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Trends and technology development in Life Science - Dr. Sheeba E

TRENDS AND TECHNOLOGY DEVELOPMENT IN LIFE SCIENCE

First Edition

Editor

Dr. Sheeba E

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Preface

Life Science can divide into many branches. In each branch, scope of research is enormous. The future of the Life Science is technology driven which help to solve many problems in the daily life. The book entitled “Trends and Technology Development in Life Sciences” provides knowledge on some recent trends and keep us updated of the same. It enhances the diagnosis; treatment of diseases and artificial intelligence perform more complex tasks. India is one of the fastest growing Life Science markets in the industry. The pandemic made this Science field to think more innovative way and to take up the challenges such as vaccine production. Manufacturing of pharmaceuticals, biotechnology-based food and medicines, medical devices, biomedical technologies, nutraceuticals, environmental science, cloud technology, etc. are important fields and research and development happening in all these branches.Collaboration is vital between academia and Life Science industry and funding is crucial for the technology development. Recent trends and technology development boost good services, simplify the processes and cost cutting. Moreover, learning the surrounding problems and interest in research can detect solution to most of the glitches. Advancement in technology is essential for the development of country and population. Study of merits and demerits are equally important in sustainable development of science.In this book, recent research techniques in different fields are included.

Editor

Dr.Sheeba E

About Editor

Dr. Sheeba E is working as Director in Rashya Centre for Learning, Palakkad, Kerala. Previously she was appointed as Associate Professor at Indian Academy Degree College – Autonomous, Bengaluru, Assistant Professor at Brindavan College, Bengaluru, Lecturer at J J College of Arts and Science, Tamilnadu and Guest Lecturer at St. Mary's College, Kerala. She has completed her Ph.D in Microbiology from Bharathidasan University, Trichy. She received Women Achievers Award for Academician's Category held in 2021 organized by Palakkad Forum, Bengaluru and second prize in poster presentation in UGC sponsored and co-sponsored KSTA National Conference "New Approaches and Concepts in Microbial Biotechnology" held on 29 -30 September 2015 organized by Department of Microbiology, Maharani's Science College for Women, Bengaluru. She has published 7 book chapters and 14 research articles in reputed journals and 17 abstracts in conference proceedings contributed as author/co-author. She was one of the editors of the book entitled "Bio entrepreneurship in Biosciences – Recent Approaches" published by Darshan Publishers, First Edition, ISBN:978-93-93942-38-8. She has organized 1 National level conference, 2 Faculty Development Programs, 2 workshops, 6 webinars and 1 quiz as organizing secretary/committee member. She acted as a resource person in National Level Conference, webinar, Faculty Development Program, Awareness program and Orientationprogram organized by different colleges in Bengaluru and Tamilnadu. She is a Life member in Indian Women Scientists Association and International Society for Infectious Diseases and Annual member in Microbiologists Society, India.

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First and foremost, I would like to thank **God**. He has given me strength and encouragement throughout to complete this endeavour successfully.

I express my sincere thanks to **Thanuj International Publishers, Tamilnadu, India** who had given an opportunity to edit this book.

I express my heartfelt gratitude to **Dr. T. Sivakumar**, Managing Editor, Thanuj International Publishers, Tamilnadu, India for his support to publish this book.

I sincerely thank **all the authors** who contributed different chapters to publish as a book entitled “Trends and Technology Development in Life Science”.

Last but not least; I owe special thanks to my **family and friends** for their all-possible wayofsupportandcooperation.

Trends and Technology Development in Life Science

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Value Added Products from Mushroom – Need for the Hour

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Introduction

Mushrooms are bell-shaped, fleshy, spore bearing Macro fungi which grow on the surface of the soil. They usually grow at temperatures ranging 15-23°C. Mushrooms are differentiated by the presence of mycelium, stem otherwise known as stalk, a fleshy and delicate round cap and gills present underneath the cap. Mushrooms are classified into fungal kingdom as they are neither a plant-like nor animal-like, but many individuals consider mushrooms as plants despite the fact that they lack chlorophyll which plants use to manufacture food and energy. Unlike plants, the cellwall of mushroom is rigid and is made up of complex polysaccharides such as chitin, glucan and glycoproteins like fungi, whereas, animals lack cell wall. Mushrooms are saprophytic which obtain their nutrition by metabolizing organic compounds (straw, wood, manure etc) as they have the ability to produce wide range of enzymes which helps to break down complex substrates and absorb the nutrients obtained during degradation. Mushrooms are perishable and highly nutritious that are considered as healthy food due to the presence of high protein, dietary fibre, vitamins, minerals and anti–oxidants. This nutrient dense food can be preferred as a substitute to meat, fish, fruits and vegetables. Mushrooms contain a compound called ergosterol whose structure is similar to that of animal cholesterol. Ergosterol can be converted to vitamin D on exposure to ultra violet radiation.Therefore, mushrooms are considered as a magnificent source of food to reduce malnutrition in the developing countries.

