europe, shrimp aquaculture has expanded rapidly in all around the world, especially in tropical areas, such as Southeast Asia and Latin America (Lombardi *et al*., 2006). India rank second next to china in shrimp production. The current market trend is for the processing of value-added products like cooked shrimp, which is very popular and widely sold in supermarkets as a chilled ready-to eat product under modified atmosphere packaging (MAP). Shrimp quality is essential to maintain not only product value but also the reputation of the farmer, processor and country
(Bari *et al.*, 2011).

The spoilage bacterial species of a packaged product depend on its endogenous microbiota, the processing undergone, the type of packaging (MAP, vacuum, aerobic, etc.) and storage temperature. Several bacterial pathogens including *Salmonella, Campylobacter jejuni, Escherichia coli 0157:H7, Listeria monocytogenes, Staphylococcus aureus* and *Clostridium botulinum* are found associated with such outbreaks (Nath et al., 2013).

Food preservation is a continuous fight which aims at either to eliminate or reduce the outgrowth potential of spoilage and pathogenic microorganisms in foods (Rasooli, 2007). Until now, approaches to seek improved food safety have relied on chemical preservatives, antibiotics or on the application of more drastic physical treatments (e.g. high temperatures or refrigeration). These preservation techniques have many drawbacks which includes the proven toxicity of the chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer demands for safe but minimally processed products without additives.

Biopreservation is an alternative natural technology used to extend the shelf life and/or control the growth of endogenous pathogenic bacteria in refrigerated products. It involves inoculating protective bacteria selected for their inhibition properties towards undesirable micro-organisms. Lactic acid bacteria (LAB) are usually chosen for these applications as they produce a wide range of inhibitory compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins or compete with other micro-organisms by nutrient depletion (Leroi, 2011). In this context, this study was carried out to determine the effectiveness of *Lactobacillus* sp. (LAB13) and their bacteriocin in preservation of economically important and exported seafood shrimp at different temperature storage condition.

Material and methods

Isolation of lactic acid bacteria (LAB)

For the isolation of *Lactobacillus* sp., 1 mL of curd sample was mixed in 99 ml of sterile distilled water, and this suspension was serially diluted up to 10^{-4} in 9 mL blank, 0.1 ml of the diluted sample were taken from 10^{-3} and 10^{-4} dilutions and spreaded in MRS agar plates and incubated for 48 h at room temperature (28±2°C). After the incubation period, morphologically different colonies were selected from the MRS agar plates and each strain were individually streaked in MRS agar plates and incubated for 48 h at room temperature (28±2°C).

Antimicrobial activity of LABs

To determine the antimicrobial activity of all the isolated *Lactobacillus* sp., were tested against five different seafood borne pathogens (*E.coli*, *V. cholerae*, *V. parahaemolyticus*, *Salmonella* sp. and *Shigella* sp. which were already isolated from seafood) using agar well diffusion assay (Schillinger and Lucke 1989).

Extraction of bacteriocin

The *Lactobacillus* sp. strains which were showed the zone of inhibition (ZOI) against all the tested sea food pathogenic bacteria are subcultured individually in MRS agar plates. The strains were inoculated separately in 50 mL of MRS broth (pH 6.8) for extraction of bacteriocin, all the culture supernatants were centrifuged at 6000 rpm for 30 minutes at 4°C. The cell free supernatant were precipitated with ammonium sulphate (40% saturation) and kept for 2 h at 4°C, and later centrifuged at 10,000 rpm for 20 minutes. After centrifugation the precipitates were obtained and resuspended in 10 mL of 0.05 M potassium phosphate buffer [pH 7.0] (Arokiyamary and Sivakumar, 2012).

Determination of bacteriocin activity

Agar plates were swabbed with 100 µl of each seafood pathogenic bacteria after growing them in a broth. Once the plates were dried aseptically, 5 mm wells were bored using a sterile cork borer and about 10 µl of (extracted bacteriocin) supernatant was placed into each well. Then the plates were incubated for 24 h at 37°C. After the incubation period the antimicrobial activity was determined by measuring the diameter of the ZOI around the wells (Arokiyamary and Sivakumar, 2012). The strain which showed the maximum inhibition zone against the tested seafood borne pathogens is taken for the mass scale production of bacteriocin.

Mass scale production of bacteriocin

The bacteriocins of *Lactobacillus* sp. (LAB13, 17 and 26) strains which showed the strongest antimicrobial activity against seafood borne pathogens was chosen for mass scale production of bacteriocin. The culture was inoculated in 1000 mL MRS broth (pH 6.8), after the incubation period the bacteriocin was isolated by following the aforesaid procedure.

Shrimp sample preparation and treatment application

Fresh shrimp (*Penaeus vannamei*) samples were collected from Kovalam landing centre, Chennai, Tamil Nadu, India. The shrimps were transferred by using icebox to the Laboratory within 1 hour. The shrimps were immediately manually eviscerated, headed and filleted. They were divided in to two groups. One group of the shrimps were stored directly and other group of the shrimps were dipped in cold distilled water containing LAB

bacteriocin, and both treatments of shrimps were packed in polyethylene bags and stored at different temperatures at –4°C and –20°C.

Microbiological analysis

Shrimps were taken randomly from both treatments at different time intervals (1st , 8th , 16th , 24th and 30th day) and homogenized using mortar and pestle, 10 g of the sample was mixed in 95 mL of sterile saline (0.85% NaCl) and this suspension was serially diluted up to 10– 4. For isolation of total heterotrophic bacteria (THB) spread plate method was followed using nutrient agar medium, for the isolation of total coliforms, *E. coli*, *Vibrio* sp., *Salmonella* sp. and *Shigella* sp. MPN technique was followed using EMB agar, TCBS agar SS agar and PALCAM agar respectively.

Results and Discussion

The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as biopreservation (De Martinis et al, 2001). In this present study morphologically 30 *Lacto bacillus* sp., strains were isolated and they were named as LAB01 to LAB30 (Fig 1). To determine their antimicrobial activity the 30 strains were tested against six different sea food borne pathogens (*E.coli*, *Vibrio cholerae*, *V. parahaemolyticus*, *Salmonella* sp., *Shigella* sp., and *Listeria* sp.,) by using agar well diffusion assay method (Table 1). Based on their zone of inhibition the strains LAB08, LAB13, LAB17, LAB23 and LAB26 were showed the maximum inhibitory activity against all the tested sea food pathogens (Fig 2 and Table 2).

Fig1: Morphologically 30 different *Lactobacillus* sp strain

Table1: Antimicrobial activity of 30 *Lactobacillus* sp., strains against sea food pathogens

LAB Strains	Zone of inhibition (in mm) against Sea food pathogens					
	E. coli	*V. cholerae*	*V.parahaemolyticus*	*Salmonella sp.,*	*Shigella sp.,*	*Listeria* sp.,
LAB01	+	++	-	+	+	-
LAB02	+	-	++	-	-	++
LAB03	+	++	++	++	++	++
LAB04	++	+	-	+	+	-
LAB05	+	+	-	+	++	-
LAB06	+	-	++	-	-	++
LAB07	+	-	-	++	+	-
LAB08	+++	+++	++	+++	+++	++
LAB09	+	-	-	+	-	-
LAB10	++	+	+	-	+	+
LAB11	+	-	+	-	-	+
LAB12	+	-	-	+	-	-
LAB13	+++	+++	+++	+++	+++	+++

LAB14	-	-	+	+	+	+
LAB15	++	-	+	+	-	+
LAB16	-	+	-	+	+	-
LAB17	+++	+++	+++	+++	+++	+++
LAB18	++	+	+	+	+	+
LAB19	++	++	++	++	++	++
LAB20	-	+	+	-	-	+
LAB21	-	-	+	+	+	+
LAB22	+	+	-	-	+	-
LAB23	+++	+++	++	+++	+++	++
LAB24	-	+	+	+	+	+
LAB25	-	+	-	+	+	-
LAB26	+++	+++	+++	+++	+++	+++
LAB27	+	-	-	+	+	-
LAB28	+	-	+	-	-	+
LAB29	-	+	-	+	-	-
LAB30	++	++	++	++	+	++

− = no inhibition zone, + = inhibition zone up to 4 mm, ++ = inhibition zone up to 8 mm; +++ = inhibition zone up to 12 mm

Among these five strains, three strains of LAB13, LAB17 and LAB26 gave good inhibitory activitis against food borne pathogens, so they were taken for bacteriocin production and to determine their bacteriocin activity against seafood pathogens. The antibacterial activity of *Lactobacillus* is of great importance in food preservation (including seafood preservation), particularly those types of foods, which are to be stored in ambient temperatures. The reduction of pathogen growth and cell density indicate that extracellular bacteriolytic products i.e., bacteriocins produced by lactic acid bacteria were responsible for this inhibition (Jamal *et al.,* 2012). These results were comparable to those obtained by Jin *et al*., (1996) found that the *Lactobacillus* cultures exhibited inhibitory effect against five *Salmonella* strains and three *E.coli* serotypes. The phenotypic and biochemical tests were performed to identify the strain (LAB13), according to the Bergey's Manual of Systematic Bacteriology (1984) guidelines; the LAB strain (LAB13) is identified as *Lactobacillus* species.

Table 2 : Screening of *Lactobacillus* sp., bacteriocin against Sea food pathogen

LAB Strains	Zone of inhibition (in mm) against Sea food pathogens					
	E. coli	*V. cholerae*	*V.parahaemolyticus*	*Salmonella sp.,*	*Shigella sp.,*	*Listeria sp.,*

LAB08	++	++	++	+	++	+
LAB13	+++	++ +	+++	++ +	++ +	++ +
LAB17	+++	++ +	+++	++ +	++ +	++ +
LAB23	++	++	++	+	++	+
LAB26	+++	++ +	+++	++ +	++ +	++ +

+++ = inhibition zone up to 12 mm

The composition of the growth medium is very important for the production of individual bacteriocins. MRS seemed to be more suitable medium. All the microbial groups increased during storage. For example, the Enterobacteriaceae reached its highest count of 110 CFU/g at the 8th day of storage in -4^{0}C and 60 CFU/g at the 4th day of storage in -20^{0}C (Table 3). But the plate counts highlighted a relevant decrease of microbial groups detected during storage at bacteriocin treated with -4°C and -20°C. The same manner was registered in coliform count and five foodborne pathogens (table 4, 5 and 6). Among the The reduction of Coliforms in bacteriocin treated shrimp treatment could be due to the acidification by lactic acid and some inhibitory compounds formed by *Lactobacillus* sp. Several studies indicated that bacteriocin can prevent the growth of undesirable bacteria in a food-grade, which is convenient for health. Kanatani *et al.* (1995) has stated that a bacteriocin (acidocin A) from *L. acidophilus* TK9201 had inhibitory effect on closely related lactic acid bacteria and food borne pathogens.

These results show the potential usefulness of these bacteriocins justifying a more in depth investigation for their identification and application as food bio preservatives. However, further work is needed to fully understand the molecular mechanisms, structure-function relationships and mechanisms of action of bacteriocins for exploration of applications

Fig 2: Antimicrobial activity of bacteriocins (LAB13, LAB17 and LAB26) against *food borne pathogens*

Left to right in **top**: *E. coli, V. cholerae, V.parahaemolyticus*,

Bottom: *Salmonella sp*, *Shigella sp.*, and *Listeria sp*.**,**

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ANTIMICROBIAL ACTIVITY OF VARIOUS SOLVENT BASED EXTRACTS OFMEDICINAL HERB *PHYLLANTHUS NIRURI* AGAINST SHRIMP *VIBRIO* PATHOGENS

Deivakumari. M, Vibin. A,Sanjivkumar. M, DharaniBalan.P and G. Immanuel
Marine Biotechnology Division, Centre for Marine Science and Technology,
ManonmaniamSundaranar University, Rajakkamangalam – 629502
Kanyakumari District, Tamilnadu.
Corresponding author E.mail: gimmas@gmail.com

1. Introduction

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. About 80% of the populations in developing countries still use traditional medicine for their healthcare (De Silva, 2005).The renewed interest in the use of medicinal plants may be attributed to cheapness, availability and accessibility by the local population,high incidence of side effects of synthetic medicines and environmental friendliness of plant extracts.Medicinal plants also serve as the starting point for the discovery of semi synthetic chemical compounds which are used in pharmaceutical industries (Sofowora, 1989).Some of the useful plants such as*Euphorbia hirta, Acalyphaindica* and *Phyllanthusniruri*etcare known as traditional healer in many rural areas throughout the world. *P.niruri* is known to be used to treat various types of ailments; almost every parts of the plant can be used as medication. The plant is much used as diuretic and other problems of the genito-urinary tract. The fruit is used to heal for wounds, scabies and ringworm remedies (Kirtikar*et al*., 1975). An aqueous extract of *P. niruri*was found to inhibit the hepatitis B virus (Thyagarajan*et al*., 1988). Medicinal plants as the alternative agents are effective to treat the infectious diseases and mitigate many side effects that are associated with synthetic antimicrobials. Extract from *P. niruri*showed promising antibacterial activity against the fish bacterial pathogens(Punitha*et al*. 2008). Considering the importance of the above, the present study was undertaken to find out the efficiency of different solvent based extracts of the medicinal plant *Phyllanthusniruri* as antibacterial agent against shrimp *Vibrio* pathogens.

2. Materials and Methods

2.1. Medicinal plant

The medicinal herb *P. niruri*was collected from local areas of Kanyakumari District, Tamilnadu, India. The collected plant was washed thoroughly and dried at room temperature. The dried plant was ground well and sieved using a nylon sieve in order to remove plant fibers (Immanuel *et al.,* 2004).

2.2.Preparation of different solvent extracts of *P.niruri*

Four different solvent (Acetone, Ethyl acetate, Chloroform and Diethyl ether) based extracts of *P.niruri*were prepared by using the standard extraction method. The extracts were kept in individual containers and stored at 4˚C until further use.

2.3. Test pathogens

The shrimp *Vibrio* pathogens such as *Vibrio harveyi, V. parahaemolyticus, V. alginolyticus, V. mimics,* and *V. vulnificus* used in this study were obtained from the MNP laboratory, Centre for Marine Science and Technology, M.S. University.

2.4. Determination of antibacterial activity through Agar well diffusion method

The antibacterial activity of various solvent extracts at different concentrations (25, 50, 75 and 100 mg/ml) were evaluated against the above shrimp pathogens by agar well diffusion method. The antibacterial activity wasassessed by measuring the diameter of zone of inhibition at mm scale.

2.5. Minimum Inhibitory Concentration (MIC)

The MIC of different solvent extracts of *P.niruri*at various concentrations (100, 50,25, 12.5, 6.25, and 3.125 mg/ml) were determined by following standard methodology.

2.6. Minimum Bactericidal Concentration (MBC)

One loopful of the above serially diluted and incubated concentrations were streaked individually on sterile Muller Hinton agar plates and incubated at 35° C for 24h. Then the plates were evaluated by comparing them with control plates containing test bacteria without any extract. The lowest concentration that has no visible growth was considered as MBC.

3. Results and Discussion

The plant based traditional medicines were proven highly effective for their utilization as a source of antimicrobial compounds. Many reports are available on the anti bacterial properties of *P. niruri*(Iwu*et al*., 1999). In the present study, different solvent extracts of *P. niruri*was evaluated for their antimicrobial activity against shrimp *Vibrio*pathogens. The susceptibility of each plant extract was tested by agar well diffusion method. The result indicated that the individual solvent based plant extracts displayed different range of inhibition against the tested pathogens. For instance, the diethyl ether extract of *P. niruri*showed the zone of inhibition of 9.33, 8.83, 9.66, 11.66, and 11.66 mm against *V. parahaemolyticus, V. harveyi, V. alginolyticus, V. mimics,* and *V. vulnificus* respectively at the highest concentration of 100 mg/ml.Similarly the acetone extract of *P. niruri* displayed the zone of inhibition of 10.33, 12.66, 10.0, 13.83, and 17.00 mm respectively against *V. parahaemolyticus, V. harveyi, V. alginolyticus, V. mimics,* and *V. vulnificus,*at the sameconcentration of100 mg/ml. However the ethylacetate extract (100 mg/ml) of *P.niruri* exhibitedthe zone of inhibition of 6.33, 10.00 and 7.33 mm against *V. parahaemolyticus, V. mimics,* and *V. vulnificus*respectively. Likewise the highest concentration (100 mg/ml) of chloroform extract of *P.niruri* showed less activity with the zone of inhibition of only 6.66mm against the only organism *V. harveyi*(Table 1). Among all the tested solvent based extracts, acetone extract exhibited comparatively better result. In accordance with these Immanuel *et al* (2004) reported the antibacterial activity of n-butanolic extract of seaweeds like *U. lactuca* and *S. wightii* against shrimp pathogen *V. parahaemolyticus* by agar well diffusion method. The zone of inhibition observed was 17.5 and 16.3mm against tested pathogen. A more recent work conducted by Palombo and Semple(2001) revealed that the methanolic extracts of various parts of *P. niruri*have antibacterial activity against five

bacterial strains such as *E. cloacae, S. aureus, P. aeruginosa, E. coli* and *S. viridians* and two fungal strains like *A. niger*and *T. viridae.* It was found a regular increase in diameter of zone of inhibitions with the advancement of concentrations in all sensitive bacterial strains. Similarly, Chotigeat*et al*. (2004) have reported for the antibacterial effect of fucoidan from *S. polycystum* against shrimp bacterial pathogens *V. harveyi. S. aureus* and *E.coli* by agar well diffusion method and they exhibited the zone size of 13, 10 and 9 mm, respectively against the above pathogens at 12 mg/ml concentration of fucoidan. Pholdaeng and Pongsamart (2010) have reported that the antibacterial activity of polysaccharide gel extracted from *Duriozibethinus* against the shrimp bacterial pathogen *V. harveyi*, who found out the inhibition zone of sharp and clear margin with the diameter of 20.43, 16.47, 12.15, 10.70 and 8.88 mm at the respective concentrations of 50.0, 25.0, 12.5, 6.3, and 3.1 mg/ml.

In the present study, the MIC and MBC of different solvent based extracts of *P. niruri* was tested against the shrimp pathogens (Table2 and 3). The MIC and MBC of diethyl ether extract of *P. niruri* was determined as25, 50, 25, 12.5, and 12.5 mg/ml against *V. parahaemolyticus, V. harveyi, V. alginolyticus, V. mimics,* and *V. vulnificus*respectively. The MIC& MBC of acetone extract of *P.niruri* observed was 25, 12.5, 25, 12.5, and 12.5 mg/ml against*V. parahaemolyticus, V. harveyi, V. alginolyticus, V. mimics,* and *V. vulnificus*respectively. The MIC& MBC of ethyl acetate extract of *P.niruri*displayed 75mg/ml each against *V. parahaemolyticus*and *V. vulnificus,* whereas 25 mg/ml against *V. mimics.* But the MIC& MBC of chloroform extract of *P. niruri* determined with 75 mg/ml against the only organism *V. harveyi*. Similar to that of the present study,Ramesthangam and Ramsamy (2007) pointed out that the MIC and MBC of ethanolic extract of *Pongamiapinnata* leaves against *Vibrio*spand *Streptococcus* sp were 1.2 and 1 mg/ml respectively. The present result showed the effective antimicrobial activity of various solvent extracts of *P. niruri* against shrimp *Vibrio* pathogens (Fig.1). Similarly, Chotigeat*et al*. (2004) have reported the minimum inhibitory concentration of fucoidan extracted from *S. polycystum* against the shrimp bacterial pathogens such as *V. harveyi, S. aureus* and *E. coli* as 12, 12, and 6 mg/ml respectively. Pholdaeng and Pongsamart (2010) observed the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of polysaccharide gel (PG) extracted from *Duriozibethinus* against shrimp bacterial pathogen *V. harveyi* and it was 6.3 mg/ml in the MHB medium. The MBC value, representing the minimal concentration in the well of no visible growth that showed no bacterial growth after sub culturing in agar medium without PG and incubating at 30˚C for 16 h, and the MBC value of 12.5 mg/ml was obtained for PG. From the study it was concluded that the acetone extract of *P. niruri*showed effective antimicrobial activity against the tested shrimp*Vibrio* pathogens.

Table1. Antibacterial activity of different solvent based extracts of *P. niruri*against *Vibrio* pathogens (zone of inhibition in mm)

Solvents	**Concentration of extracts (mg/ml)**	**Pathogens**				
		V.parahaemolyticus,	*V. harveyi*	*V.alginolyticus*	*V.mimics*	*V.vulnificus*
Diethyl ether extract	25	6.33 ± 0.527	5.16 ± 0.263	6.66 ± 0.527	7.33 ± 0.577	8.66 ± 0.527
	50	6.66 ± 0.527	7.00 ± 0.428	7.50 ± 0.366	8.66 ± 0.527	9.33 ± 0.577

	75	8.66 ± 0.577	8.0 0 ± 1.00	8.66 ± 0.577	9.8 3 ± 0.763	10.6 6 ± 0.577
	100	9.33 ± 0.577	8.8 3 ± 0.763	9.66 ± 0.577	11. 66 ±0.577	11.6 6 ± 0.577
Acetone extract	25	7.00 ± 0.526	7.6 6 ± 0.527	7.00 ± 0.527	6.0 0 ± 1.00	8.66 ± 0.577
	50	8.66 ± 0.577	8.6 6 ± 0.577	8.50 ± 0.500	7.6 6 ± 0.527	10.3 3 ± 0.527
	75	9.33 ± 0.159	9.8 3 ± 0.289	9.66 ± 0.577	9.3 3 ± 0.577	13.6 6 ± 0.577
	100	10.33 ± 0.577	12. 66 ±0.577	10.0 0 ± 1.00	13. 83 ±0.763	17.0 0 ± 1.00
Ethyl acetate extract	25	0.00±0.00	0.0 0±0.00	0.00 ±0.00	6.0 0 ± 0.428	0.00 ± 0.00
	50	0.00±0.00	0.0 0±0.00	0.00 ±0.00	7.0 0 ± 0.527	0.00 ± 0.00
	75	0.00±0.00	0.0 0±0.00	0.00 ±0.00	8.6 ± 0.527	6.00 ± 0.468
	100	6.33 ± 0.577	0.0 0±0.00	0.00 ±0.00	10. 00 ± 1.00	7.33 ± 0.577
Chloroform extract	25	0.00±0.00	0.0 0±0.00	0.00 ±0.00	0.0 0±0.00	0.00 ± 0.00
	50	0.00±0.00	0.0 0±0.00	0.00 ±0.00	0.0 0±0.00	0.00 ± 0.00
	75	0.00±0.00	0.0 0±0.00	0.00 ±0.00	0.0 0±0.00	0.00 ± 0.00
	100	0.00±0.00	6.6 6 ± 0.527	0.00 ±0.00	0.0 0±0.00	0.00 ± 0.00

Each value is the Mean ± SD of triplicate analysis.

Table 2.Minimum inhibitory concentration (MIC) of different solvent based extract of *P.niruri*against *Vibrio*pathogens.

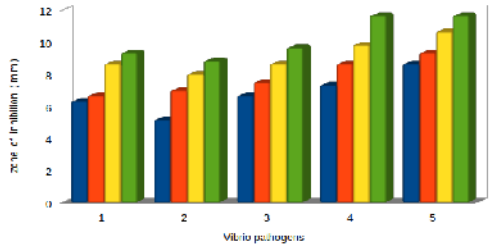
Solvent extracts	**Minimum Inhibitory Concentration (mg/ml)**				
	V. parahaemolyticus	*.hareyi*	*V.alginolyticus*	*. mimics*	*V.vulnificus*
Diethyl ether extract	25	50	25	2.5	12.5
Acetone extract	25	2.5	25	2.5	12.5
Ethyl acetate	75	-	-	25	75

extract					
Chloroform extract	-	5	-	-	-

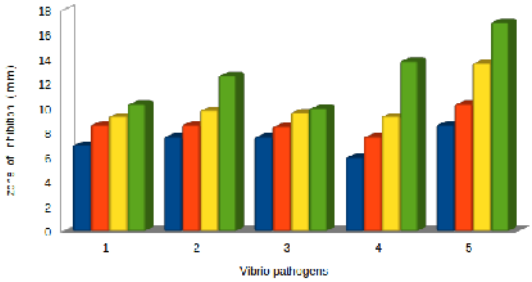
- = Not Detected

Fig. 1. Comparison between the antibacterial activity of different concentrations of diethyl ether (a), acetone (b), ethyl acetate(c) and chloroform (d) extracts of *P. niruri*against shrimp*Vibrio* pathogens

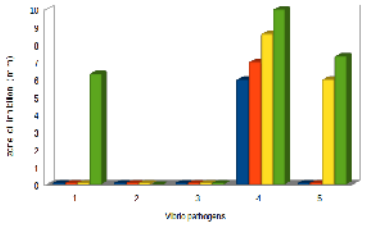
a) Diethyl ether extract



b) Acetone extract



b) **Ethyl acetate extract**



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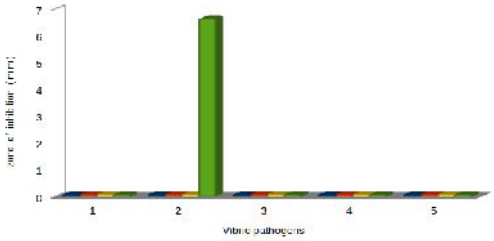
d) Chloroform extract



Acknowledgements

The authors wish to thank the University Grants Commission (UGC),Newdelhi for its financial support in the form of research grant (F.No:43-81/ 2014 SR)

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PREPARATION OF TYRINE PURPLE DYE FROM SEA SNAIL *Murex trunculus*

DR.A.S.GANGA
Assistant Professor, Department of Zoology
The MDT Hindu college, Tirunelveli – 10.

INTRODUCTION

Renewable energy resources are generated continuously in nature. They can be used again and again they are inexhaustible Eg. Solar energy, hydro power, wood, municipal waste. The renewable Energy is also called non-conventional energy. Tyrian Purplealso known as **Tyrian red**, **royal purple**, **imperial purple** or **imperial dye**, is a bromine-containing reddish-purple natural dye. It is a secretion produced by several species of predatory sea snails in the family Muricidae, rock snails originally known by the name *Murex*. Tyrian purple dye was greatly prized in antiquity because the colour did not easily fade, but instead became brighter with weathering and sunlight. Its significance is such that the name Phoenicia means 'land of purple.' It came in various shades, the most prized being that of "blackish clotted blood". This is speculation, and a matter of debate today, but according to some, in Biblical Hebrew, the dye extracted from the Bolinus brandarisis known as argaman . Another dye extracted from a related sea snail, Hexaplex trunculus, produced a blue colour which according to some is known as tekheletused in garments worn for ritual purposes.

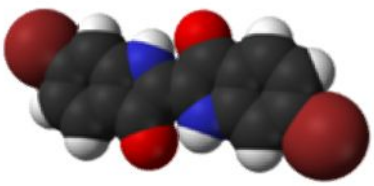
FROM SEA SNAILS

The dye substance is a mucous secretion from the hypobranchial gland of one of several species of medium-sized predatory sea snailsthat are found in the eastern Mediterranean Sea. These are the marine gastropods Bolinus brandaris the spiny dyemurex, (originally known as Murex brandaris (Linnaeus, 1758)), the banded dye-murex Hexaplex trunculus, the rock-shell Stramonita haemastoma,and less commonly a number of other species such as Bolinus cornutus. The dye is an organic compound of bromine (i.e., anorganobromine compound), a class of compounds often found in algae and in some other sea life, but much more rarely found in the biology of land animals.

Two shells of *Bolinus brandaris*, the spiny dye-murex, source of the dye

In nature the snails use the secretion as part of their predatory behaviour in order to sedate prey and as an antimicrobial lining on egg masses.The snail also secretes this substance when it is attacked by predators, or physically antagonized by humans (e.g., poked). Therefore the dye can be collected either by "milking" the snails, which is more labour-intensive but is a renewable resource, or by collecting and then crushing the snails completely, which is destructive. David Jacoby remarks that "twelve thousand snails of Murex brandaris yield no more than 1.4 g of pure dye, enough to colour only the trim of a single garment." Many other species worldwide within the family Muricidae, for example Plicopurpura pansa,from the tropical eastern Pacific, andPlicopurpura patulafrom the Caribbean zone of the western Atlantic, can also produce a similar substance (which turns into an enduring purple dye when exposed to sunlight) and this ability has sometimes also been historically exploited by local inhabitants in the areas where these snails occur. (Some other predatory gastropods, such as some wentletraps in the family Epitoniidae, seem to also produce a similar substance, although this has not been studied or exploited commercially.) The dog whelk Nucella lapillus, from the North Atlantic, can also be used to produce red-purple and violet dyes.

ROYAL BLUE



A space-filling model of 6,6′-dibromoindigo, based on the crystal structure

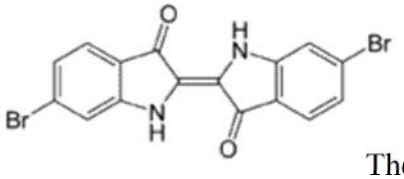
The Phoenicians also made an indigo dye, sometimes referred to as royal blue or hyacinth purple, which was made from a closely related species of marine snail.

The Phoenicians established an ancillary production facility on the Iles Purpuraires at Mogador, in Morocco. The sea snail harvested at this western Moroccan dye production facility was Hexaplex trunculus (mentioned above) also known by the older name Murex trunculus.

This second species of dye murex is found today on the Mediterranean and Atlantic coasts of Europe and Africa (Spain and Portugal, Morocco, and the Canary Islands).

CHEMICAL STRUCTURES AND METHODS

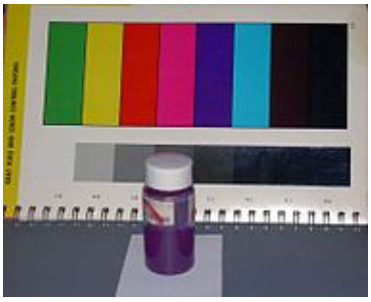
The production of Tyrian purple from shellfish. History of Animals, Aristotle described the shellfish from which Tyrian purple was obtained and the process of extracting the tissue that produced the dye.



The chemical structure of 6,6′-dibromoindigo, the main component of Tyrian Purple

The most favourable season for taking these [shellfish] is after the rising of the Dog-star, or else before spring; for when they have once discharged their waxy secretion, their juices have no consistency: this, however, is a fact unknown in the dyers' workshops, although it is a point of primary importance. After it is taken, the vein [i.e. hypobranchial gland] is extracted, which we have previously spoken of, to which it is requisite to add salt, a sextarius about to every hundred pounds of juice. It is sufficient to leave them to steep for a period of three days, and no more, for the fresher they are, the greater virtue there is in the liquor. It is then set to boil in vessels of tin [or lead], and every hundred amphoræ ought to be boiled down to five hundred pounds of dye, by the application of a moderate heat; for which purpose the vessel is placed at the end of a long funnel, which communicates with the furnace; while thus boiling, the liquor is skimmed from time to time, and with it the flesh, which necessarily adheres to the veins. About the tenth day, generally, the whole contents of the cauldron are in a liquefied state, upon which a fleece, from which the grease has been cleansed, is plunged into it by way of making trial; but until such time as the colour is found to satisfy the wishes of those preparing it, the liquor is still kept on the boil. The tint that inclines to red is looked upon as inferior to that which is of a blackish blue. The wool is left to lie in soak for five hours, and then, after carding it, it is thrown in again, until it has fully imbibed the colour.

Archaeological data from Tyre indicate that the snails were collected in large vats and left to decompose. This produced a hideous stench that was actually mentioned by ancient authors. Not much is known about the subsequent steps, and the actual ancient method for mass-producing the two murex dyes has not yet been successfully reconstructed; this special "blackish clotted blood" colour, which was prized above all others, is believed to be achieved by double-dipping the cloth, once in the indigo dye of H. trunculus and once in the purple-red dye of B. brandaris.



6,6'-dibromoindigo, the major component of Tyrian purple

DYE CHEMISTRY

A small amount of dibromindigo as a powder, and its effect on a piece of fabri

The main chemical constituent of the Tyrian dye was discovered by Paul Friedländer in 1909 to be 6,6′-dibromoindigo, a substance that had previously been synthesized in 1903. The dye was thus shown to be an organobromine compound. However, it has never been synthesized commercially.By altering the percentage of sea salt in the dye vat and adding potash, that was able to successfully dye wool a deep purple colour.

RESULT

In this Reason Recent research in organic electronics has shown that Tyrian purple is an ambipolar organic semiconductor. Transistors and circuits based on this material can be produced from sublimed thin-films of the dye. The good semiconducting properties of the dye originate from strong intermolecular hydrogen bonding that reinforces pi stacking necessary for transport.True Tyrian purple, like most high-chroma pigments, cannot be accurately displayed on a computer display."Tyrian purple" that is used in website design.

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Because of research by Benkendorff et al., the Tyrian purple precursor tyrindoleninone is being investigated as a potential antimicrobial agent with uses against multidrug resistantbacteria.

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ECOTOXNIL®: A POTENT MULTI-SPECIES AND MULTI-FUNCTIONAL PROBIOTIC CONSORTIUM FOR THE EFFICIENT REVITALIZATION OF AQUACULTURE POND BOTTOM ECOSYSTEM

G. Edward Gnana Jothi[a], J. Godfred Ponraj[a] and B. Deivasigamani[b]
[a]TIL Biosciences, The Animal Health Division of Tablets India Limited,
72, Marshalls Road, Chennai 600008.
[b]CAS in Marine Biology, Faculty of Marine Sciences,
Annamalai University, Portonovo 608502.
Email: edwardm40@gmail.com and gp@tabletsindia.com

Introduction

In recent years, aquaculture has grown immensely and due to high economic and export value, penaeid shrimps are of high significance in the global aquaculture market. The annual production of shrimp is approximately 5 million metric tons. However, the current global demand for both the wild and farmed shrimp is more than 6.5 million metric tons per annum (Karthik *et al*., 2014). The commercialisation of aquaculture has been driven by lucrative profits from export markets and fuelled by governmental support, private sector investment, and external assistance. Farmed shrimp accounts for 55 percent of the shrimp produced globally. Albeit, aquaculture farming practices have been highly sophisticated in the contemporary days, pond ecosystem is under threat due to sludge formation, ammonia formation and hydrogen sulphide formation subsequently leading to poor soil and water quality followed by decreased survival and diseases like EMS and EHP. Hence, maintaining the soil and water fertility in the pond ecosystem in addition to restoring the pond condition by reducing the sludge formation and retaining the beneficial microbial population remains as a challenging mission.

A paradigm shift is underway. Increasing numbers of farmers are focused on sustainable aquaculture. An appropriate multi-functional, eco-friendly, non-steroidal, non-antibiotic bio-fertilizer is the product of choice in the current scenario in order to revitalise the pond ecosystem and progress a sustainable aquaculture. The present study investigates the efficient probiotic strain selection, testing the efficacy of the strains against aquatic pathogens, sludge reduction, water and soil probiotic, ammonia reduction, hydrogen sulphide reduction, and characterization of the microbial strains for the multi-species probiotic product ECOTOXNIL®.

Materials and methods

The study was carried out in Minerva Aqua Farm, located at Kurru Island (14˚42'50"N and 80˚7'5"E), Alluru, Nellore, Southeast coast of India (Fig. 1) and Parangipettai (11°28'17.69" N and 79°44'20.22" E) near Vellar estuary (Fig. 2). Several hundred probiotic strains were isolated and efficient strains were selected based on the antibacterial (Boyanova *et al.*, 2005) and antagonistic activity (Lechevalier and Waksman, 1962), pathogenicity in the host animal (Karunasagar *et al*., 1994 and APHA, 1989), stability in various salinities and temperature tolerance. The competence of the probiotic consortium, ECOTOXNIL® as sludge reducer, water and soil probiotic, ammonia reducer, role in promoting growth, survival and feed conversion (FCR) of *Litopenaeus vannamei* were investigated. Three test ponds treated with ECOTOXNIL® and three control ponds were used

in the study for the duration of 119 days. The strains were characterized biochemically (Holt *et al.*, 1994) and confirmed upon molecular characterization (Kurzak *et al.* 1998).

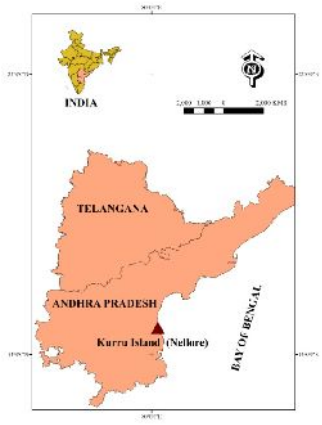

Fig. 1: Map showing the experimental area, Kurru Island, Nellore, Andhra Pradesh

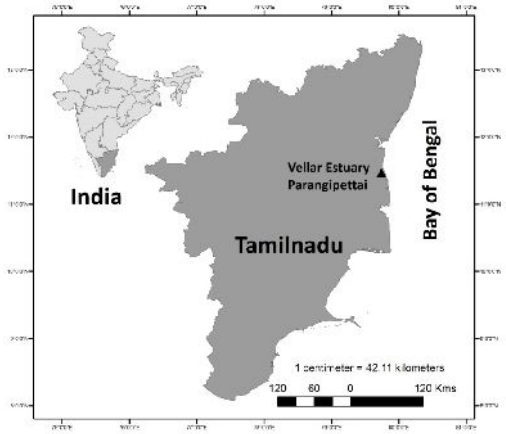

Fig. 2: Map showing the study area, Parangipettai, Tamilnadu

Results and discussion

It is vital to provide shrimp with a healthy environment and probiotics has a great deal of potential (Gomez *et al*., 2000). The strains exhibited wide range of antimicrobial activity and antagonistic activity against various aquatic pathogens were selected and tested for pathogenicity and found to be non-pathogenic in the host animal were then checked for wide range of temperature and salinity tolerance. The selected strains were then applied onto shrimp aquaculture ponds and were found to be highly effective in reducing the ammonia (Fig. 3), hydrogen sulphide (Fig. 4), bottom sludge (Fig. 5), nitrite reduction (Fig. 6) and thus enhancing the bloom (Fig. 7) and maintaining the soil and water condition in the shrimp aquaculture ponds. Subsequently leading to high survival rate (Fig. 8) and decreased FCR (Fig. 9) with lesser production cost.

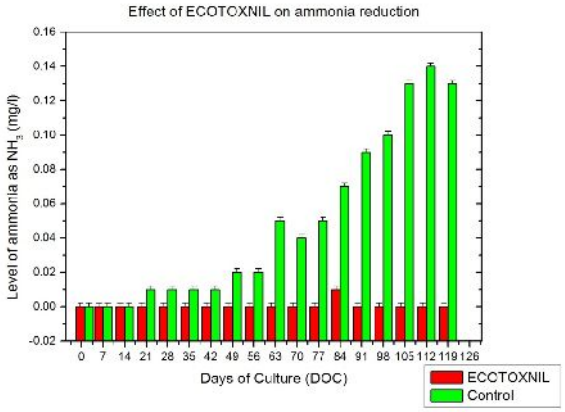

Fig. 3: Level of ammonia recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

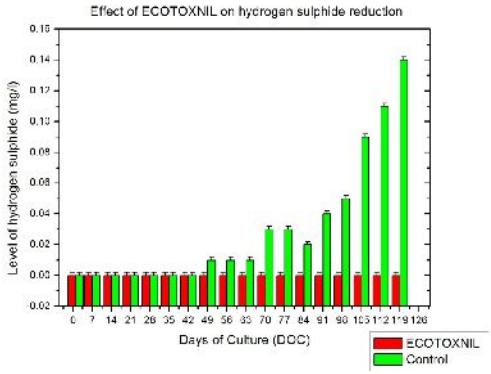

Fig. 4: Level of hydrogen sulphide recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

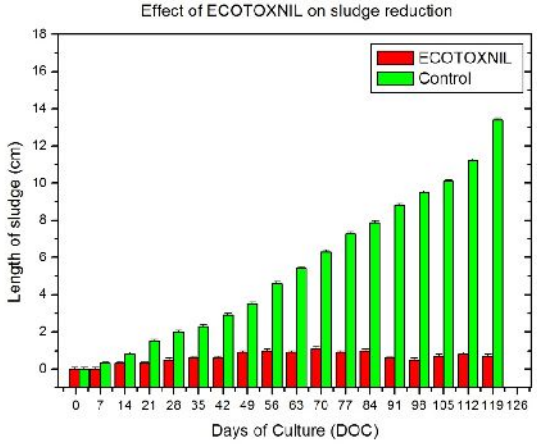

Fig. 5: Length of sludge recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

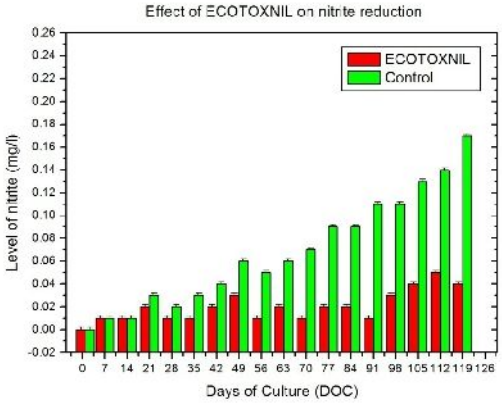

Fig. 6: Level of nitrite recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

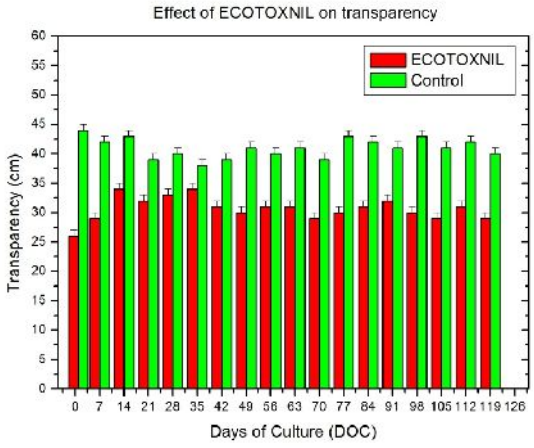

Fig. 7: Values of transparency recorded on control and test ponds for a period of 119 days at regular intervals of 7 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

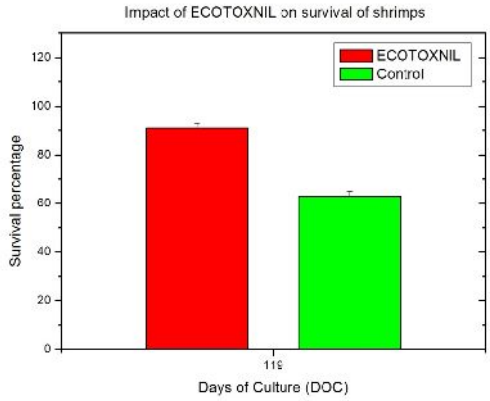

Fig. 8: Values of survival recorded on control and test ponds after a period of 119 days

*Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

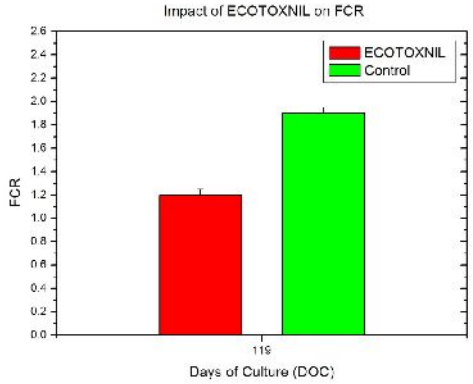

Fig. 9: Values of FCR recorded on control and test ponds after a period of 119 days *Results are presented as means with standard errors of three replicates for each experimental group (mean ± SE; n = 3)

Overall, the shrimp ponds treated with ECOTOXNIL® established remarkable output and high production to the farmers, thus promoting an eco –friendly and sustainable aquaculture.

Conclusion

Shrimps in ponds with ECOTOXNIL® usage unveiled high resistance to pathogens, improved health, higher production, survival percentage, average body weight, feed consumption with attractively lesser FCR. Thus, ECOTOXNIL® will be the product of choice for aquaculture farmers for a sustainable and eco-friendly aquaculture.

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FISH OIL – A BOOST FOR YOUNG ATHLETE

Dr.M.Elango
Associate professor & Head, Departmrnt of Physical Education
The M.D.T.Hindu College, Tirunelveli-10
Dr.J. Karthikeyan
Assistant professor, Departmrnt of Physical Education
The M.D.T.Hindu College, Tirunelveli-10

Omega–3 fatty acids are considered essential fatty acids because humans cannot make them. Therefore, they must be gained through the diet or supplementation. Fish oils are an superb source of omega-3 fatty acids that offer numerous health benefits for everybody, as well as a variety of performance-enhancing effects, such as increasing muscle growth, improving strength and physical performance, reducing exercise-induced muscle injury and delayed-onset muscle distress, combating negative immune effects of intensive training, strengthening bones, improving heart and lung functioning, and enhancing cognitive functioning

Here are the following benefits:

To increase protein synthesis

Protein biosynthesis is the process whereby biological cells generate new proteins. The correct balance of exercise and nutritional building blocks is necessary within the body for muscle growth to occur. Nutritional status is important to optimize this important process. Protein is an essential raw material that supplies the body with amino acids used to repair and build muscle and protein powders are popular supplements within the sports industry. Fish oil is not as well recognized but it may play an important role in muscle growth.

To increase muscle strength and physical performance

Strength is a measure of how much force your muscles can exert.Physical performance is mainly a function of an individual's size, shape, sex, and age, but not entirely so. Success in sport at whatever level also depends on fitness.Omega-3 fatty acids may exert an important influence on muscle function, helping to improve muscular strength, physical performance, and functional capacity.

To reduce exercise-induced muscle damage and delayed-onset muscle soreness

Exercise-induced muscle damage, which is caused by vigorous exercise, can result in delayed-onset muscle soreness and loss of physical function, which can have a profound effect on adaptations to exercise training programs. In addition to reducing exercise-induced inflammation, fish oil supplementation has also been shown to reduce exercise-induced muscle soreness and thus aid the recovery process. Fish oil supplementation may represent a possible nutritional strategy for facilitating exercise recovery and enhancing exercise training adaptations.

To help to combat negative effects of intensive training on the immune system

Regular exercise helps to boost the immune system, however, athletes can be exposed to exercise-induced stress when they consume inadequate diets and when they train too intensely.This exercise-induced stress can lead to the generation of free radicals. Omega-3 fatty acids may offer a solution to help modify blood antioxidant status after exercise and therefore protect against exercise-induced free radical damage. Omega-3 fatty acids may help to improve the body's reaction to exercise-induced stress, with potential benefits for the immune system.

To strengthen bones

Physical activity is widely known to exert positive effects on bone health. While nutritional supplements, including calcium and magnesium have received a lot of attention for their bone benefits, omega-3 fatty acids are usually overlooked despite the fact that dietary fats may play a role in bone health through the inflammatory pathway.

To improve cardio-respiratory functioning

Omega-3 fatty acids have positive effects on exercise parameters in persons with heart disease, including heart rate at rest, heart rate recovery, heart rate variability, exercise capacity, exercise time, and blood vessel dilation. Since omega-3 fatty acids seem to positively impact the cardio-respiratory system in people with heart disease, interest has been expressed regarding fish oil's potential to benefit exercise parameters in healthy individuals and athletes.Workout helps to improve cardiovascular functioning by improving the heart's ability to pump blood and consequently deliver oxygen to working muscles. Dietary fat also impacts cardiovascular performance. Exercise also strengthens and tones the lungs, which enables the pulmonary system to increase the maximum amount of oxygen that the lungs can handle. Fish oil may actually improve lung function during exercise.

To improve cognitive function

Improving complex cognitive ability during exercise may boost performance in sports that have strong cognitive demands. Omega-3 fatty acid supplementation may serve to improve decision making and reaction time efficiency in athletes by increasing communication between the brain and body. Fish oil supplementation may serve as an exciting strategy to improve athletic performance by enhancing concentration and cognitive functioning

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DIVERSITY OF EDIBLE AND NON-EDIBLE MARINE FISHES IN EAST COASTAL REGION VILLAGES AT CHENNAI, TAMIL NADU, INDIA.