There are 2 types of mushrooms namely: edible mushrooms and non-edible mushrooms. Approximately 3000 edible mushrooms were identified out of which nearly 200 species are consumed by humans and around 700 species are considered as healthy therapeutic mushrooms.Some of the edible

mushrooms which are commonly used throughout the world are button mushroom *(Agaricus bisporous),* oyster mushroom*(Pleurotus ostreatus),* shiitake mushroom *(Lentinula edodes),* paddy straw mushroom *(Volvariella volvacea),* Milky mushroom *(Calocybe induce).*White or button mushroom *(Agaricus bisporus)* contributes more than 90% of total mushroom production in India. Other mushrooms like reishi mushroom *(Ganoderma lingzhi)* are used for medicinal purposes as they are tough to eat and digest whereas few are poisonous such as amanita mushrooms*(Amanita phalloides).* Lately, the demand for mushrooms has increased tremendously due to its high protein content.

Mushrooms cannot be stored for more than 24 hours at ambient conditions due to the high moisture content. Storage of the mushroom more than the shelf life leads to microbial spoilage. Due to the perishable nature of mushrooms, drying, preservation techniques and value addition comes under the picture in order to store, retain nutrients and also to enhance the quality of the product. Value addition of mushrooms helps to intensify the savoury of the products at the same time, the amount of nutrients remains unchanged. Value can be added to a product from a readymade snack to main course. The famous value – added product of mushroom in India is mushroom soup powder. There are other value-added products of mushroom such as biscuits, nuggets, chutney powder, candies, pickles, ready to make curries etc, which are available butthese products are not popularized yet. China, USA and India are in top 5 countries in the production and cultivation of mushrooms.

Nutraceutical Aspects of Mushroom

The practice of eating mushroom is called Mycophagy. Nearly 14,000 species of basidiomycetes were identified by researchers. Approximately 7000 edible mushrooms were identified out of which nearly 2000 species are considered as high value edible mushroom and around 700 species inhabit pharmacological properties within. The energy produced by mushroom is approximately 250 – 300 cal/kg. The fruiting body of mushroom consist of about ~90% of water and the remaining is filled by secondary metabolites with biologically active compounds which are crucial and responsible for human health care. Mushrooms face a lot of biotic and abiotic stresses due to change in acidity and humidity or invasion of microorganisms. To withstand these situations, mushrooms release various types of secondary metabolites which prevent the entry of parasites and predators and prevent diseases which helps in the production of mushrooms with high yield and quality. The nutrient composition in mushroom comprises of high protein and carbohydrates, low

fat, rich in vitamins B, C, D & K, minerals such as potassium and phosphorous and fibres such as chitin.

Protein

Protein or polypeptides are a sequence of amino acid chains which help in general growth, body tissue repair, maintenance of the cell and provide structural support. The amount of protein present in dry weight (DW) of mushroom is ~3g/100g. The proportion of protein varies from species to species based on the composition of substrate, size of pileus (fungal fruiting body or cap) and harvest time. All the 9 essential amino acids namely Histidine, Lysine, Tryptophan, Leucine, Iso-leucine, Phenylalanine, Methionine, Threonine and valine are present in the form of branched chain amino acids (BCAA) which is a characteristic feature of amino acids present in animal proteins. Particularly Leucine and Lysine are present in high concentrations in mushroom. The presence of Glutamic acid and Aspartic acid is responsible for a peculiar savour called as the umami flavour which is usually present in meat. *Agaricus bisporus* has high protein when compared to other edible mushrooms. The protein content is high in a wild edible mushroom when compared with the cultivated mushrooms. Mushroom falling under Basidiomycota are considered as single cell protein (SCP) as it is rich in protein sources.

Carbohydrates and Dietary Fibres

Carbohydrates are naturally occurring organic bio-macromolecule which is made of carbon (C), hydrogen (H) and oxygen (O). Energy production and storage, constructing macromolecules, protein usage and regulation of blood glucose level are the major functions of carbohydrates. Approximately ~1g/100g of dietary fibres and ~2g/100g of sugars are included in ~3g/100g of carbohydrates content in dry weight (DW) of mushrooms. Carbohydrates in mushrooms are mostly made up of polysaccharides which are polymers of monosaccharide units. Xylose, rhamnose, fructose, glucose, sucrose, maltose, mannose and trehalose are few commonly found monosaccharide units or sugars found in mushroom which are biologically important.

Dietary fibres are the non – digestible carbohydrates which pass through stomach and intestines unchanged. Chitin (polymer of N – acetyl, D – Glucosamine), Hemicellulose (Xylans and Galactans) and β-glucans are chief dietary fibres present in mushroom. These are the major constituents of the cellwall in mushroom or any other fungi. These fibres play a crucial role in industrial purposes.