Kuppan.A, Martin. P, Kalaichelvi.N, Srinivaasu*.S, Sivamani.S
Post graduate and research department of Zoology, Government Arts college for men (Autonomous), Nandanam, Chennai-600035, India
*Zoological Survey of India, MBRC,Chennai-28

INTRODUCTION

Oceans contain the largest living volume of the ‘‘blue’’ planet, inhabited by approximately 235–250,000 described species, all groups included. They only represent 13% of the known species on the Earth, but the marine biomasses are really huge (Boeuf, 2011). The number of valid marine species is around 16,764, that is about equal to the freshwater fishes (15,170). The increase in knowledge of marine fish biodiversity over the last 250 years is commendable. Biodiversity is essential for meeting human needs. Diversity of organisms within the natural environment is important. Marine fish families fall into several groups, although some fishes are fall into more than one category (William *et al.,* 2010). The US National Oceanic and Atmospheric Administration estimated that 95% of the world’s ocean area is unexplored. Many believed that there are more than 5000 marine species (O’Dor, 2003). The overwhelming value of biodiversity as an indication of an environment health and for the functioning of ecosystems (Aarts and Nienhuis, 1999, Bengtsson *et al*., 1997, Culotta, 1996, Grime, 1997). The declining trend of deep sea fishes in the marine ecosystem is due to anthropogenic activity and overexploitation of marine resources (Alina *et al*., 2012). Progressive anthropogenic impacts on these habitats and their biota have seriously affected the sustenance of the resources and even forcing them into an endangered species. No attempt has been made in the diversity of edible and non-edible fish population in East Coastal Region villages of Chennai and hence the present investigation aimed to assess the diversity of edible fishes in the selected study sites in East Coast Region villages of Chennai, Tamilnadu, India.

MATERIALS AND METHODS

Sample collection

Fishes were collected early in the morning from different region of East Coastal Region villages (Kovalm, Kanathure, Panaiyur, and Nochikuppam) of Chennai, Tamil Nadu (Fig.1). Fishing vessels were equipped with icing systems and fish were kept at lower temperature to keep afresh. All fish samples were collected before sorting to avoid biasness on size. After collection, they were immediately preserved with ice in the ice box and transported to the laboratory. Samples were collected during the year 2015. The collected fish were transported to Zoological Research Laboratory, Government Arts College, Nanadanam, Chennai-35. The fishes were identified with the help of manual and books (Smith and

Fig. 1. Map showing the study area

Heemstra, 1986, Munro, 1955, Day, 1878)**.** The identified fishes were properly labeled and arranged in the racks of Zoology museum maintained in our Department.

RESULT AND DISCUSSION

Edible fishes

For the present investigation, 54 marine edible species were collected from Chennai East Coastal Region. They were categorized in to three groups namely food fishes, ornamental fishes, and trash fishes. Out of these 54 fishes 35 fishes were found under edible fish and the remaining 19 as non-edible category. Under the edible fishes, 25 species were found under the food fish followed by 10 species as ornamental fish. Food fishes were predominant in the order Perciformes (16 species) followed by Clupeiformes (5 species), Pleuronectiformes (2 specis), Beloniformes (1 species), Siluriformes (1 species). The total number of ornamental fishes collected under the Perciformes was 8 species and in Aulopiformes it was 2 species (Table.1). The most abundante families were Carangidae, Clupeidae, Gerreidae, Liognathidae, Sphyraenidae, Terapontidae, Lutjanidae, Siganidae and Synodontidae. The fishes belonged to the family *viz.* Clupeidae and Engraulidae, Belonidae and Scombridae were found abundant seasonally. Clupeidae and Engraulidae fishes were abundant during the period from January to May 2014, whereas Scombridae and Belonidae fish occurrence was maximum from October to December of 2014 (Kuppan and Martin, 2015).

Table.1 Edible fishes collected from East Coast Region, Chennai during year 2015.

Category	Species collected	Family name	Order	Status
Food fish	*Tylosurus crocodilus crocodiles*	Belonidae	Beloniformes	**
	Sardinella fimbriata	Clupeidae	Clupeiformes	**
	Dussumieria acuta	Dussumieriidae	Clupeiformes	**
	Pellona ditchela	Pristigasteridae	Clupeiformes	*
	Stolephorus indicus	Engraulidae	Clupeiformes	**
	Sardinella albella	Clupeidae	Clupeiformes	*
	Atule mate	Carangidae	Perciformes	**
	Sphyraena putnamae	Sphyraenidae	Perciformes	*
	Trichiurus lepturus	Trichiuridae	Perciformes	*
	Terapon jarbua	Terapontidae	Perciformes	*
	Sphyraena obtusata	Sphyraenidae	Perciformes	*
	Secutor insidiator	Leiognathidae	Perciformes	*
	Alepes djedaba	Carangidae	Perciformes	**
	Pomadasys maculates	Haemulidae	Perciformes	**

	Tripterodon orbis	Ephippidae	Perciformes	*
	Terapon puta	Terapontidae	Perciformes	**
	Mugil cephalus	Mugilidae	Perciformes	*
	Gerres filamentosus	Gerreidae	perciformes	-
	Gerres erythrourus	Gerreidae	perciformes	-
	Secutor ruconius	Liognathidae	perciformes	*
	Rastrelliger kanagurta	Scombridae	Perciformes	**
	Sillago sihama	Sillaginidae	Perciformes	*
	Psettodes erumei	Psettodiadae	Pleuronectiformes	-
	Synaptura commersonii	Soleidae	Pleuronectiformes	**
	Plotosus lineatus	Plotosidae	Siluriformes	-
Ornamental fish	*Synodus dermatogenys*	Synodontidae	Aulopiformes	-
	Trachinocephalus myops	Synodontidae	Aulopiformes	-
	Cephalopholis Formosa	Serranidae	Perciformes	*
	Acanthurus mata	Acanthuridae	Perciformes	*
	Siganus argenteus	Siganidae	Perciformes	**
	Siganus canaliculatus	Siganidae	Perciformes	-
	Monodactylus kottelati	Monodactylidae	Perciformes	-
	Lutjanus lutjanus	Lutjanidae	Perciformes	*
	lutjanus quinquelineatu	Lutjanidae	Perciformes	*
	Upeneus vittatus	Mullidae	Perciformes	*

** Most abundant; *abundant; -rare

Non- edible fishes

In the present investigation, 19 non- edible fish species belonging to 9 families and 6 orders recorded (Table 2). Maximum number of fish species were collected under the order Perciformes (52.6%) followed by Tetraodontiformes (26.3%), Beryciformes (10.5%), Scorpaeniformes (5.2%) and Syganidae (5.2%). The predominant number of species collected under the family Apogonidae followed by Pempheridae, Diodontidae, Holocentridae, and Tetraodontidae. Majority of non-edible fishes were collected under Tetraodontiformes and Beryciformes. Out of 19 non-edible fishes, maximum number of non-edible species (17 species) used for ornamental purposes which belongs to the order Perciformes. Number of fish species caught under different families *viz.* Holocentridae (2 species), Chaetodontidae (1 species), Labridae (1 speceis), and Pempharidae (3 species)

Tetraodontidae (2 species). Some species were noticed occasionally and they belong to the family Fistulariidae under the order Syngnathiformes. Most of the ornamental fishes were collected from rocky and reef area where the quantity of reef fishes were abundant and they belong to the families such as Pempharidae, Holocentridae, and Labridae. Deep-reef species were defined as those fishes which are associated with coral reefs and live below 50 meters to 500 meters (William *et al.*,2010)**.**

Table.2 Diversity of non-edible fishes collected from East Coastal Region Chennai during the year 2015.

Species	Family	Order	Status	Category
Myripristis botche	Holocentridae	Beryciformes	**	Ornamental
Sargocentron rubrum	Holocentridae	Beryciformes	**	
Apogon multitaeniatus	Apogonidae	Perciformes	*	
Apogonichthyoides nigripinnis	Apogonidae	Perciformes	*	
Apogonichthyoides pseudotaeniatus	Apogonidae	Perciformes	*	
Chaetodon decussates	Chaetodontidae	Perciformes	**	
Iniistius bimaculatus	Labridae	Perciformes	**	
ostorhinchus aureus	Apogonidae	Perciformes	*	
Pempharis mangula	Pempheridae	Perciformes	**	
Pempharis molucca	Pempheridae	Perciformes	**	
Pempheris mangula	Pempharidae	Perciformes	**	
Choridactylus multibarbus	Synanceiidae	Scorpaeniformes	*	
Diodon histix	Diodontidae	Tetraodontiformes	*	
Diodon holocanthus	Diodontidae	Tetraodontiformes	*	
Lagocephalus lunaris	Tetraodontidae	Tetraodontiformes	**	
Malichthys indicus	Balistidae	Tetraodontiformes	*	
Takifugu oblongus	Tetraodontidae	Tetraodontiformes	*	
Tryssa malabaricus	Engraulidae	Perciformes	*	Trash
Fistularia commersonni	Fistulariidae	Syngnathiformes	_	

** Most abundant; *abundant; -rare

Variety of fishes were collected using different mesh size of gill net. The predominant species in the study area are Anchovy, Sardine, and Mackerel. The seasonal fish are more

economically important for fisherfolk. Present findings are coinciding with the earlier findings of Raghu Prasad and Ramachandran Nair (1973). He reported that out of 2.5 million tonnes of fishes collected from Indian Ocean consisting of Herrings, Sardines, Anchovies and related forms contribute about 28% of the total catch from the Indian ocean. The group mackerels, billfishes, etc account for 8.6% (Anon, 1967). Veleppan Nair (1953) reported that the Clupeid fishes are second to none in the commercial fisheries of the India and they contribute more than one third of the total marine fish production of the country. The sardine, hilsa, anchovies, white baits etc. are the more important fishes of the group which support the very rich fisheries of our water. In general, the total marine fish production of India has been increasing trend, but considerable fluctuations are noticed in the annual landings in certain years. Three fisheries viz, oil sardine, Bombay duck and mackerel constitute nearly forty two per cent of the total catch and the landings of each of these show wide variations.

CONCLUSION

Aesthetic nature of marine ecosystem was reduced drastically by involving much anthropogenic activities in coastal area. The declining trend was noticed in marine fish diversity. Variety of fishes collected must be graded consumable and non consumable fishes at spot catch itself. Consumable fishes can reach consumer and non consumable fishes must be thrown back. This present findings envisage the diversity of non-edible fish in the specified catchment area is the first report. From the finding fishes at East Coastal Regions can be categorized into food fish, ornamental fish and trash fish. The present study is the preliminary attempt made by the researchers. Further extensive research is needed for the diversity and conservation of fish species in East Coastal region.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and Head of the Department of Zoology, Government Arts College, Nandanam for providing necessary facilities to carry out the work.

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GENOTOXICTY EFFECTS OF 2,4- DICHLOROPHENOXYACETIC ACID ON FRESHWATER FISH *CHANNA STRIATUS* USING COMET ASSAY

J. Anusuya, and S. Hemalatha*****
Department of Zoology(UGC-SAP Sponsored) AnnamalaiUniversity,
Annamalai Nagar-608002

Introduction

Occupational exposure to pesticides is a common and alarming worldwide phenomenon. Various industrial and agricultural activities increase pollution, particularly in the aquatic environment which is contaminated by various toxic chemicals from the discharged from waste waters and agricultural drainage (Binelli and Provin, 2004). Fish are particularly sensitive to environmental contamination of water and therefore pollutants may significantly damage certain physiological and biochemical processes when they enter into fish organs (Storm *et al.*, 2000). Bioaccumulation of insecticide and heavy metals in the tissues of aquatic organisms may be harmful for human consumption (Elia *et al.*, 2006).

2,4-Dichlorophenoxyacetic acid (2,4-D) belonging to chlorophenoxy group has been in extensive use uninterruptedly since 1947 in agriculture for broad-leaved weeds, control of woody plants and reforestation programs. In agriculture 2,4-D herbicide is used on cereal, grain crops and sugar cane to the broadleaf weed control (Colby *et al.*, 1989). Commercial formulation containing 2,4-D herbicide is a strong acid and forms water-soluble salts with alkali metals and amines, solubility (mg L_1) in water is 23-180 (pH 7), 34–196 (pH 9) (

Tomlin, 2004). On the other hand, 2, 4-D poisoning was found to provoke disruption of hematopoietic tissue cell components and protein structures in *Tinca tinca* (Gomez *et al*., 1998).

Fish erythrocytes are important biological cells for assessment of genotoxicity of environmental contaminants in fish (Ergene *et al*., 2007). Besides MN, fish erythrocytes are conveniently used for the comet assay to detect single strand DNA breakage (Ateeq *et al*., 2005). Many of the chemicals have the ability to interact with DNA and can lead to neoplasms (De Flora *et al*., 1991; Bhaskaran *et al*., 1999), gene mutations (Maccubin *et al*., 1991) or genetic disease syndromes (Kurelec, 1993) in the aquatic organisms, particularly fishes. The use of fish erythrocytes in various comet assays has further allowed finer details (Moretti *et al*.,1998) Similarly, induction of single strand DNA damage by the heat shock temperatures (34, 36, and 38^{o} C) supports these types of observation where an increase in comet tail length in kidney cells was observed in gold fish, C. auratus (Anitha *et al*., 2000). Therefore, the present study was conducted to investigate the genotoxic effects of 2,4-D using comet assay in erythrocytes of *Channa striatus* exposure *in vivo*.

Materials and Methods

Experimental fish specimens and chemicals

Healthy specimens of freshwater fish *C. striatus* were procured from the local outlets. The specimens had an average wet weight and a length of 150 ± 2.0 g and 16 ± 2 cm, respectively. Fishes were washed with 0.1% of potassium permagnate solution to avoid dermal infection. Before the experiments, they were acclimated under laboratory conditions for two weeks at a population density of 10 specimens in 100 L aquaria containing aerated. dechlorinated tap water. During acclimatization fish were fed with pieces of live earthworm on alternate days. Water in the tank was also changed once in 24h. Chemicals: 2,4-Dichlorophenoxyacetic acid, enzyme assay kit (Cayman chemicals).

Experimentation:

Three groups of fish set up which constitutes Group I: Control, Group II: 50 mg/L of

2.4-D and Group III: 100mg/L of 2, 4-D treated fish

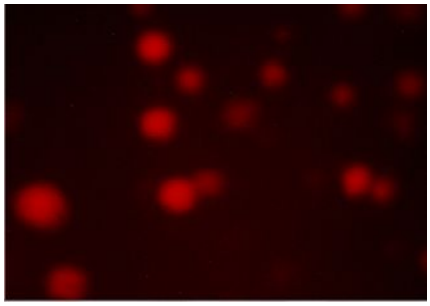
Comet Assay: Comet assay was performed according to the Tice *et al*., (2000) with some modification. Blood were collected from heart of fish. About 15 µL of the blood was mixed with 145 µL of low-melting point agarose (LMP 0.5%). A 40 µL of layered on the microscopic slide which was precoated with normal melting agarose (NMA 1%). The slides kept in refrigerator for 10 min. After solidification, the slides were immersed in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-base, pH 10, with 10% DMSO and 1% Triton X-100 added fresh) inside refrigerator at 4^{o}C for 1 hour. The slides were placed side by side in electrophoresis chamber containing fresh and cold buffer(300 mM NaOH, 1 mM EDTA, pH 13) for 45 min in refrigerator (4^{o}C). Eectrophoresis was done at 25 V and 300 mA(25-30 min at 4^{o}C). Observation slides were done with the CETI fluorescent microscope (Model: 3100.5000- Triton II) that equipped with Sony camera (Model: No.DSC-H9). Two slides per specimen were prepared and 50 cells per slide (300 cells per concentration) were scored randomly. The DNA damage was measured by visual classification of cells into five type "comets" according to the tail length (Cavas, 2011; Lee and steinert, 2003); 0: undamaged, 1: low damage, 2: moderate damage, 3: high damage and 4: complete damage. A genetic damage index (GDI), arbitrary units, was employed as below (Grisolia *et al.*, 2009 ; Silva *et al.*, 2000). GDI = (n1+2n2+3n3+4n4) / (Σ / 100) GDI: Genetic Damage Index, n1: Minimum damage, n4: Maximum damage, Σ: Total number of the cells.

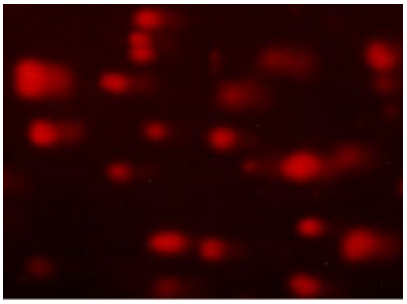
Results

The amount of DNA damage in the cells was assessed from visual grouping as degree of material hereditarily migration and appeared by Genetic Damage Index (GDI). In group I there is no GDI. But in group II the fish exposed to concentration of 50mg/l of 2, 4-D exhibited fundamentally DNA damage in their blood cells than the control groups I (Table 1; Fig. a &b). Maximum GDI noted after 14 days when compared to 7days. After that group III the fish exposed to concentration of 100mg/l of 2, 4-D exhibited fundamentally higher DNA damage in their blood cells than the control and groups II (Table 1; Fig. c).

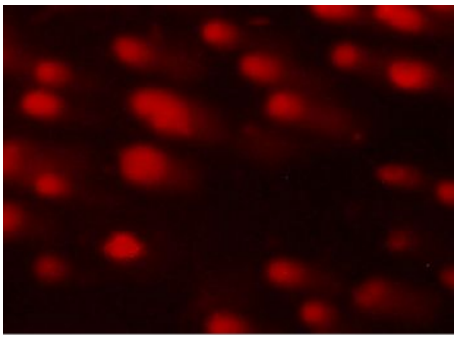
Experimental Groups	Duration (in days)		
	0d	7d	14d
Group I	0	0	0
Group II (50mg/l)	0	1	2
Group III (100mg/l)	0	4	5

Table 1.Genetic Damage Index (GDI) in blood cells of *C. striatus* in different experimental groups 0, 7 and 14 days





(a) control (Group. I) (b) **Exposure to 50mg/l of 2,4-D (Group. II)**



(c) Exposure to 100mg/l of 2, 4-D (Group. III)

Discussion

The present study demonstrated that the Comet assay can be applied effectively in fish, for evaluation of genotoxic potential of 2,4-D. The DNA damage detected in this study could have originated from DNA-single-strand breaks DNA doublestrand breaks, DNA adducts formations and DNA–DNA and DNA–protein cross links (Mitchelmore and Chipman, 1998) resulting from the interactions of herbicides or their metabolites with DNA (Fairbrairn *et al.*, 1995). Significant rise in DNA damage was measured by using various endpoints such as tail length, tail movement and percentage of migrated DNA (Pavlica *et al.*, 2001). The results indicate statistically higher values for both micronuclei and DNA migration in the form of comet tail (Russo *et al.*, 2004). Comet assay definitely offers considerable advantage over other cytogenetic methods of DNA damage detection such as chromosome aberrations, sister chromatid exchanges and micronucleus test in that the cells studied need not be mitotically active (Pavlica *et al.*, 2001).

In the present study, the DNA damage was higher in 2,4-D exposed group indicate the genotoxic potential of 2,4-D on *Channa striatus*. Mitchelmore and Chipman (1998) recommended that the DNA strand breaks, particularly as measured by the comet assay, act as a biomarker of genotoxicity in fish and other aquatic species. Insecticides/pesticides have been reported to lead to DNA damage which appears in the form of micronucleus formation, chromosome aberrations and mitotic aberrations (Sankar *et al.*, 2010). The electron-rich atoms in DNA are readily attacked by electrophiles and transfer of methyl, ethyl or alkyl group causes phosphorylation or alkylation resulting in mutagenic or clastogenic effects (Patil *et al.*, 2003). The single cell gel electrophoresis (SCGE), known as comet assay, is recognized as one of the most sensitive and reliable methodologies available for DNA strand break detection with the advantages of being fast, simple and applicable to any eukaryotic cell type *in vivo* as well as

in vitro (Kumaravel and Jha, 2006). As per a few study, comet assay has been effectively applied in blood cells of numerous fish species. Thus, fish are suitable for observing of genotoxic poisons in aquatic environment (Abdul-Farah et al., 2003; Cavas and Ergenekara, 2005).

Acknowledgement

The authors are very much thankful to the University Grants Commission (UGC-SAP) for the funding resource.

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TO STUDY THE AQUIFER CHARACTERISTIC AND SALT WATER INTRUSION BY GEOELECTRICAL AND GEOCHEMICAL METHOD AT THUTHUKUDI, TAMILNADU, INDIA

D.Muthu Raj[1] E.Kumar[2] A.Jenufa Begam[*], and S.Karthiga Devi[*].
[1]Associate Professor in Physics, The M.D.T. Hindu College, Tirunelveli, India.
[2]Assistant Professor in Physics, Tamil Nadu Open University, Chennai, India.
[*] M.Phil Scholars, The M.D.T. Hindu College, Tirunelveli, India.
[1]Corresponding author: E-mail: muthurajmdt@gmail.com

Introduction

Anthropogenic pollution of shallow groundwater resources to seawater intrusion and industrial activities is becoming a cause of concern in the east coastal belt Thuthukudi (78^0 7’30’’E and $8^0$48’45’’N**)**, Tamil Nadu, India. It is one of the most common problems in the coastal aquifers around the study area. The study area as shown in Figure (1) is the harbor city, industrial and coastal city situated along the Gulf of Mannar Bio-reserve. The coastal aquifers are often getting polluted for every moment due to several natural and the human based activities such as intrusion of saline water and Chemical weathering of the natural geological deposits.

Here, saline water displaces or mixes with freshwater in aquifers. This phenomenon can be attributed to a variety of conditions like gentle coastal hydraulic gradients, tidal and estuarine activity, sea level rise, low infiltration, environment and excessive withdrawal, and local hydrogeological conditions (Sarma et al, 1982, Nowroozi et al, 1999); Kim et al, 2006).Geophysical and Geochemical studies along the coastal zone have been extended in the last few decades due to the growing awareness of coastal pollution and its impact on the groundwater. Integrated geochemical, geophysical studies were carried out in the coastal region encompassing an industrial complex. The objective has been to gain knowledge of aquifer characteristics, which would in turn reveal the possibility of contamination of groundwater regime. Application of various geophysical techniques like Resistivity methods are also used to map the freshwater-saltwater interface and for studying conductive bodies of hydrogeological interest by various researchers (Khair and Skokan, 1998, Gnanasundar and Elango, 1999, Mukhtar et al, 2000).

Hydrogeochemical study is a useful tool to identify the processes such as weathering, dissolution, precipitation, ion exchange and various biological processes which commonly takes place below the surface. The present study is aimed mainly to understand the geophysical-chemical characteristics of groundwater and to study the resistivity distribution of groundwater in the study area by applying the Schlumberger vertical electrical sounding (VES) technique and followed by chemical analysis of water samples in and around Thuthukudi.

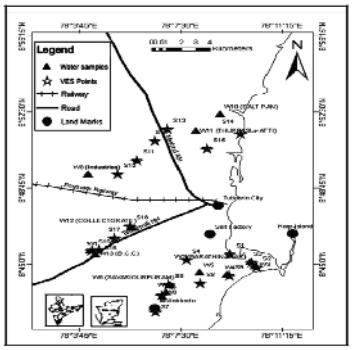

Figure (1): The study area Thuthukudi

Methodology

Hydrogeological and geophysical investigations (resistivity survey) were carried out for deciphering subsurface litho zones, understanding prevailing hydro geological conditions, evaluation of aquifer parameters in the study area. Groundwater occurs in the weathered and fractured zones of gneisses and sandy aquifers in sedimentary and alluvial formations. The resistivity sounding technique using Schlumberger method (Orellana and Mooney1966; Bhattacharya and Patra 1968 ;) was employed to delineate geoelectrical subsurface structures in the study area. To estimate the aquifer properties, chemical analysis of water sample was carried out at 13 locations in the area and the data were analyzed. The data generated was utilized for the development of aquifer characteristics.

Geoelectrical method

Application of various geophysical techniques for the investigation of ground- water pollution sites is often a rapid, cost-effective means of preliminary evaluation. Relationships between aquifer characteristics and electrical parameters of the geoelectrical layers have been studied and reviewed by many researchers (Kelly, 1993, Mazak et al, 1990, Lois et al, 2004). Resistivity methods are also used to map the freshwater-saltwater interface and for studying conductive bodies of hydrogeological interest by various researchers (Baronga and Palacky, 1991, Khair and Skokan, 1998, Gnanasundar and Elango, 1999, Mukhtar et al, 2000). From any combination of lithological types, the saltwater can be distinguished easily by the resistivity methods.

The Geophysical method consisting of vertical electrical sounding (VES) survey has been carried out in the Thuthukudi area from the coast to the land in order to know the variation of resistivity of the aquifer and to demarcate the quality of the ground water. A total of twenty Vertical Electrical Soundings (VES) were conducted using Schlumberger electrode configuration for a spreading up to 100m current electrode separation. Initially, VES data have been interpreted through curve matching technique (Orellana and Mooney, 1966) and then interpreted by computer program. An electrical investigations using vertical electrical soundings VES is conducted for solving several subsurface structural and groundwater related problems like salt water intrusion and pollution caused by industrial effluent and

characterization of ground water aquifer quality is also a one of the prime objective of this thesis.