Vitamins and Minerals

Vitamins and minerals are organic compounds which is required in minor quantity by our body to carry out metabolic processes and keep us healthy. However, these compounds are not synthesized by the body and are derived from the food consumed. There are wide range of water and fat - soluble vitamins which are present in the pileus of mushroom. Water soluble vitamins in mushrooms are composed of B complex vitamins such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), Folic acid (B9) cobalamin (B12) and ascorbic acid (vitamin C) out of which vitamin – B2, B3, B5 and B9 are abundant in mushrooms. Retinol (Vitamin A), calciferol (Vitamin D), alpha tocopherol (Vitamin E), phylloquinone (Vitamin K) are the fat – soluble vitamins present in mushrooms amongst which Vitamin D is the most salient vitamin present in mushroom. Vitamin D is existed in the form of a sterol known as ergosterol which is precursor to Vitamin D, whose structure is similar to cholesterol in animals. On exposure to sunlight ergosterol is converted to ergocalciferol (Vitamin D2) which helps in effective and easy absorption of calcium and phosphorous. The amount of Vitamin D present depends on duration of exposure to sunlight or UV light. Recent studies confirmed that the exposure of mushrooms to UV lamp has increased the content of Vitamin D when compared to the natural exposure. Using water as base for cooking or boiling mushrooms does not change the vitamin D content whereas cooking in oil will affect the content of vitamin D as calciferol is a fat – soluble vitamin.

Minerals are about 4% of DW of mushrooms.The major minerals present in mushroom are calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorous (Ph) and manganese (Mn). Copper (Cu), iron (Fe) and zinc (Zn) are present in traces. Selenium (Se) is one of the most vital and imperative Mineral which is present is mushroom. Selenium is an anti – oxidant which helps to neutralize free radicals present in the body. Mineral content in mushroom varies based on age and diameter of the fruiting body and nutrient sources used during cultivation.(Nagulwar et. al, 2020, Debasmita et.al, 2021 and Prasenjit et al. 2021).

Fats

Fats are present in insignificant amounts in mushroom. Fats are present in low quantity when compared to that of carbohydrates and proteins. Poly unsaturated fatty acids (PUFA) are the major constituents of fats which are present in the pileus of edible mushroom. Unsaturation is due to the presence

of linolenic acid in high quantities. Linolenic acid is an essential fatty acid which by its existence reduces the presence of tri – glycerides. The lipid content in mushroom depends on the oxygen availability, temperature, nutritional factors and growth conditions of mushroom.

There are other growth promoting substances such as enzymes (aromatase), alkaloids (cordycepin, lectins and lovastatin), anti-oxidants (Triterpenoids), polyphenols, glycoproteins etc. are present. These compounds are identified by efficient biotechnological methods.

Therapeutic Importance of Mushroom

Despite the nutraceutical aspects present, mushroom possess a lot of pharmacological and therapeutic importance which contribute for the wellbeing of humans. Nutritional and medicinal properties of mushrooms were recognized by our traditional folks and due to its rediscovery mushrooms are in high demand. Mushroom are known for its biological properties such as anti-microbial, antitumor, antioxidant, antidiabetic, anti-allergic, anti-asthmatic, anti-inflammatory, anti-cancerous, anti-oxidative and many more. Overall, mushrooms are health-boosters which protects body from various diseases. Here are few therapeutic properties of mushroom which are discussed below:

Mushrooms contain Host Defence Potentiators (HDP) which helps to enhance immunity by releasing macrophages at the spot of infection and develops resistance to various bacterial, fungal, parasitic and viral infections caused by microorganisms by the activation of non-specific immune stimulation. Macrophages kill the foreign antigens by a process called phagocytosis where it engulfs and destroys the invaded organism.

Vitamins present in mushrooms helps to convert food into energy by carbohydrate, fat and protein breakdown which is essential to carryout normal lifestyle. These helps to maintain healthy skin, hair and blood and also aids the release of neurotransmitters, steroids, red blood cells (RBCs) etc in consistence. Calcium and phosphorous in combination work miracles. The easy absorption of calcium (Ca) and phosphorous (Ph) with the help of calciferol (Vitamin D) ensures strong bones, teeth, nails, hair etc and also promotes cell growth, immune function and reduces inflammation. Pyridoxine (Vitamin B6) avails the formation of red blood cells, proteins and DNA.

Potassium helps in regulation of blood pressure by lowering tension of blood vessels which results in low BP. Sodium (Na) also helps in Blood pressure regulation where high consumption of sodium in diet increases fluid content and leads to high BP and vice versa. Copper (Cu) helps in absorption

of oxygen and promotes the formation of red blood cells (RBCs). Zinc (Zn) helps in immune system regulation and is responsible for optimum growth in infants and children. Iron (Fe) though present in traces aids the formation of haemoglobin in RBCs and myoglobin in muscles. Few minerals such as Mg, Mn, Mo etc are essential for muscle contractions.