The VES study is conducted at twenty locations is shown in figure 1 as S_1 to S_{20} in the study area using Schlumberger electrode configuration to understand the electrical distribution along the vertical direction. One-dimensional inversion of the VES data was carried out using an interactive inversion code IPI2Win (Bobachev, 2003) for quantitative analysis. The modeled layer parameter shows the resistivity and thickness of each layer and also the result of modeled VES data as shown in Table 1

.To obtain a more accurate resistivity image from the subsurface of the area, 2D inverse modeling was carried out by the selected Schlumberger responses of the VES curves with interpreted layer parameters. The apparent resistivity variation with respect to the current electrode separation is presented as Pseudo cross-section and resistivity cross-section as show in Figure 2

However, the pseudo section gives a picture of the subsurface of the study area. The creation of 2D model with true resistivity represents the subsurface electrical properties. The apparent resistivity variation with respect to the current electrode separation is presented as Pseudo cross-section and resistivity cross-section. However, the pseudo section gives a picture of the subsurface of the study area. The creation of 2D model with true resistivity represents the subsurface electrical properties. Here, 2D electrical resistivity pseudo cross section is prepared based on the apparent resistivity VES data (S_1 to S_{20}). Further the quality changes of the groundwater aquifer at this place is confirmed by the geochemical analysis data of chloride (Cl),Potassium (K),Electrical conductivity (EC) and Total dissolved solid (TDS)

S NO	ρ_1 **hm-m**	**hm-m**	ρ_3 **hm-m**	ρ_4 **hm-m**	ρ_5 **hm-m**	h_1	h_2	h_3	h_4	**$H=h_1+h_2+h_3+h_4$ m**
	9.69	.357	.342	6.79		.284	.528	6.02		18.83
	.049	.6363	.3221	.172		.3891	.933	.858		6.18
	3.7	.6	9.5	.05		.559	.663	.62	.11	12
	2	2.1	.446	11		.77	.802	.32		7.89
	.69	.31	6.4	.241	24	.759	.626	.51	.95	8.84
	0.1	.31	.471	24		.825	.21	0.1		12.1
	1.3	.659	00			.952	4.6			15.6
	7	.46	2.4			.24	1.2			13.4
	.232	.14	.107	.51		.02	.65	.13		4.8
										14.6

0	2.9	4.4	1.1			.86	2.7			
1	5.4	7.6	70	0.3		.403	.42	.06	1.2	21.1
2	4.9	.32	.02	5.6		.823	.22	.38		8.42
3	0.83	4.8	7.95	86.2		.5676	.385	.239		10.19
4	3.7	.6	9.5	.05	80	.56	.663	.62	.12	12
5	2.9	.75	.93	563		.457	.25	.85		9.56
6	.52	.81	3.7	.982	85	.649	.21	.26	3.6	12.2
7	3.6	.92	1.3	.86	000	.17	.72	.55	.23	14.7
8	.11	1.7	.8	09		.208		5.3		19.5
9	20	8.9	7.3	.79	21	.26	.87	.2	1.4	16.7
0	53	.83	5.6	.948		.352	.552	.72	.31	9.93

Table: 1 Results of modeled VES data at Tuticorin

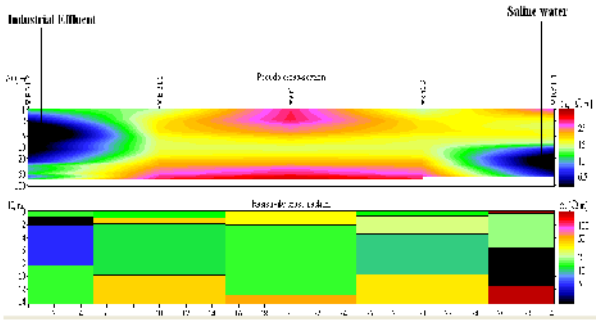

Figure 2: Pseudo cross-section and resistivity cross-section

Geochemical method

In order to get representative subsurface parameters, the local water sample collection and analysis for the evaluation of groundwater quality was carried out. A total of thirteen groundwater samples were collected from dug wells, tube wells and bore wells distributed throughout the area. To estimate the aquifer properties, water sample chemical test was carried out at thirteen locations on the area and the data were analysed. The sample locations in the study area are depicted in Figure (1). Immediately after sampling, pH and electrical conductivity were measured in the field. Total dissolved solids (TDS) were calculated from EC multiplied by 0.64. Carbonate (CO_3), Bicarbonate (HCO_3), Calcium (Ca) and Magnesium (Mg) were determined by Titrimetric Method. Sodium (Na) and Potassium (K) were

determined. The collected samples were analyzed to determine their hydrochemistry and the results obtained from the analysis were tabulated in Table 2.

Table.2. Chemical quality of ground water (Ionic concentration in mg/l)

Sl.No	Sample	pH	EC µS/cm	CO_3	HCO_3	Cl	$S0_4$	Ca	Mg	Na	K	TDS mg/l
1	W1	7.7	2100	0	45	401	-	74	100	165	73	1344
2	W2	8.2	4180	39	78	791		124	60	610	14	2675.2
3	W3	8.3	1110	18	41	38	-	16	167	120	6	0710.4
4	W4	8.2	4430	18	76	560	100	96	217	570	27	2835.2
5	W5	7.3	2680	0	52	344	50	66	26	80	54	1715.2
6	W6	8	3800	12	11	28	-	10	74	24	2	2432
7	W7	8.1	1800	39	43	25	-	72	54	150	68	1152.0
8	W8	7.9	1930	27	20	415	20	128	296	175	24	1235.2
9	W9	8.2	7360	21	67	752	--	22	900	144	10	4710.4
10	W10	6.9	8200	0	22	479	-	501	22	960	23	5248
11	W11	8.8	1080	30	28	121	4	8	36	184	5	691.2
12	W12	8.5	1110	24	35	92	-	28	472	151	6	710.4
13	W13	7.9	8400	9	43	269	-	335	100	428	6	5376.0

Table 3. Hydrochemical Parameters of water samples and WHO Guidelines

S. No	Parameter	Maximum permissible level set by **WHO**	Samples which exceed permissible limits of WHO standards
1.	TDS (mg/l)	1000	W_1, W_2, W_4, W_5, W_7,W_8,W_9,W_{10},and W_{13}
2.	EC (µS/cm)	1400	W_1,W_2,W_4,W_5,W_6,W_7,W_8,W_9,W_{10}, and W_{13}
3.	Sodium(mg/l)	200	W_2,W_4,W_{10},W_{13}
4.	Chloride (mg/l)	250	W_1,W_2,W_4,W_5,W_8,W_9,W_{10},W_{13}
5.	Calcium (mg/l)	200	W_{10},W_{13}

In the study area, the water samples W_1, W_2, W_4, W_5,W_8,W_9,W_{10}, and W_{13} contain higher chloride values than permissible limit. Like chloride the water samples W_2, W_4, W_{10}, and W_{13} collected in the study area shows high concentration of sodium than the permissible limit as show in Table 3 as the high values of these parameters than the permissible range of WHO reveal the saline nature of the aquifer. The variation of Chloride, TDS and EC at a few locations is compared with of resistivity cross-section and pseudo cross-section.

Results and discussion

The Thuthukudi city appears to have an increasing water demand as a result of continuous industrial and urban development. The alluvial nature of geological formation around the industrial and urbanized zone has a great impact on the overall hydrogeological characterization of the aquifer. The present study demarcates the salinity zone in the area based on geophysical measure relating to geochemical parameters (Choudury et.al 2001 Sherif, et.al 2006).

The ground water samples were also collected from wells or bore wells from as nearer as possible to have the correlation between electrical resistivity measured from geophysical method and electrical conductivity and other chemical parameters measured from chemical analysis of water samples. The integration of electrical resistivity method and geochemical analysis has revealed a good picture of groundwater quality in the study area.

The hydrogeological conditions and geochemical analysis were used to evolve a aquifer characteristic in the study area. The measured geophysical and geochemical data of the study area shows the characteristics of groundwater aquifer. The hydro chemical characteristic of coastal aquifers in Thuthukudi, Tamil Nadu, seems to be influenced by various processes together with seawater mixing, anthropogenic contamination, and water–rock interaction, as indicated by very wide ranges and high standard deviations of most hydro chemical parameters, such as TDS, Cl^-, $SO2^-_4$, $Mg2^+$, and Na^+ exceeding the limit of drinking water standard (WHO 1984).

The geochemical and geoelectrical properties are used to demarcate the saline baseline and contaminated water zones. The apparent resistivity measured data was interpreted qualitatively and quantitatively in the study area can be explained by the resistivity distribution. The correlation was made between chemical analysis of water samples like electrical conductivity (EC), Total dissolved solid (TDS), chloride present with electrical resistivity of the aquifer interpreted from the geophysical method. As a result, the integration of geophysical and geochemical method has distinguished different types of hydro geologic behavior, a high saline coastal aquifer, water contaminated with effluents and freshwater areas. The information consequently obtained represents a base for future geophysical and geochemical work that will help in the planning, protection, and decision-making regarding groundwater management in coastal aquifers.

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CHEMICAL ELEMENTAL CHARACTERISTICS OF WOODY BIOMASS FUELS IN COASTAL AREAS

SEETHALASHMI.A.N
Department of Physics,
The M.D.T. Hindu College, Tirunelveli 627 010, India
Email: dransmdt@gmail.com

Introduction

Biomass fuels are the first energy source harnessed by mankind. They remain the primary source of energy for more than half the world's population and account for 14% of the total energy consumption in the world (McKendry, 2002). In Andamans, farmers grow

Gliricidia sepium and *Prosopis juliflora* as live hedges. In coastal areas, *Casuarina equisetifolia* and other trees were grown in association with crops on farm lands for cash and to generate small timber (Salil K. Tewari, 2008).

Biomass is the most common form of renewable energy. The use of renewable energy sources is becoming increasingly important when it is considered to assist to alleviate global warming and provide fuel supply. It is time to explore and utilize the biomass fuels in a clean and more efficient way; however, there is a conspicuous lack of knowledge with regard to the chemical elemental characteristics of biomass fuels which is very important for utilization of them as energy sources. Modeling and analysis of energy conversion processes require adequate fuel characteristics especially average and variations in elemental compositions (Nordin, 1994).Knowledge of concentration and speciation of alkali elements in fuels is useful for biomass power generation topics. With regard to the utilization of biomass as an energy source, the investigation of chemical elemental characteristics of biomass fuels is beneficial for biomass fuels to find suitable energy conversion technologies and for various energy conversion process to utilize favorable biomass feedstock. In this paper, chemical elemental characteristics of biomass fuels in coastal areas are presented.

Materials and Methods

We developed protocol in order to characterize the chemical and elemental properties of*P. juliflora* as feed stock for energy conversion process *C. equisetifolia* and *G. sepium* are also two woody biomass species in coastal regions are taken for investigation. One to two kilograms of biomass was collected from the 5 years of energy plantation. They were oven dried at 80 °C during 48 h. The samples were then ground into powder. The sample prepared with powder by pressing it in pellet form with 1.3 mm diameter and 1.12 mm thickness at a constant pressure of 5 metric tons.

The analysis program includes proximate analysis of moisture, ash, volatile matter and calculation of fixed carbon by ordinary oven and muffle furnace. The calorific value of the sample was measured using bomb colorimeter. Elements presented in the sample were identified using EDAX (JEOL Model JED-2300) analysis. The presence of functional groups in the samples was recorded using FTIR spectrum in the range of 4000-400 cm^{-1}.

Results and discussion

Proximate analysis results

The moisture content, ash content, volatile matter and fixed carbon of woody materials *P. juliflora, C. equisetifolia,* and *G. sepium* are given in Table 1. Moisture content is of considerable importance with regard to selection of energy conversion process technology while biomass fuels with high moisture content are more suited for biochemical process such as fermentation conversion (McKendry, 2002). Moisture content can also affect the combustion efficiency. High moisture content in biomass results in lower overall efficiency of the combustion process, since a considerable amount of energy is needed to remove the moisture (Porteiro, *et al.,* 2010; Tumuluru, *et al.,* 2011).On this basis from Table 1, it can be seen that woody biomass fuels under this investigation are most favorable biomass feedstock for thermal conversion technology with their moisture content in the range 5-6%. The ash content of biomass affects both the handling and processing costs of overall biomass energy conversion cost. The chemical composition of the ash is closely related to

operational problems such as slagging, fouling, sintering and corrosion(Jenkins, *et al.,* 1998; Vamvuka, *et al.,* 2003).. From Table 1 it can be seen that ash content ranged from 1-5%. Volatile matter is in the range of 78-85%. While the ash content and volatile matter in bituminous coal is 20% and 28% respectively. The results of ash content obtained in our work (Table 4) for *P. juliflora* are found to be in agreement with the reported value (Patel et al., 1986; Rajput and Tewari, 1986; Khan, *et al.,*1986; Madan and Tandon, 1991; Goel and Behil, 1992). The volatiles in biomass promote better burnout of the fuel and lower NO_x emissions (Hein and Bemtgen, 1998). So the three woody fuels have the advantages of low ash and high volatile that make the ideal feedstock for pyrolysis and gasification.

The calorific value is one of the most important characteristics of a fuel, and it is useful for planning and controlling of the combustion plants. It indicates the amount of heat that develops from the mass (weight) in its complete combustion with oxygen in a calorimeter standardize.Higher proportion of carbon content leads to high calorific value 4200 kcal/kg (Khan, *et al.,* 1986).The calorific values of the selected samples (Table 1) are in the range 3500-3800 kcal/kg. The calorific value of bituminous coal achieves 3500 kcal/kg. The lower content of nitrogen and sulphur in biomass fuels compared with coal is especially important for environment protection.

Table 1. Proximate analysis of woody biomass materials *P. juliflora, C.equisetifolia* and *G. sepium*

BIOMASS	MOISTURE (%)	ASH (%)	VOLATILE MATTER (%)	FIXED CARBON (%)	CALORIFIC VALUE kcal/Kg
P. juliflora	5.35	1.01	77.95	15.69	3891
C.equisetifolia	6.00	1.44	84.39	8.17	3602
G. sepium	4.80	4.58	79.64	10.98	3540

Elemental Composition Analysis

The good heat of combustion of all the biomass materials is due to their higher proportion of carbon and lower proportion of oxygen (Goel and Behil,1992) is agreement with *P. juliflora, C. equisetifolia* and *G. sepium.* EDAX analysis (Table 2) of *P. juliflora* and *G. sepium* has low concentration 0.39 % and 0.29 % of Mg 2.32 % and 2.22 % of Ca respectively (Gomes and de Muñiz, 1986) which is the major ash forming elements in the biomass fuels. For the two (*P. juliflora* and *G.sepium*) biomass fuels the sequence is Cu>Ca>Mg , the exceptional case is that potassium which is major obstacles for an efficient utilization of biomass fuel for power production.

Table 2. Elemental analysis of biomass materials *P. juliflora*, *C. equisetifolia* and *G. sepium*

ELEMENTS PRESENTED Ka	*P. juliflora* Mass (%)	*C. equisetifolia* Mass (%)	*G. sepium* Mass (%)
C	75.3	77.78	74.38
O	17.92	22.22	20.62
Mg	0.39	-	0.29
Ca	2.32	-	2.22
Cu	4.05	-	2.49

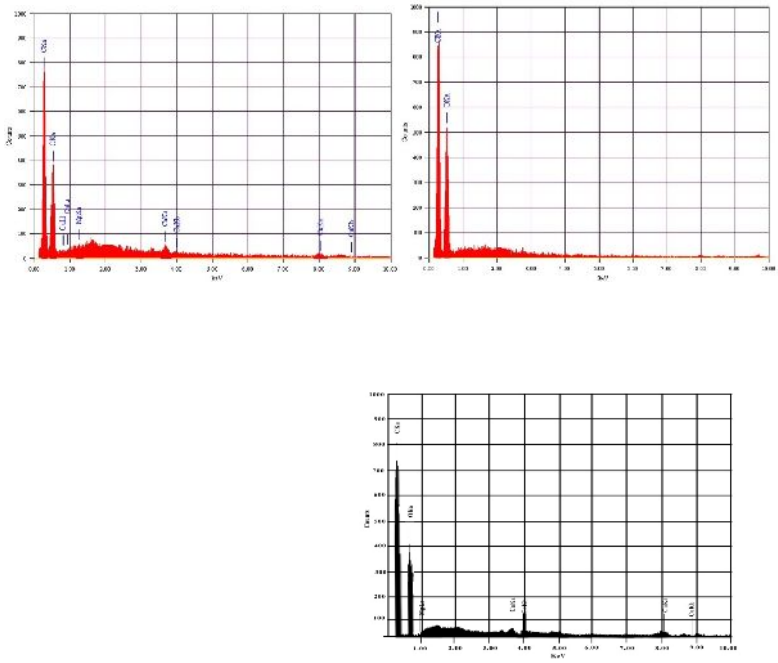
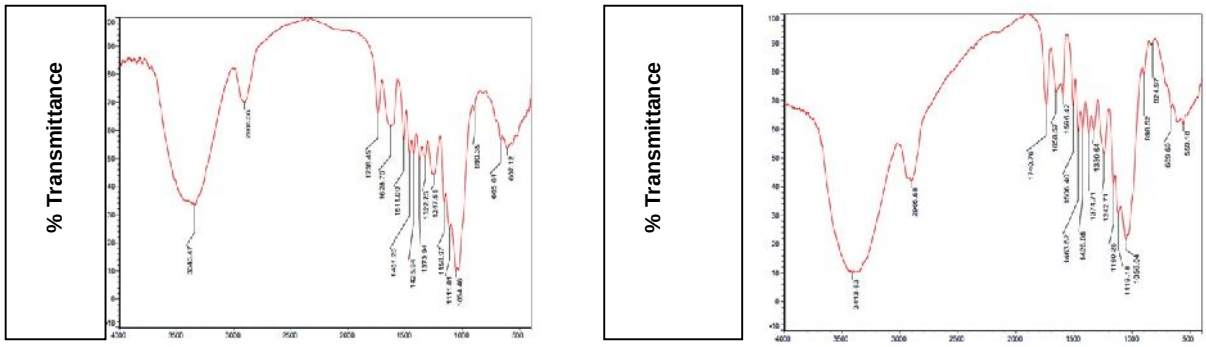


Fig. 1 Elemental analysis of *P. juliflora*, *C. equisetifolia and G. sepium* wood

3.4FT-IR Spectral Analysis

The recorded FT-IR spectrum of species is shown in Fig.2. The FT-IR spectral assignments are tabulated in Table 3. The FT-IR spectrums of the biomass materials (Fig 2) have O-H stretching vibrations at around 3200-3600 cm^{-1}. The O-H stretch carboxylic acids appear at 2908.36 cm^{-1} for *P. juliflora*, at 2905 cm^{-1} for *C. equisetifolia* and 2923 cm^{-1} for *G. sepium.* The C=O stretch appears at 1738.45 cm^{-1}, 1740 cm^{-1} for *P. juliflora* and *C. equisetifolia.* The peak at 1373 cm^{-1}, 1374 cm^{-1} and 1376 cm^{-1} for *P. juliflora, C. equisetifolia* and *G. sepium* respectively are assigned for strong C-N stretching of aromatic amino group. The peaks obtained at 1158 cm^{-1}, 1111 cm^{-1}, 1054 cm^{-1} for *P. juliflora* and 1160 cm^{-1}, 1119 cm^{-1}, 1058 cm^{-1} for *C. equisetifolia* and 1163 cm^{-1}, 1113 cm^{-1} and 1059 cm^{-1} for *G. sepium* are C-N stretching

alcohols assignment. The region from 1400-600 cm^{-1} is called finger print region because the pattern of absorptions in this region is unique to any particular compound, just as a person's fingerprints are unique. The presence of carboxylic acids, amines and hydroxy-substituted compounds which are responsible for volatility (John Coates, 2000).



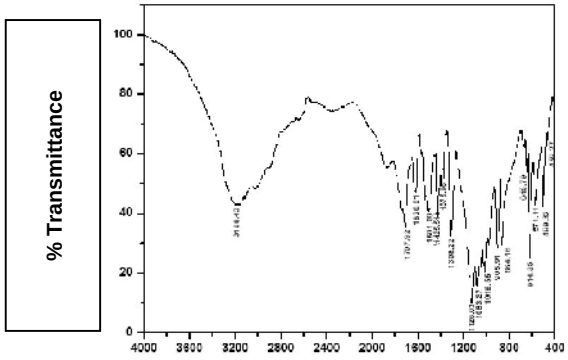


Fig 3.FT-IR spectrum of*P. juliflora, C. equisetifolia andG. sepium* wood

Table 3. FT-IR spectral assignments for *P. juliflora, C. equisetifolia* and *G. sepium*

Wave Number Cm^{-1} *P. juliflora*	Wave Number Cm^{-1} *C. equisetifolia*	Wave Number Cm^{-1} *G. sepium*	Assignments
3343	3413	3419	O-H Stretch
2908	2905	2923	O-H Stretch Carboxylic acids
1738	1740	-	C=O Stretch
1628	1658	1648	N-H bend primary amines
1461	1463	-	CH_2& CH_3 bend

1373	1374	1376	C-N Stretching of aromatic amino groups
1247	1242	-	C-O Stretch
1158	1160	1163	C-N Stretching alcohols
1111	1119	1113	C-N Stretching alcohols
1054	1056	1059	C-N Stretching alcohols

Conclusion

Thermal and EDAX analyses showed that *P. juliflora, C. equisetifolia* and *G. sepium* have low moisture content; the low proportion of oxygen indicates that all are appropriate to meet the requirements of thermochemical process. FTIR analyses reveal that the presence of carboxylic acids, amines and hydroxy-substituted compounds confirm the volatility of three species in coastal areas. "Agroforestry is a land-use that involves deliberate retention, introduction, or mixture of trees or other woody perennials in crop/animal production field to benefit from the resultant ecological and economical interactions". Thus this study investigates the energy value of the woody biomass in coastal areas.It could also serve to establish a database of biomass fuels or feedstock in coastal areas, which would support decision making in terms of energy conversion technology, selection and operating conditions setting.

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MARINE SCIENCE PUBLICATIONS IN GLOBE – AN ANALYSIS OF SCHOLARY INFORMATION

**Dr.S.Arumugam & **G.Vigneshwaran*
** Assistant Professor, Department of Physical Education and Sports, Manonmaiam Sundaranar University, Tirunelveli, Tamilnadu, India-627 012.*
*** Assistant Professor, Mother Teresa College of Physical Education, Pudukkottai, Tamilnadu, India-622 102.*

Introduction

Marine Science is the study of the ocean, its ecosystems and its life forms as well as the study of coastal environments, oceanic currents and the sea floor. Marine Science can be defined as the process of discovering the facts, processes, and unifying principles that explain the nature of the oceans and their associated life forms. Marine science consists of four branches of oceanography. Oceanography is the science of recording and describing the ocean's contents and processes.

Though there was countless number of publications in marine science in various forms, only limited publications are found in the electronic form. This study attempts to analyse of global marine science publications, which is available in the electronic form.

Bibliometrics is a statistical analyze of scientific and technical publications. In other words it is the statistical analysis of texts and information. Bibliometrics is the term first coined by Alan Pritchard in 1969. This paper attempts to give us complete profiling of the publications on 'Marine science'. Increasingly bibliometrics are being used as a measure of research impact or research influence. Bibliometrics analyses quantitative and qualitative data to describe publication patterns within a field of research. This information can be used to evaluate the influence/performance of a researcher and to provide a comparison between researchers. These results also help us to determine university rankings and have an impact on university funding. The purpose of the present study when will to bibliometrically analyse the global marine science publications.

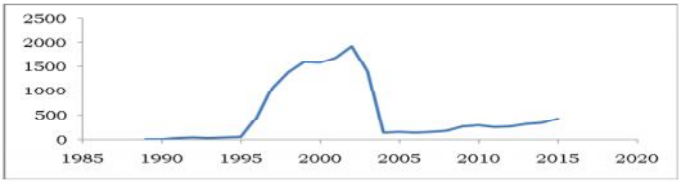
Objectives

- To identify the year-wise publications of marine science publications.
- To find out country-wise distribution of publications.
- To list the top ten institutions with high productive publications in marine science.
- The document type wise classification of marine science publications.
- To know the top ten contributing authors.
- To analyse the subject area wise distribution of marine science publications.
- To know the top ten contributing journals.
- To know the top ten funding agency.