Polysaccharides are anti-tumor and immunomodulating compounds which play vital biological roles in our regular lifestyles. The glycemic value of mushroom is zero therefore regulates the blood glucose levels. Homo and heteropolymers of these polysaccharides act as antitumor compounds although they don't interact with tumor cells directly however reduces the size of tumor cells by 50%.

Carbohydrates helps in the stimulation of growth of gut microbiota. Unlike other compounds carbohydrates do not rupture the mucosa lining of stomach and are not broken down by stomach acids instead they reach colon comfortably and revamp the gut health. Consumption of mushrooms along with regular exercise and change in life style aids weight loss due to the presence of dietary fibres.

The presence of poly unsaturated fatty acids (PUFA) in the pileus of mushrooms helps in the reduction of serum sterols such as serum cholesterol therefore decreasing the changes of occurrence of various cardio vascular diseases (CVDs) such as atherosclerosis which occurs due to the deposition of fats or cellular waste products etc in the inner lining of arteries which ultimately leads to blockage of blood flow and results instroke. PUFA also helps to prevent hypercholesterolemia which is a condition due to increase in the amount of low-density lipoprotein (LDL) in the body.

The compounds which are responsible for antioxidant properties in mushroom are Selenium (Se), Polysaccharides such as chitin and β Glucans, α tocopherol (Vitamin E) and ascorbic acid (Vitamin C). These compounds mainly help in preventing cancers, neutralizing unstable molecules which causes damage and helps to boost immunity. β Glucans play a significant role in activation of both innate by releasing dendritic cells, natural killer (NK) cells, macrophages, mast cells, neutrophils, basophils and eosinophils and adaptive immunity by stimulating the release of white blood cells (B – cells and T – cells) and lymphocytes. Selenium is a prominent metal as it helps to reduce hypertension, neutralize free radicals, prevents cell damage and plays a vital role in the treatment of metabolic disorders such as alzheimer's and parkinson's diseases. α tocopherol (Vitamin E) also helps in the treatment of

alzheimer's and also prevents the damage of cells, proteins and lipids. Steroids such as triterpenes inhibits the release of histamine and reduces inflammation resulting in anti-inflammatory effect.

Button mushroom inhibits the activity of an enzyme called as aromatase which helps to release estrogen. Inhibition of this enzyme reduces cell proliferation which further decreases the risk of breast cancer.

(Priyanka etal, 2022, Virginia, 2021, Fakhreddin, 2019, Mohd et al, 2017, Abdul et al, 2021, Hassan et al, 2022, Prasenjit et al, 2021, Javad et al, 2020, Sushila et al, 2011 and Muhammad et al, 2021).

Preservation of Mushroom

Mushrooms are highly perishable in nature hence they tend to deteriorate very easily and cannot be stored for long period of time. This leads to loss of weight, veil-opening, browning, liquefaction and finally results in microbial spoilage of the product. Preservation methods play a crucial role to encounter serious post – harvest problems. Preservation of mushroom helps to prevent microbial spoilage, permit longer shelf life, low chances of contamination due to presence of chemicals, no loss of quantity and quality of the product while on contrary it enhances the flavour, texture, colour and the nutrient content of the product. Drying and canning are the most commonly used preservative methods. Steep preservation prevents the product from spoilage only for a short span of time. Low doses of γ – radiation delays maturation process in mushrooms therefore reduces loss of water, colour and flavour and quality of the product. There are 5 main types of preservation techniques used:

1. Smoking
2. Drying
3. Canning
4. Steep preservation and
5. Value addition of mushrooms(R.D. Rai and T. Arumuganathan, 2008).

Smoking

Presence of water content is prone to easy spoilage as microorganism favour moist conditions therefore leads to proliferation of cells by utilization of nutrients which results in the spoilage of the product. Mushrooms contain about 90% of water in them. Smoking is one of the ancient methods of preservation which is done to remove excess moisture making the product less prone to spoilage. The heat produced during this method helps to remove the

moisture content. This preservative method is carried out by burning of wood which results in heat production. This gives a characteristic flavour to the food when cooked. Smoking is done only when there is no enough sunlight for a really long time.

Drying

Drying is one of the oldest techniques used to decrease the moisture content in commodities. As mushrooms are rich is moisture content, drying of the product brings down the moisture content from 90% to 10% making it free from spoilage due to microbial activity or biochemical activity. This dehydrated product contains low moisture content with a longer shelf life. These dehydrated mushrooms are stored for a longer period and are used in various food formulations such as salads, soups, snack items, rice dishes and etc. These dried mushrooms can be consumed after rehydration according to the convenience of the consumer. Different types of drying methods are followed such as:

Sun – Drying

Sun – drying is one of the oldest and cheapest technique followed in all the drying methods. Sun – drying is important and natural method where care has to be taken while performing. Even though environmental affect causes damage to the product, sun – drying is still considered as cheapest method as it doesn't depend on any fuel or mechanical energy. Mushrooms are cleaned and are placed on flat trays. These flat trays are placed in dust free zone and are exposed to sunlight for few days which is totally based on degree of temperature (above 25°C and ≤ 50% relative humidity and high wind velocity). These mushrooms are usually placed upside down where gills are exposed to sunlight. The moisture content of sundried mushrooms is about 10 – 12%. However, the sun – dried mushrooms are further oven dried at 55 – 60°C to prevent further spoilage and promoting longer shelf life of the product. Sun – dried mushrooms can be stored for about a year in air tight containers.

Cabinet Air Drying

The conventional method such as oven drying method resulted in change of colour from white to dark brown especially in the case of Button mushrooms. As the air movement inside the oven occurs in limited spaces due to lack of ventilation, the evaporated water is condensed on the pileus of mushroom leading to deteriorating qualities of the product. To overcome this scenario, cabinet air drying method with the help of tray – dries or cabinet air dries are used. Cabinet air dryers contains a series of trays arranged in the

plenum chambers. The hot air is circulated through mushroom to accomplish the removal moisture content. Ventilation of hot air is done through electrical or mechanical powers and also requires fuel. Cabinet air drying is usually done at 55 - 60°C. However, the temperature and the amount of hot air passing through mushroom is regulated and adjusted.

Fluidized – Bed Drying

Fluidized bed drier is used in this method which helps to remove the moisture content present in the commodity by means of high velocity of hot air. Mushrooms are exposed to high velocity hot air in such a way that the hot air does not dispense the product and keep them at fluidized condition. Fluidized bed drying not only provides high quality product but also reduces the time duration of the preservative process.

Osmo – Air Drying

Osmotic – air drying is also known as osmotic dehydration which is usually applicable for delicate or highly perishable commodities such as fruits and vegetables. The main principle behind osmotic dehydration is to avoid thermal treatments and ensure removal of moisture content at low temperatures and to get a desirable product with flavour, texture and colour which is similar to that of the natural form. Osmotic dehydration occurs in 2 stages,where it includes pretreatment of commodities in osmotic syrup (high salt or sugar concentration) as first step and the second step in this method includes the removal of stabilized product from the osmotic syrup and production of product after proper air drying of the commodity is done.

Freeze –Drying

The main principle behind freeze – drying is sublimation where the water content is removed from frozen to vapour state directly instead of converting to liquid state. Usually mushrooms are freeze dried at -20°C and the product is sublimated at a very low vacuum for about 12 – 16 hours. Freeze dehydration is carried out in 3 steps: Firstly, the moisture present in the commodity forms ice crystals due to very low temperatures, this is now sublimated for the removal of ice crystals and then left in the freeze dryer for the removal of left over water by evaporation. The size, shape and flavour of the product are retained and the freeze – dried product is applicable for storage upto 6 months without deterioration. Freeze – drying is very expensive and requires excessive utilization of energy.

Canning

Mushroom can be preserved for a year or two by a method known as canning.To produce good quality mushrooms, the products should be processed immediately. In case of any delay for processing, mushrooms should be stored at 4 - 5°C until processed.Usually, the canning provokes shrinkage and weight loss of the product which is a major problem during the process of the method of preservation. Canning of mushrooms involves a series of steps to be followed such as:

- Sorting of mushrooms based on the diameter of cap, colour, diseased etc is done and then it is washed 3-4 times to remove soil adhered around the mushroom. Iron free water with 0.1% of citric acid is used for washing to prevent discolouring.
- Blanching is done for 4 – 6 minutes at 95 - 100°C in a stainless kettle which contain brine solution with a combination of 1% salt and 0.1% citric acid. Inhibition of enzymatic activity and inactivation of microbes is usually done by blanching
- Filling of the blanched products into cans of various sizes is done. Cans contain brine solution (2% salt + 0.1% of citric acid) and are washed, sterilized and are passed through the exhaust box for about 10 - 15 mins at atemperature where cans reach 85°C.
- After sterilization in autoclave for about 30 mins at 15lbs pressure, the cans are cooled undergo labelling and packing of the product.