Database and Methodology

Web of science is a bibliographic database containing abstract and citations of peer-reviewed publications. Web of science, is a bibliographic database which contains abstract and citations for academic journal articles. According to web of knowledge database, it covers nearly 60,000 titles from over 10,000 international publishers, of which 20,500 are peer-reviewed journals in the scientific, technical, medical and social sciences. Web of science is owned by Thomson Reuters and is available in online by subscription. The data for this study were retrieved from the Web of science database. Using search string in Web of science, the term 'Marine Science' in 'article title, abstract and keywords' published in years and all types were analyzed. In all, 14,290 documents results were found in web of science database as January 1, 1989 to December 31, 2015. With the assistance of analyse result option, the data for analyse were collected. Using illustrations in the form of diagram and table the result of the study were discussed broadly.

I. Year-wise classification of Marine Science publications



In Web of science, publications pretended to marine science were limited to the period from January 1, 1989 to December 31, 2015 only. From the above line graph, it was found that the total numbers of document available was 14,260. In the year 2002, maximum of 1925 documents are published in marine science. The analysis also reveals that steady increase in the productive was observed since 1996 to 2003.

II. Country-wise distribution of publications marine science

S.No	Country	Record Count	%
1	USA	4227	29.580 %
2	England	1503	10.518 %
3	France	1284	8.985 %
4	Germany	1261	8.824 %
5	Canada	1087	7.607 %
6	Australia	997	6.977 %
7	Japan	990	6.928 %
8	Spain	759	5.311 %
9	Italy	715	5.003 %
10	Netherlands	546	3.821 %

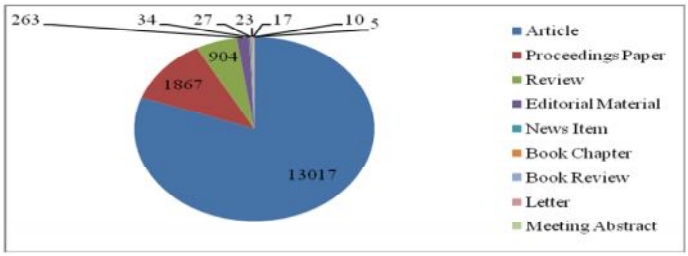
In present study, out of 152 countries, which had published atleast one publications in marine science were analysed and top ten 'Nation List' along with the productivity in terms of number of publications are presented in the above table. United States of America top the list with 4227 publications, followed by England with 1503 publications. No Asian nation was in the top ten list. India were placed in 19th position with 250 publication in marine science.

III. Top ten institutions in publishing marine science publications

S.No	Institution	Record Count	%
1	National Oceanic and Atmospheric Administration	216	1.512 %
2	Consejo Superior de Investigaciones Científicas	208	1.456 %
3	WOODS Hole Oceanog Instiyution	201	1.407 %
4	University of Washington	166	1.162 %
5	University of California SAN DIEGO	161	1.127 %
6	Russian Academic Science	158	1.106 %
7	Chinese Academic Science	147	1.029 %
8	Oregon State University	144	1.008 %
9	CNRS	142	0.994 %
10	CNR	140	0.980 %

The analyses of institution-wise distribution have been contributed by 7093 institutions around the globe. The researchers had taken top ten productivity institution for this analysis. By seeing the analysis, National Oceanic and Atmospheric Administration (NOAA) (216) top the list followed by CSIC (208). Among the top ten institutions in the globe, only one Asian nation institutions were in the top ten lists pertaining to the research publications output in marine science.

IV. Document-wise distribution of publications



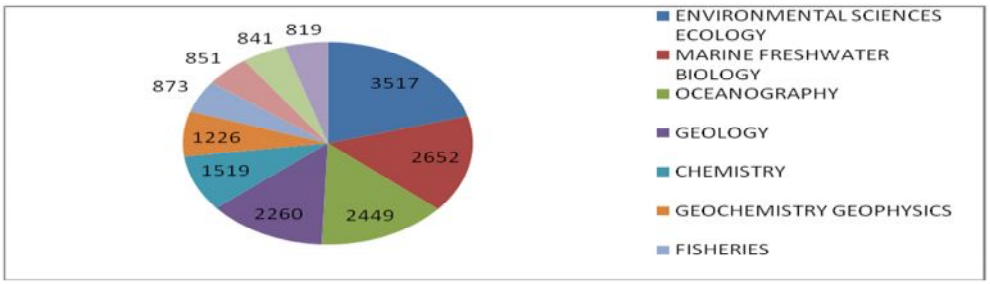
The result indicates that marine science publications have been classified into many types of documents. The above diagram indicates the numerical values of each document. The analysis reveals that 'article' has constituted 13,017 of total documents of source, followed by 'proceedings paper'. Out of 14,260 documents on marine science, more than 91.28% were in the form of 'article' and 'proceedings paper'' type. The remaining documents of source were in review, editorial materials, news item, book chapter, book reviews, letter, meetings abstract and correction are having 7.72%.

V. Top ten contributing authors

S.No	Authors	Record Count	%
1	Damste JSS	46	0.322 %
2	Tanabe S	39	0.273 %
3	Muller We G	32	0.224 %
4	Kobayashi J	29	0.203 %
5	Schouten S	28	0.196 %
6	Fusetani N	25	0.175 %
7	Kobayashi M	24	0.168 %
8	Tsuda M	24	0.168 %
9	Cimino G	23	0.161 %
10	Depledge MH	23	0.161 %

By analyzing the authorship pattern, at global level more than 35,000 authors have participated in publishing in marine science. The above table shows the top ten authors productivity on marine science. It also indicates the number of works done by each of them, Damste has published 46 items in marine science publications.

VI. Subject-area wise distribution of marine science publications

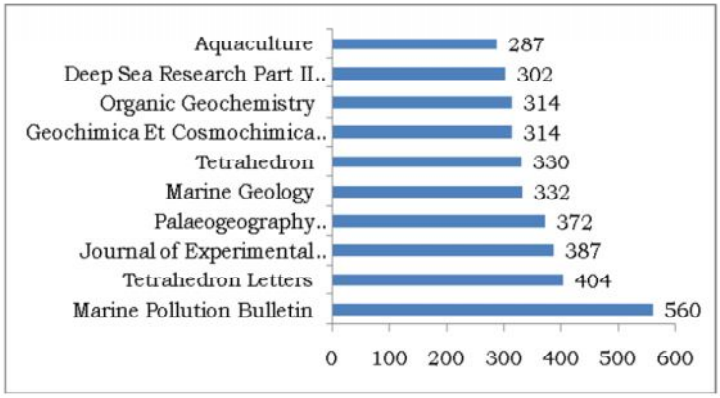


The result indicates that marine science publications were found in 125 subject areas. The subject 'Environmental Sciences Ecology' is having the highest number of output of 3517, followed by 'Marine freshwater biology, Oceanography, etc.,'

VII. Funding agency-wise distribution of publications

Funding agency	Record Count
National Natural Science Foundation of China	61
National Science Foundation	58
NSF in India	28
European Union	26
Australian Research Council	22
David and Lucile Packard Foundation	22
European Commission	20
Gordon and Betty Moore Foundation	16
Natural Sciences and Engineering Research Council of Canada	15

The analyses of funding agency-wise distribution have been contributed by 2,552 agencies around the gl obe. The top 2 funding agencies during the study period which were contributed highest number of publications are National Natural Science Foundation of China (61) from China and National science foundation from United States (58) were giving more fund in marine science publications.



VIII. Journal-wise distribution of publications

It could be noted that marine science scientists were to bring out their publication in different type of source. The present investigation had taken top ten published journals, which published marine science publications. Out of 1472 Journals, Marine Pollution Bulletin has published 560 publications and stood first among the all source title.

Findings

The large number of marine science publications was in the year 2002and triennial growth rate was observed since 1996 to 2003.

The study indicates that publications in marine science was observed in 152 countries and United States of America top the list and it was followed by England.

The result reveals that National Oceanic and Atmospheric Administration had contributed high number of publications in marine science.

The analysis reveals that 'article' has constituted 13,017 of total documents of source, followed by 'Proceedings Paper'.

Among all authors globally Damste has published 46 items in marine science publications.

The results indicates the subject 'Environmental Sciences Ecology' is having the highest number of output of 3,517, followed by 'Marine freshwater biology, Oceanography, etc.,'.

Among all funding agencies globally National Natural Science Foundation of China has given more funding 61 items in marine science publications.

Marine Pollution Bulletin has published maximum of 560 publications in marine science.

Conclusions

From this study, it was concluded that more works on marine science should be encouraged nationally throughout the country. To preserve the information and to enhance the academic excellence globally, publications in marine science should be in electronic form

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A STUDY ON IMMUNOMODULATORY EFFECT OF *Justicia gendarussa*(Burm) and *Cardiospermum halicabum* (Linn)

M. Andrew Pradeep*, G. Dhivya, D.Ramya, R. Sethu Nagarajan
Post Graduate Department of Immunology & Microbiology
The American College, Madurai – 2.
Corresponding Author Email: micpradeep@gmail.com

Introduction

Transplantation is the most effective treatment for end-stage organ failure. Graft rejectionis a normal process in which the functional immune system of a transplant recipient attacks the transplanted organ or tissue. T-cells are central to the process of transplant rejection through allorecognition of foreign antigens leading to their activation and the orchestration of an effector response that result in organ damage.

Allograft rejection is mediated primarily by T-cells, with B-cells. Depending on the transplant type, Rejection can be Hyper-acute, Acute and Chronic. Hyper-acute rejection is a complement-mediated response within recipients with pre-existing antibodies to the donor. It occurs within minutes and the transplant must be immediately removed to prevent a severe systemic inflammatory response.

Allogenic transplantation will not succees unless the recipient immune system is down regulated. The immunosuppressants are a class of drugs capable of inhibiting the body's immune system, such immunosuppressive drugs are Calcineurin inhibitors (Cyclosporin, Tacrolimus), mTOR inhibitors (Sirolimus, Everolimus), Anti-Proliferatives (Azathioprine, Mycophenolic acid), Corticosteriods (Perdnisolone, Hydrocortisone), Antibodies (Monoclonal anti-IL-2R receptor antibodies, Polyclonal anti-T cell antibodies). Glucocorticoids suppress the cell mediated immunity. They act by inhibiting genes that codes for the cytokine, Interleukin and TNF. They also suppress the humoral immunity, causing B-cells to express smaller amount of IL and IL-receptor. This diminishes both B-cell clone expansion and antibody synthesis. These drugs are used in organ transplant patients to

decrease the body's own natural defense to foreign bodies (such as the transplanted organ) and thereby attempt to prevent the organ's rejection by the body.

Immunosuppressive drugs are used to treat autoimmue disease or diseases that are most likely to be of autoimmune origin. In rheumatoid arthritis, Cyclosporin helps to reduce inflammation and thus reduce pain and swelling. It also limits damage to the joints and helps to prevent disability. Because cyclosporine reduces the damage to the joints rather than just relieving the pain. They also treat some other non-autoimmune inflammatory diseases(e.g., long term allergic asthma control).

Immunosuppressive drugs are not without side-effects and risks because majority of them act non-selectively, the immune system is less able to resist infections and the spread of malignant cells. There are also other side-effects such as hypertension, dyslipidemia, hyperglycemia, peptic ulcer, liver and kidney injury. The Immunosuppressive drugs also interacts with other medicine and effects their metabolism and action with known side-effects.

Modulation of immune response is a possible therapeutic measure, by using medicinal plants are commonly used for the treatment of various ailments and are considered to have advantages over the conventionally used drugs which are expensive and known to have harmful side effects (Khare et al., 2004). Besides primary metabolites with immunomodulatory activity, several plants *(Lepidagahis cristata, Tripterygium wilfordii, Lagenaria sicearia, Achillea talagonica*) have secondary metabolites that have been found to interfere with immune system functions (Wagner et al., 1993). Several studies have previously demonstrated the immunosuppressing effects of medicinal plants on lymphocyte proliferation in the presence of mitogens, allogenic cells ad specific antigens (Wong et al., 1994).

In our present study, two medicinal plants were selected that they traditionally used for treating ailments such as rheumatism, inflammation, autoimmune disease.They are *Justicia gendarussa* (Burm), *Cardiospermum halicacabum* (Linn).

The present study was undertaken to screen the extract of the medicinal plants for its immunosuppressive property on T-cell proliferation and Cytotoxicity assay

Materials and Methods

Plant :*Justicia gendarussa (Burm)*

Taxonomy:

Family	:	*Acanthaceae*
Genus	*:*	*Justicia*
Species	:	*gendarussa Burm*
Botanical name	:	*Justicia. Gendarussa (Burm)*
Vernacular name	:	Karunochi (Tamil)
Plant parts used	:	Leaves, Roots

Properties:Leaves contains a bitter alkaloid (justicine) rich in potassium salts.Phytochemical analysis showed the presence of alkaloids, flavonoids, steroids and amino acids.

Uses: Fresh juice from leaves cures rheumatism, asthma, fever, cough, head ache, eczema and analgesic action.

Plant :*Cardiospermum halicacabum (Linn)*

Taxonomy:

Family	:	*Sapindaceae*
Genus	:	*Cardiospermus*
Species	:	*halicacabum*
Botanical name	:	*Cardiospermum halicacabum (Linn)*
Vernacular name	:	Mutakkarran, Modakathon (Tamil)
Plant parts used	:	Entire plant

Properties: Phytochemical analysis showed that the plant contain saponins, flavonoids, phenolic compounds, alkaloids and tannins.

Uses: The leaf extract used as a allergy relief liquid and used as a medicine for hay fever, burning pains, swelling. The plant extract treat rheumatoid arthritis.

Bleeding

Human blood containing EDTA used for Lymphocyte separation.

Separation of blood mononuclear cells

One ml of Hisep Lymphocyte Separation Medium (LSM) was aseptically transferred into a centrifuge tube. It was overlayed with 3.0ml of diluted blood and centrifuged at 1500rpm for 30 minutes. The inter layer (Lymphocytes and monocytes) was collected using a clean glass Pasteur pipette and transferred into an eppendorf tube.

Isolation and Separation of T-lymphocytes

Pre-washed 0.7g of nylon wool was packed into a 1ml of syringe, soaked with PBS and autoclaved for 20minutes. The column was mixed with 1-3ml of pre-warmed RPMI (without phenol red) supplemented with 10% FBS (Fetal Bovine Serum). Previously collected lymphocytes were suspended in 1ml of pre-warmed RPMI and incubated at 37 degree Celsius for 45 minutes. The non-adherent T-lymphocyte were collected by passing about 3.5ml of pre-warmed RPMI-FBS.

Cell proliferation assay

T-cell proliferation assay is an InVitro method commonly employed to assess the cell mediated immune response of the host. Various stimulants such as mitogens and antigens can be induce the lymphocyte to undergo mitosis.(Using MTT).

Cytotoxicity assay

Cytotoxicity assay is an InVitro method to assess the viability of cells following incubation with some immunosuppressant. 100microlitre of cell suspension was added in a microtitre well containing RPMI (without phenol red).Stock solution of drug was prepared (100ng/ml).

The cytotoxicity effect was calculated by the following formula

$$\text{Cytotoxicity (\%)} = 1 - \frac{\text{OD of cells treated with cytotoxin}}{\text{OD of untreated cells}} \times 100$$

Result:

Table 1 Standard cell preparation for MTT assay

Number of cells per ml	Absorbance at 570nm
10	0.263
100	0.289
1000	0.322
10000	0.356
100000	0.405
1000000	0.414
10000000	0.456
100000000	0.479

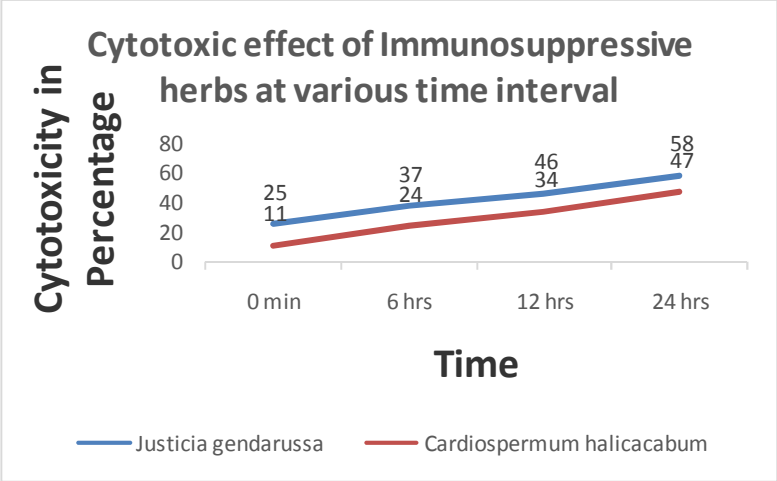
Table 2 Cytotoxic effect of Immunosuppressive herbs at various time intervals

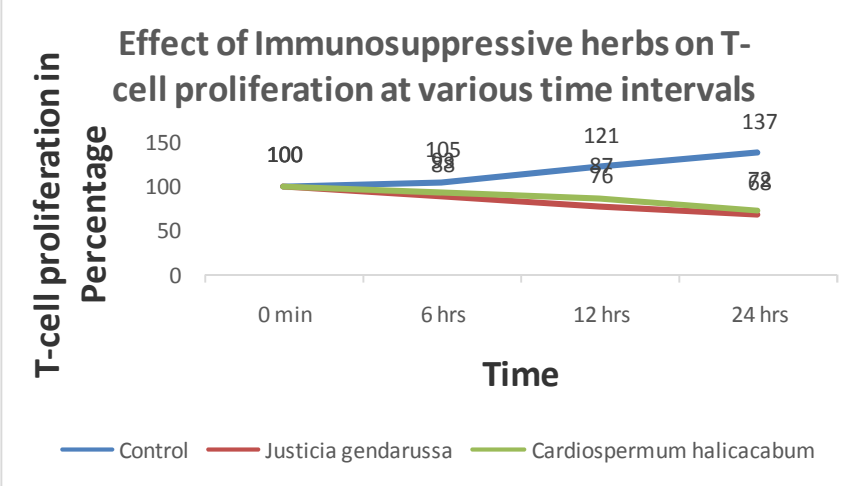
DRUGS	Cytotoxicity in Percentage			
	0 min	6 hrs	12 hrs	24 hrs
Justicia gendarussa	25	37	46	58
Cardiospermum halicacabum	11	24	34	47

Table 3 Effects of Immunosuppressive herbs on T-cell proliferation assay at various time intervals

HERBS	T-cell proliferation in percentage			
	0 min	6 hrs	12 hrs	24 hrs
Control	100	105	121	137

Justicia gendarussa	100	88	76	68
Cardiospermum halicacabum	100	93	87	72





Discussion

Allograft rejection is a normal process in which the functional immune system of a recipient attacks the transplanted organ or tissue. Immunosuppressive drugs are administered to recipients in order to minimize the risk of rejection. A wide range of immunosuppressive drugs have now been used to control transplant rejection and autoimmune disease. The clinical application of immunosuppressants has significantly improved 90% of patient survival with renal transplant (Puri et al., 1993). Unfortunately immunosuppressants (Cyclosporin and Tacrolimus) have number of adverse effects like Nephrotoxicity, Hepatotoxicity, Induction of diabetes, Induction of Hypertension and Neurotoxicity (Waldmann et al., 2003). The

Immunosuppressants without any side effects are still a challenge to the medical fraternity. Suppression of immune response by medical plant products as a possible therapeutic measure has become a subject of scientific investigation (Serkova et al., 1996).

This study focuses on two medicinal plant *Justicia gendarussa* and *Cardiospermum halicacabum*. The effect of Immunosuppressive drugs on T-cell functions assessedby cytotoxicity assay, T-cell proliferation assay.

Medicinal plants have various active components like alkaloids and steroids which are significantly act as immunosuppressive agent by inhibiting CD-28 co-stimulated T-cell proliferation and cytokine production (Lai et al., 1999).

Here the *Justicia gendarussa* and *Cardiospermum halicacabum* also have the specific active component like alkaloids-Justicine, Cardiospemin (Wahi et al.,1974, conn et al., 1980), Steroids-Beta-sitosterol, 1-cyano-2-hydroxymethyl-prop-2ene-1-ol (Chakravaty et al., 1982, Chrisholm et al., 1958) shows the similar immunosuppressive activity which involves the inhibition of T-cell proliferation.

The cytotoxicity effect of immunosuppressive drugs on T-cells is determined by MTT assay. The viability of the cells decreased with increase in the time intervals. The decrease might be due to the toxicity of the extracts (Chen et al., 1999).

Mitogen stimulated lymphocyte proliferation has been studied by MTT assay. The clonal proliferation of activated T-cell depend upon the expression of CD25 (IL-2R) receptor in the early phase after T-cell activation (Taniguchi et al., 1993). When used Concanavalin A as a mitogen know to signal through T-cell receptor (TLR) causing release to intracellular calcium (Komada et al., 1996). It binds to the CD2 and activate protein kinase.

Justicia gendarussa and *Cardiospermum halicacabum* inhibits T-cell proliferation quantified by colorimetric assay based on the reduction of MTT dye by the T-cells. The inhibitory activity of leaf extracts mediated by the interaction between the active components of the extracts with the cell surface molecules or growth factors involving mitogen activation (Arockiyaraj et al., 2007).

Cyclolinopeptite A, active immunosuppressive component which is present in *Justicia gendarussa* and *Cardiospermum halicacabum* inhibits the calcium dependent activation of T-lymphocytes through direct binding of Cyclophilin A and consequence inhibition of calcineurin action (Brown et al., 1994). Furanoditerpinoids is a specific component which is also present in *Justicia gendarussa* and *Cardiospermum halicacabum* inhibits mitogen response in T-cell proliferation (Hang et al., 1996).

The use of commercial immunosuppressive drugs like cyclosporine in transplantation involves the risk of life-threatening host toxicity. The present study shows that the medicinal plants (Justicia gendarusaa and Cardiospermum halicacabum) has the immunosuppressive activity by the presence of active components in the. Therefore this *Justicia gendarussa* and *Cardiospermum halicacabum* may act as an effective and safe therapeutic tool in the transplantation.

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USE OF MARINE RESOURCES INSIDDHA MEDICINAL SYSTEM

*Dr. S.L. Subha, M.D., Dr. S.L. Chithra, B.S.M.S., and
**Dr. K. Subramonian
*Siddha Medical Practisioners, Tirunelveli
**Department of Plant Biology and Plant Biotechnology,
The M.D.T. Hindu College, Tirunelveli.

Introduction :

Siddha System is one of the oldest medical system in the world. Siddhars use plant and animal sources for medicine which is in land and water. Sea is one of the biggest source of not only food material but various health care substances. Research on marine organism began in the last century, but a number of marine products are used in siddha system of medicine since immemorial. Siddhars, the founders of siddha medicine prepared a wide range of products from marine and document their preparation in literature and also utilize them for people.

Coral (பவழம்)

Coral referred as "Tropical rain forest of the leep". Since they are the beautiful marine organism providing valuable medicinal uses.

It comes under five sea wealth in siddha literature. Thirumoolar compared pavalam with "Shakti" (i.e. energy)

சுரதோடம் ஜயமுத் தோடசுரங்காசம்

அருசிகீ டத்தாலாம் ஆலம் – பெருவிந்து

நட்டம் அதிதாகம் நாவறட்சி போமொளியுங்

கிட்டும் பவழத்தாற் கேள்.

Coral is used in the form of parpam. It is a good sources of calcium carbonate. So its is used for low bone density and calcium deficiency. Pavala parpam is a definite solution for bronchial asthma and kaba diseases in siddha. Pavala powder also used as a tooth powder for strengthening teeth gums.

Pearl (முத்து)

Like coral, pearl also been used for centuries as remedies not only for bone problems also for other diseases like acidity, skin problems and to decrease the aggressive mood.

…….. சுத்தத்

தயிரிய நட்டத்தோடு தாது நட்டமும்போம்

உயிறுருநன் முத்திருந்தா லோது

Chelonia Turtle (ஆமை)

Tortoises live in both land and water, but water living tortoise is used for medicine it is the best pediatric medicine.