Steep Preservation

Steep preservation is a short – term storage (3 – 6 months) method which can be easily transported avoiding spoilage and browning of mushrooms. Mushrooms are dipped in steeping solution which contains 2% of NaCl, 2% citric acid, 2% sodium bicarbonate & 0.15% KMS (potassium meta bisulphite) along with 2% salt, 2% sugar, 0.3% citric acid, 1% ascorbic acid and 0.1% KMS solution which is a concentrated solution of salts or acid. For steeping, blanched mushrooms for 8 – 10 days at 21 - 28°C were used. Unexhausted steep preservation and exhausted steep preservation are the two techniques which are followed in this method. (R.D. Rai and T. Arumuganathan, 2008).

Value Addition of Mushrooms

Even today the focus of the Indian mushroom industry is to produce fresh products as an alternative than the products with real value addition.

Value addition in mushroom has a huge impact on humans as many preferably look out for products which has high nutrient content in them. Only product export is in the preserved form whereas the domestic trade is in fresh form. There is a high demand for value added products especially for ready to make or instant products as they are rich in nutrients and makes it easy for the consumers to cook. Value addition of the commodities helps to overcome post-harvest damages caused, leading to microbial spoilage making it unfit to consume. Attractive packaging of the product is included under secondary value addition which also plays an important role that allures customers to purchase the product. Mushroom biscuit, Mushroom nuggets, Mushroom soup powder, ready to make curry etc are some of the value – added products of mushrooms. Value addition completely changes the physical state of the product but preserves the nutrients of the commodity.

(G C Wakchaure, 2011, R. D. Rai et al, 2007, Priyanka Priyadarsini, 2020).

Here are few benefits of value addition of mushroom as listed below:

i. Time saving method which is easy to prepare or cook.
ii. Value added products are easy to transport.
iii. Gives longer shelf life to the product and prevents loss of nutrients.
iv. It helps in better utilization of resources.
v. Enhancement of the value of the product according to the business plan.
vi. Helps to generate employment.

Value added products of mushrooms which are commercially produced are discussed below:

Mushroom Powder

Mushroom powder is a dried form which is rich in nutrient content and can be preserved for a longer period of time. Different types of mushroom powders are available which are made of a wide range of varieties. Mushroom powder can also be prepared at home by dehydrating and later pulverizing them. Fresh mushrooms which are not damaged were picked and cleaned properly to remove adhered soil particles. These cleaned mushrooms are further chopped into small pieces and are pretreated with blanching (2% salt + 0.01% citric acid) in water at 100°C for 3 minutes. Blanched mushrooms are dehydrated at 65°C in a tray drier for 6 hours. The mushrooms are kept for drying until the moisture content is brought down to approximately 5-10%. The dried mushrooms are cooled and are converted to powder form. This powder is sieved and are stored in air tight containers. Nutrient content in all the bakery

products such as biscuits, cakes can be enriched by the addition of high protein powder which is made from dehydrated mushrooms. Mushroom powders can be added to many recepies such as soups, salads, curries, beverages etc and enhance the nutrient content. Mushroom powder has high protein, minerals and carbohydrate content and has low fat. Approximately 10 % of moisture content is present in mushroom powder.

Mushroom Bread

Mushroom bread is prepared by the addition of mushroom powder in specific concentration along with the other ingredients which are used in the preparation of white bread. Usually, 5% of mushroom powder is preferred over other concentrations as it gives best results for texture and total acceptability. The addition of 5% mushroom powder to the normal white bread increased the nutrient content when compared to that of white bread and also has the umami taste. List of ingredients used in mushroom bread preparation are listed below:

Ingredients: (Fakhreddin, 2019)

Mushroom powder	Salt
Wheat flour	Vegetable oil
Milk powder	Yeast
Sugar	Distilled water

Mushroom bread contains compounds such as ergothioneine which exhibits antioxidant properties that helps to replenishes cells and γ – aminobutyric acid that acts as primary inhibitory neurotransmitter and helps to reduce neuronal excitability. Addition of mushroom powder enhances dietary fibre, antioxidant and phenolic content and helps in starch degradation.

Mushroom Biscuits

Biscuits are usually rich in carbohydrates and fat but are low in vitamins, fibres and minerals which is unhealthy for the regular consumption. Addition of mushroom powder to these biscuits will enhance the flavour, enrich the essential nutrient content and also has low calorific value. The powder made from white button mushroom, oyster mushroom and shiitake mushroom are the commonly used mushroom powder for the preparation of mushroom biscuits. Addition of high concentration of mushroom powder in biscuits will proportionally increase protein, ash i.e., minerals, total dietary fibres and β Glucan levels which promote low calorie content. Mushroom

biscuits can be made from both fermented and unfermented batter also increases the nutrient levels such as dietary fibres, minerals and various amino acids which turn biscuits as gluten free formulations. Researchers found that addition of 15% of mushroom powder gave effective results when compared to the other concentrations. The colour, texture, flavour, appearance and the aroma of the biscuits concentrated with 15% of mushroom powder was accepted rather than the other concentrations. Ingredients used for the preparation of mushroom biscuits are listed below:

Ingredients: (Jyothi etal, 2016)

Refined wheat flour	Ghee
Mushroom powder	Ammonia
Milk	Baking powder
Sugar	

Refined wheat flour and mushroom powder should be measured and taken in a ratio of 80:20 or 90:10 for best results. The proximate composition of the mushroom biscuit includes enormous amounts of proteins, carbohydrates which are reducing & non – reducing sugars and starch, soluble & insoluble dietary fibres, polyphenol and minerals such as Fe, Zn and Ca. Presence of these constituents will boost the immunity and minimize the risk health conditions such as atherosclerosis, cancer, diabetes etc.

Mushroom Cake

Approximately 4 – 10 percent of mushroom powder can be added to regular ingredients which are used for cake preparation. Addition of mushroom powder amplifies the nutrient and sensory properties present in cake. Addition of mushroom powder reduces the volume and increases the firmness of the cake. Researchers proved that the increased concentration of white button mushroom powder in cakes not only escalated the levels of protein and ash but also improved viscosity of cake batter, volume and cohesiveness value of the baked cakes. The ingredients used for the preparation cakes incorporated with mushroom powder includes:

Ingredients: (M. A. M. Sheik etal, 2010)

Mushroom powder	Salt
Wheat flour or maida	Baking powder
Sugar powder	Butter or vegetable oil.
Egg	Vanilla essence
Milk powder or milk	

Addition of 15% of mushroom powder increased the flavour, colour and texture of the cake whereas 10% is the widely accepted concentration for sensory properties. 15% of mushroom powder increases the quantity of moisture (~19%), protein (~15%), carbohydrates (~50%), fats (~14%) and minerals (~0.8%).

Mushroom Nuggets

Powders such as black gram powder, soybean powder and urad dhal powder are used in for the preparation of mushroom nuggets. The following ingredients are used for the preparation of mushroom nuggets:

Ingredients:(G.C. Wakchaure, 2011)

Mushroom powder
Urad dhal powder
Salt
Red chilli powder
Sodium bicarbonate
Water

Mushroom powder is mixed with urad dhal powder in 1:8 ratio and this mixture is prepared into paste by addition of required amount of water, salt and other spices. Round balls with 2-3 cm are prepared and are sundried by spreading them on a tray. These can consume in two ways: these can directly be deep fried and can be used as a snack or can be used in curries alone or in a combination with other vegetables. Nuggets made out of white button mushrooms have high levels of protein and that made of oyster mushrooms are rich in other nutrients such as fat, vitamins, minerals etc.

Mushroom Soup Powder

Mushroom soup is frequently used as an appetizer and also consumed as main course food item for the people who strictly follow diet. White button mushrooms and oyster mushrooms are the commonly used mushrooms for the preparation of mushroom soup powder. Button or oyster mushrooms are dehydrated in de-humified cabinet air dryer for the production of good quality mushroom powder. The main ingredients used for the preparation of mushroom soup powder are:

Ingredients: (G.C. Wakchaure, 2011 and R.D. Rai and T. Arumuganathan, 2008.)

Mushroom powder	Salt
Milk powder	Black pepper
Corn flour	Cumin powder
Refined oil	Ajinomoto
Sugar	

Mix the ingredients with equal volume of water enhances the taste and aroma of the soup. Mushroom soup can also be customized by addition of spices like garam masala and other ingredients such as ginger, garlic, tomatoes, roasted bread, oregano etc. Mushroom soup powder contains prebiotic fibres, antioxidants and polysaccharides which help to support daily health and body functions. Ajinomoto is rich in glutamic acid which is responsible for rich umami flavour. 20% of Oyster mushroom powder along with 40% milk, 25% corn flour and other ingredients has the standard properties on the basis of nutrient content and organoleptic properties.

Mushroom Pickle

Indian thalis devoid of pickles are considered to be futile. They give the best taste when consumed along with parathas, chapati and rice. Pickles made with mushrooms are healthy and savoury when compared to other pickles. Mushroom pickle is a very popular and frequently prepared product that helps to preserve mushrooms for a longer period of time. Preparation of mushroom pickle is an alternative as canning is not preferable due to weather conditions especially in rural areas. Milky mushroom, shiitake mushroom and oyster mushroom are the most routinely used for the preparation of pickle. For the preparation of pickle, mushrooms are pretreated with 0.03 – 0.05% of KMS solution and are blanched at 85°C for 3 – 5 minutes. Mushrooms are cooled

after pretreatment and are chopped into halves or graded. After this, mushrooms are undergone salt curing process where 20g of NaCl is added per kg of mushrooms and are left overnight. This process helps to remove the water content from mushrooms. Mushrooms are dried for 2 to 3 hours and all the other ingredients are addedto enhance the quality and taste of the product.