Use :

1. Shell – Shell subjected to high temperature become ash and is given with honey is highly effective for children's loose motion vomiting & indigestion problem.
2. Flush & Skin : Tortoise flush & skins heals external wounds when taking internally
3. Egg: Egg of tortoise used for whooping cough of kids.
4. Blood : Blood is used for respiratory diseases. It is also used as a home medicine in areas of coastal areas.

Ostrea Edults Cinn

Oyster Shell (கிளிஞ்சல்)

Oyster have been part of the human diet since pre historic times, also used as a medicine before many decades. Paste of the shell can be used for foot crack.

Turbinella rapa - Conch Shell (சங்கு)

Conch shell is a major object used in hindu prayer. It is used as a trumpet to get rid of regative energy and qil spirits. It is also used as a container for holywater.

Siddha literature highlights that medicine prepared from this shell gives strength like mountain and beauty like manmadhan.

கசிவா மிரத்த பித்தங் கண்ணோய் களேகும்

பசியாறும் வாதம் பிறக்கு.....

In siddha it is used in all kinds of eye diseases like night blindness and conjunctivitis. Parpam made from sangu is a best medicine for gastritis.

Squlus charcharius (சுறா)

Many types of shark are found in sea. But milk shark is used for medicine. It has very good nutritional value. It should be given for pregnant and lactating women. It gives strength and also excrete the delivery waste.

The liver of shark is rich in Vitamin – A and used for night blindness. Ghee taken from liver contains Vitamin A & D, used for primary complex & boost the immune system. Recent investigations show that shark cartilage (tough elastic tissue) is used for various cancer.

Helix Aspera (நத்தை)

Snail drinks the impurities of water and clean them They contain tremendous amount of protein and nutrients.

Flush of shell can be used for anorectal diseases mainly piles.

நத்தைக் கறிதனக்கு நாடாது மூலமுத

லொத்தமல ரோகமெலா மோடுங்காண்

Cyprala moneta Linn (பலகறை)

Marine shell also one of the important wealth of sea in siddha. There are 3 colours available in sea, among them white shell is best for medicine.

Powdered shell are used as a topical eye medicine and scientifically proved to have some anti – inflammatory action in conjunctivitis.

Ash form of *Cyprea moneta* known as pavala parpam contain phosphate, calcium, carbonate & manganese. This is used for jaundice, Spleen & liver enlargement.

This parpam mixed with various birds egg, yolk and applied for external wounds. (Eg) knife cut – dove egg; for thorn prick - parrot egg.

Mutilus margaritiferus (முத்துச்சிப்பி)

This Pinctada oyster produces pearls. Some people cooked this shell with salt and pepper. “Humans and oysters share the capacity of self repair” A human bone’s health is as does a cracked oysters shell. Now we believe that this shell can be used to stimulate the bone growth. Oyster shell also used as a best aphrodisiac. The high zinc content of this shell stimulate testosterone production.

Conclusion :

The Marine eco system offers a huge variety of medicine for humans. However clinical trials and scientific approach is needed in order to transfer innovative discoveries from traditional medicine into active clinical therapeutics.

PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF HALOPHILIC ORGANIC SOLVENT TOLERANT PROTEASE FROM MARINE CRUSTACEAN SHELL WASTE AND ITS APPLICATIONS

Thirumalai Maruthiah[1], Arunachalam Palavesam[2]*

[1]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam - 629 502, Kanyakumari District, Tamilnadu, India
[2]*Department of Animal Science, Manonmaniam Sundaranar University, Tirunelveli – 627 012, Tamilnadu, India
* Corresponding Author Email: plavesh06@gmail.com
Phone: +91 09443545411 Fax: +91 04652 253078

1. Introduction

Marine industry generates 50–60% of the total weight of shellfish as waste consists of protein (35–50%), chitin (15–25% of dry weight) and inorganic compound (calcium carbonate) which are considered as major environmental pollutants due to uncontrolled dumping (Islam et al., 2004; Sachindra et al., 2005). Bioconversion of these materials has been proposed as waste treatment alternate to the disposal of shellfish wastes. The utilization of these wastes not only solves the environmental problem but also decreases the production costs of microbial proteases (Annamalai et al., 2014). Among the microbial proteaases, alkaline protease places 40% of the total world enzyme production, with specific applications in bakery, brewing, detergent, diagnostic reagents, feeds modification, leather finishing, laundry additives, pharmaceuticals, and peptide synthesis, silk, silver recovery from X-ray / photographic film, soy processing, and waste treatment (Gupta et al., 2002).

The production and purification of HOSP through the bioconversion of marine shell wastes by various marine coastal proteolytic bacterial species were reported by several authors (Annamalai et al., 2012, 2013, 2014; Maruthiah et al., 2014, 2015a,b]. However, the application of *Bacillus* sp. for the deproteinization of marine crustacean wastes is rarely seen. In this study, the protease-producing *Bacillus* sp. can be used for deproteinization of crustacean wastes. Generally, the preparation of chitin from various crustacean shells involves demineralization and deproteinization with the use of strong acids or bases. Typically to overcome the scarcity of the chemical treatments, microorganisms and proteolytic enzymes were effectively used to deproteinze the crustacean wastes (Maruthiah et al., 2015a,b; Jellouli et al., 2011; Hadder et al., 2011; Ghorbel-Bellaaj et al., 2012, 2013). Considering the above facts discussed in the present study, an attempt has been made on the prodction and purification of the HOSP from the coastal sedimentary bacterium using marine shell wastes. Further, the candidate strain also performed maximum deproteinization and antioxidant efficiency using shrimp shell waste.

2. Materials and Methods

2.1. Marine fish waste powder preparation for HOSP production

The shrimp shell powder (SSP), crab shell powder (CSP) and lobster shell (LSP) wastes used in this study were purchased from local fish processing unit, washed thoroughly with tap water and sun dried. The dried materials were milled and sieved (100 µM) to get

uniform fine powder and used as sole carbon sources for protease production (Maruthiah et al., 2015b].

2.2. Isolation of HOSP bacterium and culture conditions for protease production

The candidate bacterium was isolated from the sediment sample of manakudi coast, Kanyakumari District, India and it was identified based on the morphological, physiological, and biochemical characteristics as wellas 16S rRNA sequencing. The isolation and identification of HOSP bacterim using solvent enrichment method (15% cyclohexane and 10% sodium chloride) (Maruthiah et al., 2014, 2015a,b).

2.3. Enzyme assay and Protein estimation

The protease assay was carried out by the method of Takami et al., (1989) using 1% casein as a substrate. The amount of protease produced was measured with the help of a tyrosine standard graph. The protein content in all the samples was estimated by using Bradford method. For this study readily used Bradford reagent was used (Sigma, USA).

2.4. Effect of various marine wastes on proteaseproduction

In the investigation of suitable carbon source for protease production, growth was carried out in 250 ml Erlenmeyer flaskswith 50 ml of basal medium containing 0.1% K_2HPO_4 and 0.05% $MgSO_4 \cdot 7H_2O$ (pH 9) and supplemented with 0.1–2% (w/v) of marine wastes to be investigated such as SSP, CSP, SPP andSCSP (Shrimp and Crab shell powder at 1:1, 1:3 and 3:1 ratio,w/w).

2.5. Production and purification of HOSP

In the investigation of suitable carbon source for protease production, growth was carried out in 500 ml Erlenmeyer flasks with 100 ml of basal medium containing Shrimp and Crab shell powder (3:1 w/w), 0.1% K_2HPO_4 and 0.05% $MgSO_4 \cdot 7H_2O$, NaCl (100.00 g/L), calcium choride 3.0 (g/L) were seeded with 5% inoculum and incubated in shaking incubator (125 rpm) for 48 h. After incubation, the culture broth was centrifuged (4°C at 12,000 × g for 20 min) and the crude enzyme was used for further purification.

2.5.1. Purification of HOSP from *Alcaligenes faecalis* APCMST-MKW6

The purification starts with 75% ammonium sulphate precipitation and kept at 4°C for 24 h. Ammonium sulphate fractions were resuspended in minimal volume of 50 mM Tris–HCl buffer (pH 7.2). The precipitates were collected through centrifugation at 6000 × g for 15 min and dissolved in 50 mM Tris–HCl buffer (pH 9.0) and dialysed against same buffer (4°C) for 12 h. Then it was loaded on a DEAE-Sepharose fast-flow column, pre-equilibrated with 50 mM Tris–HCl (pH 8.0). The unabsorbed protein fractions were eluted with the same buffer at a flow rate of 2 ml/min. The protease activity of individual eluted fractions was determined. Further the fractions showing the highest protease activity were pooled together and concentrated by using ultra filtration unit (Amicon 10 kDa molecular weight cut-off device, Millipore, USA). The homogeneity and the molecular weight of the purified protease were determined by SDS-PAGE and further confirmed by zymogam gel analysis (Laemmli, 1970; Garcia-Carreno et al., 1993).

2.5.2. Biochemical properties of purified protease

The effect of different pH (4-10) and temperature (30°C - 80°C) on protease activity were studied. The effect of metal ions ($MgCl_2$, $CaCl_2$,$ZnCl_2$, $MnCl_2$, $HgCl_2$, $ZnSO_4$, $MnSO_4$ and $BaCl_2$ at 5 ppm), surfactants (Poly ethylene glycol, SDS, triton X 100, Tween 20, 40, 60 and 80 at 5 mM), NaCl (5–25 %) inhibitor (PMSF, DTT, iodoacetamide, mercaptoethanol and

EDTA at 5 mM), solvents (ethanol, methanol, hexane, acetone, DMSO, chloroform and benzene) and detergents (Ariel, Tide, Rin, Surf, Sunlight, Henko and surfexcel) were studied by following standard assay procedure.

2.7. Chitin extraction biological method

2.7.2. Biological method of deproteinization using crustacean shells

In the deproteinization experiment, the powdered shells from the crustaceans were individually mixed with above said halophilic production medium taken in flasks (solid to liquid ratio was 1:3 w/v) except casein acid hydrolysate, peptone and yeast extract. Then the flasks were sterilized at 121°C and cooled. Further the candidate proteolyitc bacterial strain (*Alcaligenes faecalis* APCMST-MKW6) was inoculated in to the flasks. The flasks were incubated at 50°C for 3 h. After incubation the reaction was stopped by heating the flasks for 20 min at 90°C. Then, it was centrifuged at 5000 rpm for 20 min to separate soluble and insoluble fractions. The solid fraction was washed with distilled water and dried for 3 h at 60°C. After that, the mineral contents (demineriliz‌ation) were removed by using the 2N HCl for 24 hrs. Further, the samples were dried at 90°C for 20 min. From the dried materials, the protein content was estimated by using the standard protocol (Yang et al., 2000). Results were presented as means of experiments performed in triplicate.

3. Results and discussion

3.1. Microorganism

The HOSP strain MKW6 was isolated from marine water of manakudi coast, tamilnadu, india and screened for their protease activity.The morphological and biochemical, 16S rRNA gene sequence analysis were confirmed the identity of the strain MKW6 as *Alcaligenes faecalis* APCMST-MKW6 (GenBank Accession No. KF009687).

3.2. Effect of various marine wastes on proteaseproduction

The effect of various marine wastes on protease produc-tion was carried out in basal medium containing various concentrations of (3:1%, w/v) SCSP (2872 IU/ml) were most suitable carbon sources for protease production than other marinewastes used such as 3:1 SCSP (4186.65 U/ml), 1:1% SCPP (3277.11 U/ml) and 1:3% SCPP (2700.55 IU/ml) after 48 hrs incubation of maximum cell growth. Likewise, shrimp shell powder was found to be the more suitable inducer for protease production by *Bacillus cereus* TKU006 (Wang et al., 2009), *Chryseobacterium taeanense* (Wang et al., 2008) and *Bacillus amyloliquefaciens* V656(Wang et al., 2002).

3.3. Purification of HOSP by *Alcaligenes faecalis* APCMST-MKW6

The protease (HOSP) from *Alcaligenes faecalis* APCMST-MKW6 was purified to the homogeneity by a combination of ammonium sulphate precipitation, dialysis, ultra filtration and ion-exchange chromatography. The protease was purified at final purification step, i.e. 16.39 fold purity with an overall yield of 21.67%. The specific activity of the purified enzyme was 70.34 U/mg. The homogeneity of the purified protease was confirmed by a monomeric band of 49 kDa obtained by the SDS–PAGE analysis. More or less similar range molecular weight proteases were reported by several marine halophilic organic solvent tolerant starins such as *B. flexus* APCMST-RS2 (44.3 kDa), *Bacillus* sp. APCMST-RS7 (32 kDa), *Bacillus* sp. APCMST-RS3(40 kDa), *B. alveayuensis* CAS 5 (33 kDa).

3.4. Characterization of HOSP

In the present study, the effect of optimum temperature, pH and NaCl displayed that, the halophilic organic solvent tolerant protease showed maximum activity at 60°C, pH 9.0 and 2.5 M after 1.30 h of incubation time. Thus this protease was classified as a moderately thermoactive alkaline halophilic protease. In accordance with the present study, similar kind of previously reported purified halophilic organic solvent tolerant protease from marine bacterial spcies such as *Bacillus* sp. APCMST RS3 (Maruthiah et al., 2015b), *B. alveayuensis* CAS 5 (Annamalai et al., 2013) and *B. firmus* CAS 7(Annamalai et al., 2014). The influence of different chemicals on protease activity was also screened in the present study. Initially, Among the tested metal ions ($MnCl_2$, $MgCl_2$, CaCl2, and $BaCl_2$), surfectants (tween 80 and SDS), substrates (casein), detergents (ariel, rin and tide) and solvents (hexane, isopropanol, acetonitrile, methanol, N-Butanol and ethanol) were found to activate much on protease activity compared to the control. In accordance with these, our previous study also stated that the protease from *B. subtilis* AP-MSU 6 showed enhanced enzyme activity above mentioned chemicals (Maruthiah et al., 2014, Maruthiah et al., 2015a,b, Annamalai et al., 2013, Annamalai et al., 2014).The proteases are classified based on their sensitivity to various inhibitors. In the present study, the protease activity was effectively inhibited by serine (PMSF) and metalloprotease (EDTA) inhibitors and thus it indicates that this enzyme is serine metallo protease**.** This result suggested the presence of serine residue near the active site of the metalloprotease. Previously, our authors confirmed that, β - mercaptoethanol and dithiothereitol (DTT) stimulate the enzyme activity suggesting that both are thiol-dependent serine proteases (Maruthiah et al., 2014, 2015a,b, Annamalai et al., 2013, Annamalai et al., 2014).

3.5. Marine fish waste based applications

3.5.1. Deproteinization by *Alcaligenes faecalis* APCMST-MKW6

Deproteinization is one of the most important industrial applications which was successfully carried out in the present study by using two different approaches (chemical and biological). Suggesting the efficiency of the bio-deproteinization process, the culture supernatant from *Alcaligenes faecalis* APCMST-MKW6 was effectively deproteinized the shrimp shell as maximum of 86.46%within 7 days of fermentation, followed by crab (63.08%) and lobster (59.07%) shells respectively. Hence, the biological deproteinization method was registered almost similar results compared to the chemical method. In accordance with the present findings, the marine isolate *Bacillus* sp. APCMST-RS7 and *Bacillus* sp. APCMST-RS3 also effectively deproteinized the crustacean shell wastes in the preparation of chitin (Maruthiah et al., 2015a,b). The reclamation of processing waste of crustacean shells by bioconversion comes to an alternative solution to reduce the environmental problems associated with crustacean processing industries.

4. Conclusion

Considering the ecofriendly, low cost protease production by microbial reclamation of marine wastes seems to be a judicious approach. The production of inexpensive proteinolytic enzymes not only solves environmental problems, but also promotes the economic value and utilization of marine wastes. The remarkable properties of halophilic organic solvent tolerant protease such as temperature, pH, NaCl, surfactants, metals will make this enzyme as a potential candidate for the development of sustainable waste based industrial process. In addition, deproteinization of this enzyme showed its found to be the improved industrial application property.

Length Weight Relationship for Pelagic Marine Fishes in East Coastal Region, Chennai, Tamil Nadu

Martin. P, Kuppan. A and Kalaichelvi.N
Post graduate and research department of Zoology, Government Arts college for men (Autonomous), Nandanam, Chennai-600035, India

INTRODUCTION

Length-Weight Relationship (LWR) has important role in fishery resource management and also useful for comparing life history and morphological aspects of populations inhabiting in different regions (Ferhat *et al*., 2007; Goncalves *et al*., 1997). Condition factor studies were taken into consideration for the health and general well-being of a fish as related to its environment; hence it represents how fairly deep-bodied or robust fishes (Reynold, 1968). The relationship indicates the taxonomic differences and events in the life history, such as metamorphosis and the onset of maturity. It also denotes the fatness and general well-being of a fish or groups of fishes. Obtaining the relationship between total length and other body weight are also very much essential for stabilizing the taxonomic characters of the species.

Length-weight relationship is important in fishery science, notably to raise length frequency samples to total catch, to estimate biomass from underwater length observations, to evaluate fish growth and body condition etc. The length-weight relationship of fish is important in population assessments (Ricker, 1968). Length-weight relationship (LWR) is a very important parameter to understand the growth dynamics of the fish population. Length and weight data are useful to standard results of any fish sampling program (Morato *et al.,* 2001). LWR of fishes are important in fishery biology because they allow the estimation of average weight of fish at given length group by establishing mathematical relation between the two parameters (Beyer, 1987). LWR is particularly important in parameterzing yield equations and in estimations of stock size (Abdurahiman *et al*., 2004). The exact relationship between length and weight differs among species of fish according to their inherited body shape, and within a species according to the condition (robustness) of individual fish (Schneider *et al*., 2000). The study of morphometric characters in fishes is important because they can be used for the differentiation of taxonomic units (Ambily *et al*., 2010). No attempt has been made on the morphometric study on edible fishes in East Coastal Regions. Hence the present study aimed to study the length weight relationship of the chosen edible marine fishes in ECR at Chennai, Tamil Nadu.

MATERIALS AND METHODS

Sample collection

Fishes were collected early in the morning from East Coastal Region, Chennai, Tamil Nadu. Fishing vessels with gill nets, hook and line were used to catch marine fishes. Fishing vessels were equipped with icing systems and fish were kept at lower temperature to keep fresh. In this experiment, all fish samples were collected before sorting to avoid biasness on size. After collection, they were immediately preserved with ice in the ice box and transported to the laboratory. Samples were collected during the year 2015.

Sample measurement

After its arrival to the Zoological Research Laboratory, Government Arts College, Nandanam, Chennai-35, total length (L) and standard length (SL) of fishes were measured

using a special measuring board with a meter rule calibrated in centimeters. Fish length was measured to the nearest centimeter .Body weight (W) was measured by using Infra Digital (model IN 600) monopan electronic balance after bolt drying with a piece of clean tissue correct to two decimal places. The length-weight relationship was calculated using the equation (Le Cren, 1951; Pauly,1983; 1993) $W=_a L^b$ where W is the weight of fish in grams, Coefficient 'a' is the intercept in the y-axis ,regression Coefficient 'b' is the exponent and L is the total length of fish in cm. The value of 'b' indicates isometric growth when close to 3. The growth is positive allometric when the value of 'b' is more than 3 and negative allometric when 'b' is less than 3. The statistical significance level of R^2 was estimated and the parameters 'a' and 'b' were estimated by linear regression analysis based on the natural logarithms:

$$\text{Log } W = \log a + b \log L$$

Additionally the coefficient of determination r^2 was estimated. The Fulton's condition factor (K) for each experimental fish has been calculated using the formula: $K= (W/L^3) \times 100$

Where K is the condition factor

W is the weight of fish (g)

L is the length of fish (cm).

RESULTS AND DISCUSSION

Length –weight relationship

Length weight relationship was carried out for 19 marine fishes belonging to different family from pelagic, epipelagic, neritic and oceanic zone during the year 2015. Totally 1,110 fishes were collected and each fish existed at the average number 58. The relationship between length and weight was significant one to analyse marine fishes because this relationship determines the fish growth and productivity of marine water. The b values for all fishes were ranged from 1.99 to 4. In some fishes length weight ratio was greater than 3 and some other fishes it was less than 3. In epipelagic fishes b value was greater than 3 whereas in *Sardinella gibbosa* b=3.42, in *Stolephorus commersonni* b=3.2, in *Stolephorus indicus* b=4, in *Sarda chiliensisb*=3.06, and in *Saurida tumbil* b= 3.2. In pelagic region fishes b value was close to 3, value in *Lutjanus fulvus* b= 2.91, in *Tylosurus crocodiles* b=2.92 and in *Silage sihama* b=2.9. In other fishes b value was less than or close to 3 *viz. Rastrelliger brachisoma, Gerres filamentosus* , *Parastromateus niger* etc. The r^2 value was greater than 0.8 in all fishes of *Sardinella fimbriata* r^2=0.92, *Thryssa mystax* r^2=0.92, *Mugil cephalus* r^2=0.91 etc. The comparative study on LWR of marine fishes in east coastal region of Chennai are presented in the table 1 and figures 1 and 2. The feeding habit, feeding ground, spawning period and the seasonal availability of chosen marine fishes were also quoted in the table 1.

Fig. 1 Length weight relationship of marine fishes in ECR of chennai

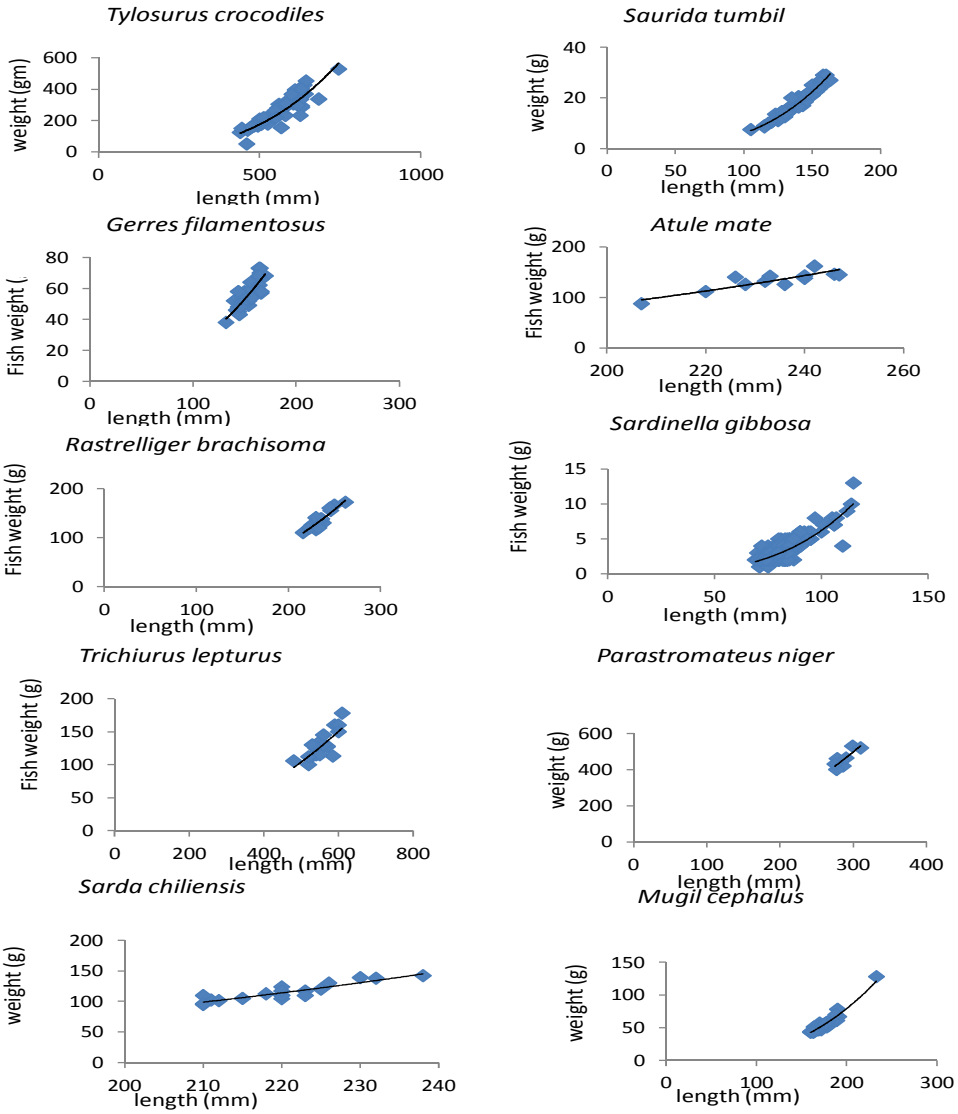
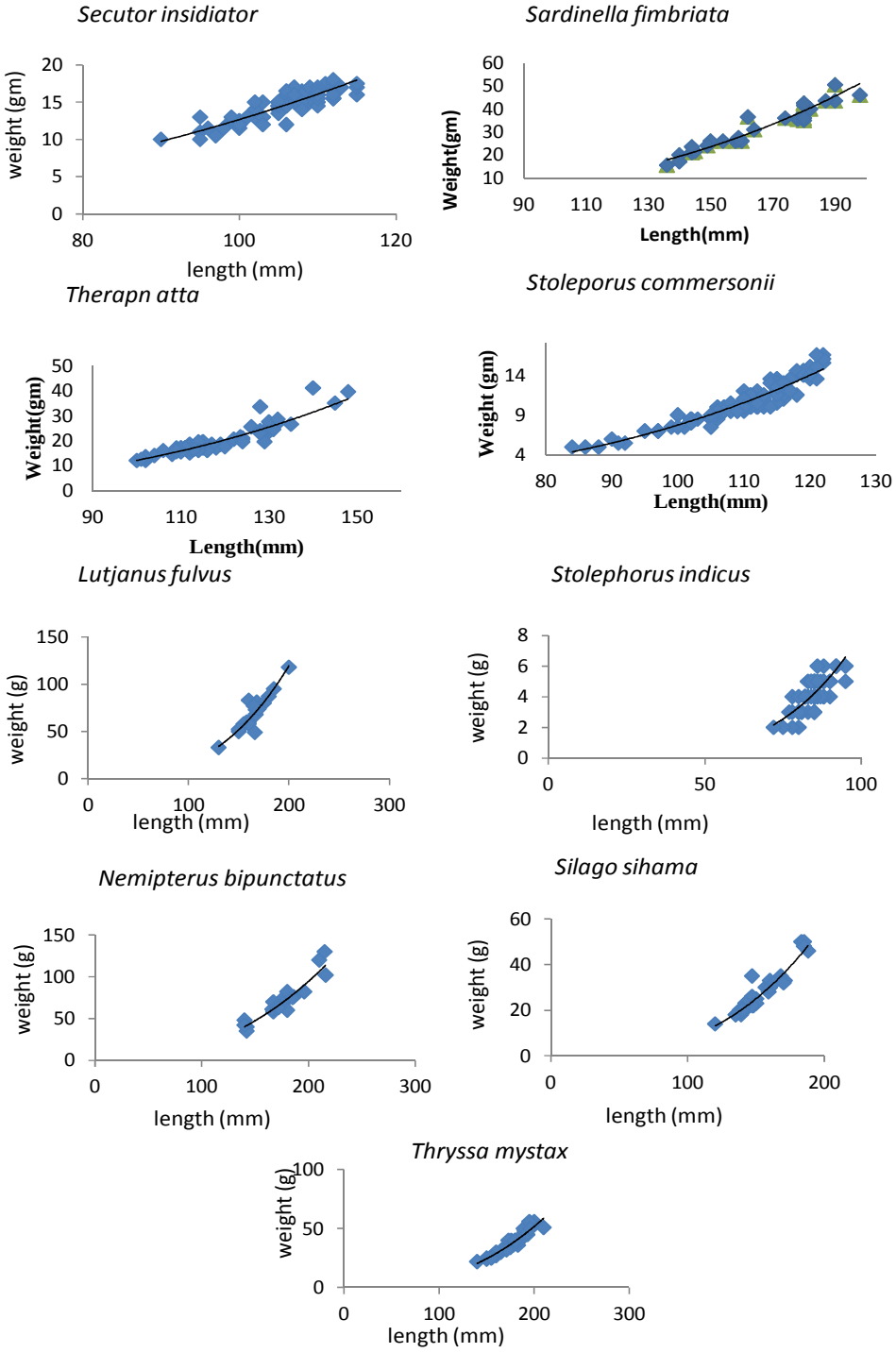


Fig. 2 Length weight relationship of marine fishes in ECR of Chennai



pecies name	F amily	o	2				eeding habit	eeding ground	pawnin g	Distribu tion	S eason
ylosurus crocodil es	B elonidae	5	.7 3	. 9 2	.00E -06	. 1 4	arnivor ous	elagic	ug and Feb- Marc	South east coast of India and indo pacific region	Y ear round
tule mate	C arangidae	2	.7 9	. 7	.00E -05	. 9	arnivor ous	elagic	ummer	Indo- Pacific: Red Sea and the east coast of Africa	Y ear round
arastrom ateus niger	C arangidae		.6 9	. 9 9	.005	. 9 3	arnivor ous	pipelagic	une to August	West and east coast of India	A ug to Dec
richiurus lepturus	C hirocentrid ae	4	.6 2	. 0 2	.000 4	. 0 7	arnivor ous	elagic	pril to Aug	West and east coast of India	J uly to April
ardinella gibbosa	C lupeidae	8 3	.5 8	. 4 2	.00E +00	. 5 8	ooplank ton	pipelagic	pril to Oct	South- west coasts of India	M ay to July
ardinella fimbriata	C lupeidae	8	.9 2	. 7 2	.00E -05	. 7	ooplank ton	pipelagic	ainy	South- west coasts of India	A ug to Dec
tolephor us commers onii	E ngraulidae	2 6	.9 2	. 2	.00E -06	. 8	mnivore s	pipelagic	ainy	East and west coast	O ct to April
tolephor us indicus	E ngraulidae	5	.5 5		.00E -08	. 6 9	mnivore s	oastal pelagic	ainy	East and west coast	O ct to April
hryssa mystax	E ngraulidae	2	.9 2	. 6	.00E -05	. 6 8	arnivor ous	elagic	une to July	Through out Indian ocean	A pril to jun
erres filament osus	G erreidae	0	.7 3	. 1 4	.001	. 2	mnivore	ub- littoral	ec to April	India, China, Japan, Indonesia etc	S ep to Jan
ecutor insidiato r	L eiognathid ae	3	.7	. 5	.000 1	. 2	arnivor ous	emersal	ct to Dec	Red Sea and the Gulf of Aden, along the Indian coasts	M ar to June
utjanus fulvus	L utjanidae	9	.8 3	. 9 1	.00E -05	. 5 2	arnivor ous	elagic	ec to april	South west and south east coast of India	S ep to Jan
ugil cephalus	M ugilidae	2	.9 1	. 7 8	.00E -05	. 0 2	ooplank ton	enthopel agic	ct to Dec	East and west coast of India	A ug to Feb
emipteru s bipuncta tus	N emipterida e	9	.9	. 4	.000 3	. 2	arnivor ous	pipelagic	pril to Sep	East coast of India	A ug to Feb
	S									South,	A

astrelliger brachisoma	combridae	8	.77	.45	.0002	.94	arnivorous	elagic	arch to September	middle-west and south east coast of India	ug to Nov
arda chiliensis	Scombridae	9	.82	.06	.00E-06	.07	arnivorous	pipelagic	onsoon	East coast of India	Oct to May
illago sihama	Sillaginidae	3	.9	.9	.00E-05	.74	mnivores	eritic sone	ec to April	East coasts of India	May to Dec
aurida tumbil	Synodontidae	6	.94	.2	.00E-06	.65	arnivorous	enthic	ct-Mar	East coast of India	Throughout the year
erapon puta	Terapontidae	9	.88	.82	.00E-05	.2	mnivores	enthopelagic	ec to April	Indo west pacific, northern Indian ocean and	Sep to Jan

The length weight relationship of marine fishes in East Coastal regions of Chennai was supported by many authors. Jaikumar *et al*., (2011) has reported that the length – weight relationship in *Lambis lambis* is in allometric growth (b=2.3765). Maria Yankova (2014) has reported the co-efficient of determination (r^2) of different samples showed high degree of correlation between length and weight of horse mackeral for female ,male and both sexes is 0.8571,0.9716 and 0.994 respectively.

Subodha Kumar and Sudarsan (2012) has reported parameters of 'a' and 'b' of the LWR of 20 fish species .The calculated 'b' value of all the species ranged between 2.5 and 3.5. Kurma Rao and Ramesh Babu (2013) has reported the regression values of juveniles (2.16), adults (2.81), males (2.66) and females (2.74) of *Mugil cephalus*. Alex Nehemia *et al*.(2012) has reported that the value of exponent 'b' and the condition factors (K) for *Tilapia Zillii* in fresh water (FW) and full strength sea water (FSSW) (in the bracket) were found to be 2.94 (3.3)and 2.07 (0.74) respectively. On the other hand the value of exponent 'b' and condition factor (K) for *Oreochromis urolepis* in FW and FSSW (in the bracket) were found to be 2.81 (3.46) and 0.86 (0.53) respectively.

CONCLUSION

Overall length weight relationship varied between families because all fishes having different behaviour and different feeding habit. The range also varied among the carnivore and herbivore fish. In carnivores, value had ups and down whereas omnivore and herbivore fish value was in linear range the carnivore fish utilized the food the when food availability was more in open sea, whereas in herbivorous and omnivorous fishes can get the food where the plankton productivity was more. The length weight relationship was determined by factor such as availability of food, water quality or productivity

ACKNOWLEDGEMENT

The authors are thankful to the Principal and Head of the department of Zoology, Government Arts College, Nandanam Chennai -35 for providing necessary facilities to carry out the work.

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WATER POLLUTION - A SILENT KILLER TO CLAM, *Perna viridis*

[1]Sivagami, K. and [2]Dr. Ronald, J.

[1]Department of Zoology, Devi Kumari Amman Womens College, Kuzhithurai.

[2]Department of Zoology, St.Xaviers College, Palayamkottai, Tamilnadu, India

INTRODUCTION

Although water pollution is an age old problem, but in this modern age, the problems like population increase, sewage disposal, industrial waste, radioactive waste, etc, have polluted our marine water resources. According to a study by the National Institute of Oceanography, Goa, Bombayites discharge more than 2,000 million cubic meters of sewage into sea each year. This is enough to fill a building about 500 meters long, 500 meters wide and over 29,000 high. Additionally 25 million cubic meters of individual effluents are basically thrown into the sea. Although several drastic measures have been taken during the last few years, the problem of pollution continues to pose a serious challenge as the threat of nuclear war. In the evolution of the human race man has reached a stage when he has acquired the power to harness and transform nature in various ways. Unless their power is used discretely the whole human race and environment stand in danger of annihilation. Ocean Acidification is formed due to the disposal of sewage into the sea. Ocean Acidification could severely impact the commercial and restoration effects by reducing viability of shell fish larvae, setting success, growth and survival. It is therefore, critically important that resource managers, local industries, restoration programs and the general public understand the extent to which ocean acidification will

affect the survival of shellfish population along the west coast. In this study, we discussed about the impact of sewage disposal to shellfish. Sewage is defined asthe water supply of a community after it has been fouled by wastes from residences, business buildings and other sources. Disposal of domestic sewage into the sea cause a major problem to the marine fauna.

MATERIALS AND METHODS

"**Clam**" is an informal term used to refer to any molluscus within Class Bivalvia. *Perna viridis* ranges from 80 to 100 millimeters in length and may occasionally reach 165 mm. Its shell ends in a downward-pointing beak. The smooth periostracum is dark green, becoming increasingly brownish towards its point of attachment (umbo), where it is lighter. Younger mussels are bright green and that becomes darker as it ages. The shell's interior has a pale-blue sheen. The mussel has a large mobile foot which it uses to climb vertically should it be covered by sediments. It also produces byssus to help it attach to its substrate. The Asian green mussel is found in the coastal waters of the Indo-Pacific region. The mussel inhabits estuarine habitats and is found in densities as high as 35,000 individuals per square meter in any submerged marine object. Although vivid green in appearance, the mussels are shrouded with overgrowth and are often hard to find. The mussels live in waters that are 11-32°C with a wide-ranging salinity of about 18-33 ppt.[3] *P. viridis* grows fastest at 2 meters below the surface, in high salinity and high concentration of phytoplankton although it can tolerate a range of salinity and turbid water. They are hermaphrodites.

We have chosen two coastal areas, Kanyakumari and Muttom. Kanyakumari is a place located in the coastal region, where Indian Ocean, Arabian sea and Bay of Bengal joins. It is a famous picnic spot, where so many picnic resorts and hotels are present. Samples of the experimental organism, *Perna viridis* were collected from Kanyakumari and Muttom and were examined visually. The healthy and malformed organisms were visually checked and separated.

RESULTS AND DISCUSSIONS

From 100, 45 organisms were affected by infectious diseases in Kanyakumari. The bacterial genus vibrio is causing the disease in stressed *Perna viridis.* This genus of bacteria are heterotrophic, they may attack the living tissues as energy sources there by acting as pathogens. Our best examples of such opportunism, associated with polluted habitats, can be found among the chitin – degrading micro organisms, some of which are enough to attack living Bivalves causing shell diseases or exoskeletal diseases or shell erosion. This abnormality can be considered in some ways as the invertebrate analogue of fin erosion. Shell erosion seems to involve activity of chitin destroying (chitinoclastic) micro organisms with subsequent secondary infection of underlying tissues by pathogens. These pathogens are from the sewage released by hotels in coastal areas. The samples of *Perna viridis* were collected from Muttom, a coastal region near Kanyakumari. In Muttom hotels, resorts are not present. From 100 only 10 were affected by exoskeletal diseases. *Perna viridis* species found in Kanyakumari are affected more than that of *Perna viridis* species found in Muttom. In Kanyakumari the hotels and resorts the waste water from laundry, kitchen,

bathroom, etc, are directly discharged into sea through pipes. This is the major waste pollutant in the sea and is responsible for the infection of *Perna viridis.* The study shows that how sewage affects marine animals at the individual or population levels. We are learning more all time about the infectious diseases of shellfish and about the mechanics utilized by invertebrates to resist infection. We have also achieved considerable understanding of how pollution induced stress can play in the development of infectious diseases in shellfish. Sewage-contaminated water causes eutrophication, which is the increase in concentration of chemical elements required for life. The nitrates, phosphates, and organic matter found in human waste serve as a food for algae and bacteria. This causes these organisms to overpopulate to the point where they use up most of the dissolved oxygen that is naturally found in water, making it difficult for other organisms in this aquatic environment to live. The bacteria are basically strangling the other organisms. Some of the organisms that do overpopulate from this can also be disease-causing microorganisms. Phosphates are also found in soaps and detergents, but there are other household products that we use every day that can be toxic to many animals and humans if they are dumped directly into a water body.

CONCLUSION

Open experiments conducted in the West coast of India have shown distribution of sewage pollutants on surfaces and of measuring their volumes in all weathers. The studies were conducted at different temperatures. The national institute of oceanography in India and central marine fisheries research institute has carried out studies in investigating various aspects of marine pollution and their effects on living beings. Finally suggested that the treatment process which is to remove all the undesirable impurities, to that extent where they do not cause any trouble and water is available to the consumers as per health standards. The municipalities in big cities either unaware of health hazards of water pollution or care little about the ecological balance in their respective territories. The radioactive contamination of oceans has posed a serious threat to the aquatic life. Besides radioactivity, toxic metals, pesticides, plastics, mustard gas and chemical warfare agents, have been dumped into the oceans causing severe ecological damage and physiological changes in the water life. If such a state continuous then whole of the aquatic life will be disappeared from this world due to human sins. Now the environmental ministry has been set up, the foremost task of the ministry would be to prepare comprehensive pollution control legislation. Simultaneously strong machinery for enforcement should be set up at the local and national level. Since pollution does not know boundaries, there should be extensive coordination of all the agencies at different levels. Every one of us should try to check the pollution and misuse of water because every drop of water is costly. Even man's important diet milk which is called amrit contains 87% of water in natural way. The lives of plants, animals and human beings are not possible without water. It is the duty of all of us to try our utmost to make proper use of water as every drop of water speaks "water is life", It is the soul of nature and hope of future".

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POSTER PRESENTATION

THE EFFECT OF POLYSACCHARIDE EXTRACT OF MANGROVE *RHIZOPHORA MUCRONATA* (LAMK) ON WSSV DISEASE RESISTANCE AND IMMUNE ACTIVITY IN SHRIMP *PENAEUS MONODON*

M. Sanjivkumar, M. Deivakumari, S. Paul Backer and G. Immanuel
Marine Biotechnology Division, Centre for Marine Science and Technology,
M.S. University, Rajakkamangalam – 629502, Kanyakumari District, Tamilnadu.

Abstract

The role of mangrove polysaccharides to control aquatic diseases will become particularly important in the future of aquaculture, especially with regard to their increasing number of synthetic antibiotic resistant strains of bacteria and viruses. The use of mangrove polysaccharides in aquaculture is now an applicable practice for the treatment of shrimp disease. The present investigation was carried out to study the immuno-stimulant effect of polysaccharide extracted from mangrove plant *Rhizophora mucronata* on *Penaeus monodon* through oral administration against WSSV. Initially the polysaccharide was extracted from *R. mucronata* and the yield was calculated as 2.68 ± 0.12%. The basic components of the extract were analyzed through UV- visible spectrophotometer and FT-IR analysis. In the UV- visible spectrophotometer, the extract was scanned at the range of 180 – 800nm and the functional groups were denoted respectively at the absorption peak of 199 and 473nm, which was confirmed that the presence of polysaccharide and hexose monosaccharide in the extract. Similarly in FT-IR analysis, 10 major groups (O-H, C-H, C=O, C=C, C-OH, C-O-H, S=O, -C-O, C-H-, C=C-H) were observed at the wavelength of 603 to 3414. Further the extracted polysaccharide was supplemented in pellet diet at three different concentrations (0.1, 0.2 and 0.3%). These prepared pellet diets were fed to shrimp *Penaeus monodon* for 45 days; a control group of shrimp was also maintained and fed with polysaccharide free diet. After 45 days of feeding experiment, the growth performance of shrimp was determined. In control group of shrimp, the weight gain and SGR were observed as 4.43g and 6.77% respectively, whereas the weight gain (4.76 to 5.60 g) and SGR (6.93 to 7.27%) were significantly increased with respect to the increasing concentrations of (0.1 to 0.3%) polysaccharide supplemented diets fed group of shrimp. After the feeding experiment, the shrimp were challenged with WSSV and the mortality percentage was recorded daily up to 21 days and the cumulative mortality index was calculated for all the tested groups of shrimp. During the challenge experiment with WSSV, the immunological parameters such as total heamocyte count (THC), prophenoloxidase activity and respiratory burst activity were analyzed at every 10 days intervals up to 21 days. The total haemocyte count of the control group at the beginning of the experiment was observed as 54.60×10^5cells/ml and it was increased (72.50 to 86.70×10^5cells/ml) with increasing the concentrations (0.1 to 0.3%) of polysaccharide supplemented diet. During 10^{th}day of experiment, the THC was decreased in both control (26.50 $\times10^5$cells/ml) and experimental groups (52.40 to 78.50×10^5cells/ml). But, at the end of the challenge experiment (21^{st}day), the THC was increased (74.37 to 88.92 $\times10^5$cells/ml) with increasing concentration (0.1 to 0.3 %) of the extract. At the beginning of the prophenoloxidase challenge experiment (0day), the activity of the control group exhibited as 0.1418 OD, whereas it was increased from 0.1504 to 0.1738 OD in the experimental groups fed with the respective concentrations

(0.1 to 0.3%) of polysaccharide supplemented diets. When the duration of challenge experiment increased, the prophenoloxidase activity was decreased in both control (0.0720 OD) and experimental groups (0.1002 to 0.1198 OD) and at the 21stday, the PPO activity was gradually increased i.e. 0.1576, 0.1682 and 0.1752 OD in respective concentrations of the diet. The respiratory burst activity of control group was observed as 0.0350 OD at the beginning of the study and it was increased from 0.0473 to 0.0682 OD at the respective concentration of supplemented diets. When the duration of challenge experiment increased to 10 days, the RB activity was also increased (0.0502 to 0.0690 OD), whereas in control group, it was decreased to 0.0072 OD. In variably at the end of the challenge experiment (21stday), the RB activity was gradually decreased to 0.0451, 0.0573 and 0.0676 OD respectively at 0.1 to 0.3% of extract concentration.

Key words: *R. mucronata* - *P. monodon* – WSSV – polysaccharide

STUDIES ON ANTIBIOTIC ACTIVITY OF BACTERIA ISOLATED FROM COW DUNG

Dr.P.Raja*, E.Peratchi Selvi
PG Student Department of Zoology, St. Xavier's College.

ABSTRACT

The prevalence of antimicrobial resistance among key microbial pathogens is increasing at an alarming rate worldwide. In this context, microorganisms offer great scope. Many microorganisms, especially gut associated bacteria provides promising source. They possess characteristic antagonistic mechanism especially chemical defense, to ward of other bacteria. In the present study enumeration of total bacterial density in fecal samples of cow and buffalo was done. Based on different morphology 20 bacterial strains were selected for antibiotic screening by applying cross-streaking method against six non-pathogenic bacteria. Then the supernatant of active three bacteria was collected and further screened for their efficiency in inhibition of growth of test bacteria. In next level, the supernatant was extracted by ethyl acetate, the crude extract was screened. This study concludes that fecal samples are good source for the isolation of antibiotic producing strains with promising activity. The active supernatant and crude solvent extracts can also be subjected to column fractionation, HPLC, mass spectroscopy, IR spectroscopy and NMR for the identification of active compounds. This work warrant further scope to explore fecal samples from cattle for the isolation of active antibiotics and to assess possible usage as probiotics.

MARINE Vibrio- A SOURCE OF BIOACTIVE SECONDARY METABOLITE

***G. RAMANATHAN, G. R.BALAJI, S.MAHARAJA, and M.RAMAMOORTHY**

Research Department of Microbiology,
V. H. N. Senthikumara Nadar College,Virudhunagar-626001,
TamilNadu, India

*****corresponding author saranbag@gmail.com

Abstract

The culturable, heterotrophic bacteria Vibrio is widely distributed in aquatic environments from brackish to deep sea waters and commonly found associated with marine organisms. Marine bacteria serve as a source of many new bioactive compounds with interesting properties. Among them Vibrios are underexplored for their ability to produce bioactive secondary metabolites. The majority of these metabolites have been isolated from three major species namely *V. parahaemolyticus*, *V. anguillarum*, and *V. vulnificus*. The metabolites include Andrimid**,** Aqabacamins, Prodigiosin, Kahalalides, Solonamide, Diketopiperazines and Siderophore-antibiotic conjugates**.** The Metabolites produced by these organisms has antibacterial, antifungal, anticancer and antiprotozoan activity. Their production has been linked to antagonism, intraspecies communication, and pathogenicity. This article focused on vibrio derived metabolites and its potential biological activity.

FIN FISH RESOURCES

*Dr. V. SARAVANAN, [1]PRIYANKA, [2]G. SUGANYA, [3]J.SUGANYA

*Associate Professor, [123]UG Students, DEPARTMENT OF ZOOLOGY,

THE MDT HINDU COLLEGE, PETTAI -10.

Abstract

Abundance, bathymetric distribution and diversity of deep sea demersal finfish resources along the south-west coast of India were studied. A total catch of 4255 kg (96.7 kg h-1) was recorded by sampling in the depths ranging from 100 to 1100 m. The major constituents were finfishes i.e., teleost fishes (67%) followed by elasmobranchs (14%), crustaceans (13%), cephalopods (2%) and others (4%). Maximum abundance (113.81 kg h-1) of finfishes was observed in the depth zone of 100-300 m, followed by 500-700 m (86.91 kg h-1), 700-900 m (67.71 kg h-1) and 900-1100 m (7.58 kg h-1). *Nettastoma* sp. (12.13 kg h-1), *Lamprogrammus exutus* (5.22 kg h-1), *Chlorophthalmus bicornis* (4.19 kg h-1), *Bembrops caudimaculata* (5 kg h-1) and *Uranoscopus* sp. (4.95 kg h-1) were the dominant fish species. The bathymetric distribution of the fishes which occurred more than one time were analysed using measures of the centre of gravity (COG) and the habitat width (HW). The analysis revealed that most of the species exhibited a wide distribution range, although a few were restricted to the greatest depths. Species richness index was maximum (8.25) at 500-700 m depth zone and minimum (3.20) at 900-1100 m depth zone. Species diversity did increase up to the depth of 900 m and declined beyond this depth, whereas, the species evenness increased with depth. Out of the total estimated

harvestable potential of 3.92 million t of marine fish from the exclusive economic zone (EEZ) of India, the present exploitation is about 3 million t, which is mainly from the inshore waters (Anon, 1991; Somavanshi, 1998; Dehadrai, 2006). The remaining potential of about 0.92 million t is largely from deep sea and oceanic regions which remains untapped (Somavanshi, 1998; Dehadrai, 2006). Although the deep sea shrimps and lobsters from Indian waters are being exploited by several private trawlers, the finfishes remain unexploited (Jayaprakash et al., 2006; Rajan et al., 2001). This is mainly due to the lack of knowledge on their abundance, distributionand diversity.

KEY WORDS

Exploitation, shrimps, bathymetric

BIO MASS RESOURCES

*Dr. A. SIVAGURUNATHAN, [1]R.CHITRA, [2]K.GOMATHI, [3]A. SARANYA

*Assistant Professor, [123]UG Students, DEPARTMENT OF ZOOLOGY,

THE MDT HINDU COLLEGE, PETTAI -10.

ABSTRACT

This paper discusses biomass as a renewable energy source. The paper defines the resources as well as the ways biomass energy is converted into electricity, technologies involved in extracting power from biomass as well as the advantages and the disadvantages of using of biomass as a source of energy. The paper also reviews a few biomass projects in the United States and some other parts of world and discusses the future of biomass. Biomass is a term used to describe all organic matter produced by photosynthesis, existing on the earth's surface. They include all water- and land-based vegetation and trees, and all waste biomass such as municipal solid waste (MSW), municipal biosolids (sewage), and animal wastes (manures), forestry and agricultural residues, and certain types of industrial wastes. The world's energy markets have relied heavily on the fossil fuels. Biomass is the only other naturally occurring energy-containing carbon resource that is large enough in quantity to be used as a substitute for fossil fuels.

The exploitation of energy from biomass has played a key role in the evolution of mankind. Until relatively recently it was the only form of energy which was usefully exploited by humans and is still the main source of energy for more than half the world's population for domestic energy needs. One of the simplest forms of biomass is a basic open fire used to provide heat for cooking, warming water or warming the air in our home. More sophisticated technologies exist for extracting this energy and converting it into useful heat or power in an efficient way. In the mid-1800s, biomass, principally wood biomass, supplied over 90% of U.S. energy and fuel needs, after which biomass energy usage began to decrease as fossil fuels became the preferred energy resources. This eventuality of fossil fuel and the adverse impact of fossil fuel usage on the environment are expected to be the driving forces that stimulate the transformation of biomass into one of the dominant energy resources.

KEY WORDS: Biosolids, residues, exploitation, sophisticated

NON RENEWABLE ENERGY RESOURCES

*Dr. V. SARAVANAN, [1]M.ESAKKI@BABY MARI, [2]P.SELVARANI, [3]P.RANJITHAM

*Associate Professor, [123]UG Students, DEPARTMENT OF ZOOLOGY,

THE MDT HINDU COLLEGE, PETTAI -10.

ABSTRACT

A **non-renewable resource** (also called a finite resource) is a resource that does not renew itself at a sufficient rate for sustainable economic extraction in meaningful human time-frames. An example is carbon-based, organically-derived fuel. The original organic material, with the aid of heat and pressure, becomes a fuel such as oil or gas. Earth minerals and metal ores, fossil fuels (coal, petroleum, natural gas) and groundwater in certain aquifers are all non-renewable resources.In contrast, resources such as timber (when harvested sustainably) and wind (used to power energy conversion systems) are considered renewable resources, largely because their localized replenishment can occur within timeframes meaningful to humans. The localized deposits of metal ores near the surface which can be extracted economically by humans are non-renewable in human time-frames. There are certain rare earth minerals and elements that are more scarce and exhaustible than others. These are in high demand in manufacturing, particularly for the electronics industry.Most metal ores are considered vastly greater in supply to fossil fuels, because metal ores are formed by crustal-scale processes which make up a much larger portion of the Earth's near-surface environment, than those that form fossil fuels which are limited to areas where carbon-based life forms flourish, die, and are quickly buried.

Keywords: fossil fuels, timber, exhaustible, manufacturing

MARINE PHYTOPLANKTON – A SUPPLEMENTARY FOOD FOR HEALTH & DISEASE PREVENTION

*Subramonian.K, [1]Ramasubramanian.S., [2]Dhinakaraselvam.M., [3]Muthupattan.P.

*Department of Plant Biology & Plant Biotechnology,

The M.D.T Hindu College,Tirunelveli-627 010.

[123]UG Students, Department of Zoology, The M.D.T Hindu College, Tirunelveli-627 010.

Abstract :

Marine phytoplankton is also known as marine micro algae, are microscopic single cell plant that are most abundant in the ocean , they are capable of turning water and a light energy from the sun into nutrients and oxygen through photosynthesis, a marine algae responsible for producing up to 90% of the earth oxygen supply. The marine phytoplankton in the ocean that cover 80% in their earth surface are by far the greatest produces of the water which sustains human life. Marine phytoplankton are considers to the be basis of all others life forms on earth. Marine phytoplankton are the basic food sources which directly are indirectly are life in the ocean. Based on nutritional research,

phytoplankton provides nutritional elements and phytochemicals that can help to prevent, treat and cure all of the following health condition. Rheumatoid arthritis, Type - 2 diabetes, Autoimmune disorders such as lupus, Eczema and skin disorders, Breast cancer, prostate cancer and other cancers, Heart disease and atherosclerosis, Dementia and Alzheimer's disease, Chronic fatigue syndrome , Parkinson's diseases and other neuromuscular disorders Liver diseases and hepatitis , Depression, mood swings and behavioral disorders, Eye diseases , Infertility and reproductive system disorders , Infections and common colds , Asthma and respiratory disorders , Kidney and bladder disorders, Osteoporosis and skeletal disorders , Chronic pain and joint pain .The nutritional micro algae are rich in vitamins, antioxidants, omega 3 fatty acids, minerals, and a wide verity of functional compounds including the master antioxidant enzyme super oxide dismutase (SOD), all in which improve health and well-being . In addition, these compounds play a preventative role because they reduce the risk factors associated with certain diseases.

Keywords : phytoplankton, Dementia, atherosclerosis

SEA WEEDS- A POTENT SOURCE OF ANTIOXIDANT

*****Subramonian.K., [1]Aishwarya . K ., [2]Vaishnavi .A , [3]Rajalakshmi.M.
*Department of Plant Biology & Plant Biotechnology,
The M.D.T Hindu College,Tirunelveli-627 010
[123]UG Students, Department of Zoology, The M.D.T Hindu College, Tirunelveli-627 010.

Abstract

Free radicals have been claimed to play an important role in affecting human health by causing many diseases (*e.g*., heart diseases, cancer, hypertension, diabetes and atherosclerosis). In the past decade, anti-oxidants have shown their relevance in the prevention of various diseases, in which free radicals are implicated. According to the previous studies, terrestrial plants are rich sources of phytochemicals possessing important properties such as antioxidant activity. Many investigators have found several types of anti-oxidants from different parts of various plant species such as oilseeds, cereal crops, vegetables and spices. Recently, a polyphenolic compound including flavonoids is known as safe and non-toxic anti-oxidants. Many studies have shown that a high dietary intake of natural phenolics is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, various types of cancer, diabetes, obesity, improved endothelial function and reduced blood pressure. Phenolic compounds are commonly found in plants and seaweeds. Like other plants, seaweeds contain various inorganic and organic substances, which can benefit human health. Algae generally has higher antioxidant activity due to a higher contents of non enzymatic antioxidant components, such as ascorbic acid, reduced glutathione, phenols and flavonoids. As a result, many marine bio-sources in the last decades have attracted attention in the search for natural bioactive compounds to develop new drugs and healthy foods. Compounds with antioxidant, antiviral, antifungal, antimicrobial, antitumor and anti-inflammatory activities have been found in brown, red and green algae. The present study aimed to list out the antioxidant properties of Marine resources for future applications in medicine, dietary supplements, cosmetics or food industries.

Keywords : antioxidant, Free radicals, Algae

SEAWEED RESOURCES

* Dr. A.S. GANGA, [1]M.KALAIARASI, [2]M.MAHESHWARI, [3]M.RANJITHA

*Assistant Professor, [123]UG Students, DEPARTMENT OF ZOOLOGY,

THE MDT HINDU COLLEGE, PETTAI -10.

ABSTRACT

The utilization of marine biomass as an energy resource in Japan. A marine biomass energy system in Japan was proposed consisting of seaweed cultivation (Laminaria japonica) at offshore marine farms, biogas production via methane fermentation of the seaweeds, and fuel cell power generation driven by the generated biogas. We estimated energy output, energy supply potential, and CO2 mitigation in Japan on the basis of the proposed system. As a result, annual energy production was estimated to be 1.02×109kWh/yr at nine available sites. Total CO2 mitigation was estimated to be 1.04×106tonnes per annum at the nine sites. However, the CO2emission for the construction of relevant facilities is not taken into account in this paper. The estimated CO2mitigation is equivalent to about 0.9% of the required CO2mitigation for Japan per annum under the Kyoto Protocol framework.

CO2 mitigation, Fuel cell power generation, Laminaria japonica, Marine biomass, Seaweed. warming has become one of the most serious environmental problems. To cope with the problem, it is necessary to substitute renewable energy for nonrenewable fossil fuel. Biomass, which is one of renewable energies, is considered to be carbon-neutral, meaning that the net CO2concentration in the atmosphere remains unchanged provided the CO2emitted by biomass combustion and that fixed by photosynthesis are balanced. Biomass is also unique because it is the only organic matter among renewable energies. In other words, fuels and chemicals can be produced from biomass in addition to electricity and heat. Marine biomass has attracted less attention than terrestrial biomass for energy utilization so far, but is worth considering especially for a country like Japan which has long available coastlines.

Keywords Seaweed, combustion, coastlines.

SHELL FISH RESOURCES

*Dr.A.S. GANGA. [1]P.GOBI,[2] M.ESWAR, [3]PUTHIYA KUMAR

*Assistant Professor, [123]UG Students, DEPARTMENT OF ZOOLOGY,

THE MDT HINDU COLLEGE, PETTAI -10.

ABSTRACT

Shellfish aquaculture is prominent in many coastal and estuarine environments. Filter feeding by cultured shellfish connects the benthic and pelagic environments in coastal ecosystems. Bahía San Quintín is a reverse estuary in Baja California, Mexico, where Pacific oysters (*Crassostrea gigas*) are cultivated. While oysters likely feed heavily on phytoplankton especially during upwelling periods, we hypothesized that other forms of organic matter available in high quantities such as seagrass (*Zostera marina*) and macroalgae (*Ulva* spp.) must also be used by the oysters, especially in the most inshore portions of the bay. We measured the carbon and hydrogen stable isotope composition of oysters and their potential food resources at upper, mid, and lower bay sites during upwelling and non-upwelling seasons and applied a Bayesian mixing model to

evaluate resource use. Hydrogen isotopes provided a large separation between potential food resources. Although we did not find any strong seasonal effects due to upwelling, there was a strong spatial gradient in resource use. Phytoplankton were most important at the lower (oceanic) site (median resource use for two sampling times, 68 and 79 %) and decreased up the estuary as macroalgae became more important (43 and 56 % at the upper site). At all sites for both sampling times, seagrass was an unimportant resource for oysters. The contrast between high phytoplankton use at the lower site and increased macroalgal use at the upper site is likely due to available resource biomass. Our results illustrate the adaptability of oysters to varying resource availability and the possibility of a higher system carrying capacity than that based on phytoplankton alone given multiple potential food sources. **Shellfish** is a culinary and fisheries term for e*xoskeleton*-bearing aquatic invertebrates used as food, including various species of *molluscs*, *crustaceans*, and *echinoderms*. Although most kinds of shellfish are harvested from saltwater environments, some kinds are found in freshwater. In addition a few species of land crabs are eaten, for example *Cardisomaguanhumi* in the Caribbean.

KEYWORDS Coastal , oysters , phytoplankton , isotopes

HEALTH BENEFITS OF SEA VEGETABLES

*Subramonian.K, [1]Esakkisaravanan.G., [2]Krishnakumar.G.[3] Saravanan. S.

*Department of Plant Biology & Plant Biotechnology, The M.D.T Hindu College,Tirunelveli-627 010.

[123]UG Students, Department of Zoology, The M.D.T Hindu College,Tirunelveli-627 010.

Sea Vegetables offer one of the broadest ranges of minerals of any food, containing virtually all the minerals found in the ocean and not surprisingly, many of same minerals found in human blood. The also offer a variety of unique phytonutrients, including their sulfated polysaccharides (also called fucoidans). Unlike some other categories of vegetables, sea vegetables do not appear to depend on carotenoids and flavonoids for their antioxidant benefits, because in additional to these two important categories of antioxidants, sea vegetables contain several other types, including alkaloid antioxidants. Sea vegetables are an excellent source of iodine, vitamin C, manganese, and vitamin B2. They are also a very good source of vitamin A (in the form of carotenoids) and copper as well as a good source of protein, pantothenic acid, vitamin B6, niacin, vitamin B1 , calcium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, vanadium, and zinc . It also has anti-inflammatory, anti-cancer, anticoagulant, antithrombotic, and antiviral properties. sea vegetables contain a variety of enzymes called haloperoxidases. It appears to play a multi-faceted role in regulation of carbohydrate metabolism and blood sugar. For women who are at risk of estrogen-sensitive breast cancers, sea vegetables may bring a special benefit in this regard. The present study aimed to list out the Minerals and Vitamins of Sea Vegetables and their related Health Benefits.

Keywords : Sea Vegetables, antioxidants, anti-inflammatory, anti-cancer

RENEWABLE ENERGY RESOURCES

* ELANGO SUBRAMANIAN.S., [1]RAMAIYA. A., [2]SASI KUMARI .M., [3]JEYA CHITHRA.S.
*Associate Professor, [123] UG Students, DEPARTMENT OF ZOOLOGY,
THE MDT HINDU COLLEGE, PETTAI -10.

ABSTRACT

Renewable energy systems can provide clean, reliable, secure and competitive energy products and services to help meet the rapidly increasing global energy demand. In a carbon constrained world of the future, renewable energy sources with zero net greenhouse gas emissions will have an increasingly important role to play. Being widely distributed, renewable energy sources have the potential to provide electric power, heating, cooling and vehicle transport fuels for the millions of people currently with limited or no access to them. Progress towards including the full externality costs relating to the use of fossil fuels in comparative economic analyses of energy supply systems, together with the rate at which the costs of renewable energy technologies can be reduced as a result of mass production of energy conversion devices and greater project experience, will determine how significant the contribution of renewable energy to the global energy supply mix will become over time. This paper outlines the socioeconomic potential for a range of renewable energy technologies. A source of renewable energy, such as from the sun or tides or wind, is one which is naturally occurring and which is theoretically inexhaustible. This, by definition, excludes energy derived from fossil fuels or nuclear fuels. Renewable energy fluxes can be intercepted and the captured energy used for the benefit of the human race with minimal environmental impacts. Renewable energy resources provide a source of energy that is not depleted when used, for example solar radiation, the motion of the wind, rivers, waves or tides, and biomass from forests and crops that after harvest are subsequently replanted.

KEY WORDS : Competitive, reliable, constrained, impacts

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *CAULERPA MEXICANA* SONDER EX KUETZING (GREEN SEAWEED) IN THOOTHUKUDI, TAMIL NADU, INDIA

JOHN PETER PAUL, J*. AND SAKUNTHALA, M.
*Research Department of Botany, St. Xavier's College (Autonomous),
Palayamkottai – 627 002, Tamil Nadu, India. E.mail: johnarock2008@yahoo.com

Metallic nanoparticles have been synthesized using different chemical methods, producing hazardous materials. Environmental issues of nanotechnology are becoming increasingly prominent area of research. Therefore there is a need to synthesize nanoparticles that are safe and environmental friendly. Biosynthesis of nanoparticles could be an alternative to traditional chemical methods for the production of metallic nanomaterials in a clean, nontoxic and ecologically sound manner. Recently, biosynthesis of nanoparticles using plant extract has emerged an easy and viable alternative to traditional chemical and physical methods. In the present study silver nanoparticles were

synthesized using the aqueous extract of green seaweed *Caulerpa mexicana* as the reducing agent. The formation of silver nanoparticles was confirmed by colour change, UV-Visible Spectroscopy, FT-IR, X-Ray Diffraction Method (XRD), Scanning Electron Microscope (SEM) and Energy Dispersive Spectrophotometer (EDS). The nanoparticles showed an absorbance at 441nm on UV-Vis spectroscopy. The presence of proteins was identified by Fourier Transform-Infra Red spectroscopy (FT-IR).The presence of elemental silver was characterized by X-Ray Diffraction Method (XRD), Scanning Electron Microscope (SEM) and Energy Dispersive Spectrophotometer (EDS). From the present study, it was concluded that the size of the silver nanoparticles were 45 to 56nm. These results not only provide a green approach for the synthesis of nanoparticles but also open a door for new pharmaceutical leads.

Keywords: Green synthesis, Green seaweed, *Caulerpa mexicana*, silvernanoparticles.

SCREENING OF DIURETIC ACTIVITY OF METHANOL EXTRACT OF *GRACILARIA CORTICATA* J.AG. (RED SEAWEED) IN KOOTHANKUZHI COAST, TAMIL NADU, INDIA

JOHN PETER PAUL, J. AND INIYA UDHAYA, C.

Research Department of Botany, St. Xavier's College (Autonomous),

Palayamkottai – 627 002, Tamil Nadu, India. E-mail: johnarock2008@yahoo.com

ABSTRACT

Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites and their discovery has significantly expanded in the past few decades. The seaweeds synthesize a variety of compounds such as carotenoids, terpenoids, xanthophylls, chlorophyll, vitamins, saturated and polyunsaturated fatty acids, amino acids, antioxidants such as polyphenols, alkaloids, halogenated compounds and polysaccharides such as agar, carrageenan, alginate, laminaran, rhamnan sulfate, galactosyl glycerol and fucoidan. In the present study, the screening of diuretic activity of *Gracilaria corticata* J.Ag**.** collected from Koothankuzhi coast in the south east coast of Tamil Nadu, India was analyzed. Dry powdered plant materials were subjected to methanol extract and studied at the dose of 200mg/kg and 400mg/kg body weight on mice. Methanol crude extract showed potential significant diuretic activity on mice in both doses from 1 hour to 4 hour as compared to the standard drug furosemide. The results showed for the first time that *Gracilaria corticata* J.Ag**.** exhibited significant diuretic activity, a property that could lead to the application in one of many useful healthcare or related products as well as in chemoprevention of the diseases

Keywords: Red seaweed, Diuretic, *Gracilaria corticata*, Furosemide. Electrolyte

DISCOVERY, DIVERSITY AND STATUS OF THE CRITICALLY ENDANGERED MANGROVE LUMNITZERA RACEMOSA WILLD. IN INDIA

K Sampath Kumar[1,3,*] and K Kathiresan[2]

[1] – Research & Development Centre, Bharathiar University, Coimbatore- 641 046, India. (E-mail: rksambath@gmail.com)

[2]- Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai – 608 502, India**.** (E-mail: kathiresan57@gmail.com)

[3]**- Present Address:** Asst Professor (T), Department of Plant Science, Manonmaniam Sundaranar University, Tirunelveli – 627 012, T.N., India.

Abstract:

Mangroves continue to disappear globally at an alarming rate, and the loss of individual mangrove species is of great concern. It is important to collect information especially the presence of threatened species for refining conservation priorities (Polidoro *et al*., 2010). *Lumnitzera racemosa* Willd. (Combretaceae), is a true mangrove species. The type specimen was collected from the coastal village Alamparai, near Villupuram, Tamil Nadu in 1799 by J P Rottler. The mangrove species, however, is getting rare and threatened along both East and West Coasts of India. The discovery, diversity, distribution and present status of the species in the region have been discussed and the first record of occurrence of the eumangrove in the Palk Bay, Southeastern coast of India is also highlighted. The threat factors affecting *L. racemosa* have been identified and strategies suggested for conserving this vanishing yet valuable mangrove species.

Keywords: Mangroves, *Lumnitzera racemosa*, India, Distribution, Conservation.

THE EFFECT OF POLYSACCHARIDE EXTRACT OF MANGROVE RHIZOPHORA MUCRONATA (LAMK) ON WSSV DISEASE RESISTANCE AND IMMUNE ACTIVITY IN SHRIMP PENAEUS MONODON

M. Sanjivkumar, M. Deivakumari, S. Paul Backer and G. Immanuel

Marine Biotechnology Division, Centre for Marine Science and Technology, M.S. University, Rajakkamangalam – 629502, Kanyakumari District, Tamilnadu.

Abstract

The role of mangrove polysaccharides to control aquatic diseases will become particularly important in the future of aquaculture, especially with regard to their increasing number of synthetic antibiotic resistant strains of bacteria and viruses. The use of mangrove polysaccharides in aquaculture is now an applicable practice for the treatment of shrimp disease. The present investigation was carried out to study the immuno-stimulant effect of polysaccharide extracted from mangrove plant *Rhizophora mucronata* on *Penaeus monodon* through oral administration against WSSV. Initially the polysaccharide was extracted from *R. mucronata* and the yield was calculated as 2.68 ± 0.12%. The basic components of the extract were analyzed through UV- visible spectrophotometer and FT-IR analysis. In the UV- visible spectrophotometer, the extract

was scanned at the range of 180 – 800nm and the functional groups were denoted respectively at the absorption peak of 199 and 473nm, which was confirmed that the presence of polysaccharide and hexose monosaccharide in the extract. Similarly in FT-IR analysis, 10 major groups (O-H, C-H, C=O, C=C, C-OH, C-O-H, S=O, -C-O, C-H-, C=C-H) were observed at the wavelength of 603 to 3414. Further the extracted polysaccharide was supplemented in pellet diet at three different concentrations (0.1, 0.2 and 0.3%). These prepared pellet diets were fed to shrimp *Penaeus monodon* for 45 days; a control group of shrimp was also maintained and fed with polysaccharide free diet. After 45 days of feeding experiment, the growth performance of shrimp was determined. In control group of shrimp, the weight gain and SGR were observed as 4.43g and 6.77% respectively, whereas the weight gain (4.76 to 5.60 g) and SGR (6.93 to 7.27%) were significantly increased with respect to the increasing concentrations of (0.1 to 0.3%) polysaccharide supplemented diets fed group of shrimp. After the feeding experiment, the shrimp were challenged with WSSV and the mortality percentage was recorded daily up to 21 days and the cumulative mortality index was calculated for all the tested groups of shrimp. During the challenge experiment with WSSV, the immunological parameters such as total heamocyte count (THC), prophenoloxidase activity and respiratory burst activity were analyzed at every 10 days intervals up to 21 days. The total haemocyte count of the control group at the beginning of the experiment was observed as 54.60×10^5cells/ml and it was increased (72.50 to 86.70×10^5cells/ml) with increasing the concentrations (0.1 to 0.3%) of polysaccharide supplemented diet. During 10^{th}day of experiment, the THC was decreased in both control (26.50 $\times10^5$cells/ml) and experimental groups (52.40 to 78.50×10^5cells/ml). But, at the end of the challenge experiment (21^{st}day), the THC was increased (74.37 to 88.92 $\times10^5$cells/ml) with increasing concentration (0.1 to 0.3 %) of the extract. At the beginning of the prophenoloxidase challenge experiment (0day), the activity of the control group exhibited as 0.1418 OD, whereas it was increased from 0.1504 to 0.1738 OD in the experimental groups fed with the respective concentrations (0.1 to 0.3%) of polysaccharide supplemented diets. When the duration of challenge experiment increased, the prophenoloxidase activity was decreased in both control (0.0720 OD) and experimental groups (0.1002 to 0.1198 OD) and at the 21^{st}day, the PPO activity was gradually increased i.e. 0.1576, 0.1682 and 0.1752 OD in respective concentrations of the diet. The respiratory burst activity of control group was observed as 0.0350 OD at the beginning of the study and it was increased from 0.0473 to 0.0682 OD at the respective concentration of supplemented diets. When the duration of challenge experiment increased to 10 days, the RB activity was also increased (0.0502 to 0.0690 OD), whereas in control group, it was decreased to 0.0072 OD. In variably at the end of the challenge experiment (21^{st}day), the RB activity was gradually decreased to 0.0451, 0.0573 and 0.0676 OD respectively at 0.1 to 0.3% of extract concentration.

Key words: *R. mucronata* - *P. monodon* – WSSV – polysaccharide