Ingredients: (Jyoti etal, 2016)

Rehydrated mushrooms
Black Mustard seed Powder
Salt
Turmeric powder
Red Chilli powder
Cumin seed powder
Fennel seed powder

The amount of sodium present in pickle that helps to regulate blood pressure. Excess quantity of salt and oil present in pickle helps to preserve pickle for a long period of time as it oozes out excess moisture content and prevent contamination. Vinegar helps to regulate blood glucose levels. Picking of mushrooms helps to reduce weight and get rid of constipation and gas.

Mushroom Preserve (Murabba)

Murabba is usually referred to the fruits and vegetables which are cooked in high concentrations of sugar syrup. This recipe is popular in Middle east, south & central Asian countries.The main ingredients used in the preparation of mushroom preserve is sugar solution. Mushrooms can be used as it is or can be chopped into pieces for preparation of murabba. The first step of preparation of murabba is grading of mushrooms followed by cleaning of mushrooms to remove adhesive dust present. Mushrooms are pricked for better absorption and then blanched in 0.05% KMS for 10 minutes. These blanched mushrooms are treated with sugar which is 40% of mushrooms weight for 3 days. Then mushrooms are taken out and are treated with 0.01% of citric acid and remaining 40% sugar syrup. After the concentration reaches 65° Brix, the mushrooms are taken out and are dipped into a different container which has freshly prepared sugar syrup and are stored in air tight container.

Mushroom Ketchup

Ketchups are highly viscous in nature that are usually prepared with concentrated pulp of fruits or vegetables. Mushrooms are washed in 0.05% of KMS solution and are cooked in 50% of water which are later blended into a mixture. Mushroom paste along with other blended ingredients are cooked until TSS is brought to 38°Brix.

Ingredients:(R.D. Rai and T. Arumuganathan, 2008.)

Mushroom paste	Black pepper
Acetic acid	Red chilli powder
Sodium benzoate	Ajinomoto
Onion	Arrarote
Ginger	Salt
Garlic	Sugar
Cumin	

Mushroom ketchups are used as a side dish for snacks. Unlike other ketchups, mushroom ketchup act as high sources of nutrients and also enhances the flavour due to the presence of other ingredients. Mushroom ketchups also contain low amounts of acids and are rich in sugar content.

Mushroom candy, Jam, Chutney powder, Mushroom papad, Mushroom chips, ready to serve mushroom curry and other fortified corn extrudates are the other value-added products which can be prepared by including mushroom as a key component.

Conclusion

Most of the localities throughout the world sell mushrooms without proper packaging and unspecified quantities. Therefore, value addition comes into the picture. Awareness about the value addition of mushroom should be given as it is highly nutritious with a lot of medicinal properties in it. Consumption of mushrooms or the value - added products derived from mushroom can be a solution to a lot of deadly diseases and helps in the survival of individuals. Now a days mushroom cultivation is in high demand due to the extraction of by products from mushroom which mainly contains bioactive components that includes – polysaccharides, alkaloids, glycosides, phenols, tocopherols, enzymes, organic acids and other nutrients (proteins, fats,

minerals). The cell wall of mushroom contains chitin, hemicellulose, branched non – cellulosic β – glucans and mannans. The nutrient content is mushroom is affected by the change in environmental conditions and also during processing, harvesting and post harvesting of mushrooms. Therefore, farming ways need to be standardized to overcome all the challenges.

Dehydrated mushrooms in the form of powder are incorporated in many of the bakery products such as cakes, biscuits, etc which increases its consumption in household level. As mushrooms contain enormous amount of protein it, it helps to overcome and fight against protein – energy malnutrition. The value-added products which are made with mushroom were found superior in nutrients than the other products. Irrespective of age, mushrooms can be consumed by everyone and can be used as nutritional reservoirs to fulfil daily nutritional requirement. Other than this, preparation of value – added products from mushrooms benefit the farmers from the loss of money.

References

Abdul Rehman Niazi and Aneeqa Ghafoor, 2021, Different ways to exploit mushrooms: A review, All Life, 14(1): 450 – 460.

Afifa Jahan and Bhoopendra Kumar Singh, 2019. Mushroom value chain and Role of value addition, International Journal of Botany and Research, 9(1): 2319 – 4456.

Anuradha Srivastava, B L Attri and V P Sharma. 2020, Value addition in mushroom, Field Crops Production Foodgrain Crops, 31 – 34, ISBN No: 978-81-7164-116-1.

Anuradha Srivastava, BL Attri and Shailja Verma. 2019, Development and Evaluation of instant souppremix using oyster mushroom powder, Mushroom Research, 28(1): 65 – 69.

Barshalin Das, Saswati Sahu, Priyadarsini Sahoo, Prof. Monali Madhusmita, Dr. Satyananda Swain. 2022, Value addition in Oyster Mushroom, International Journal for Research in Applied Science and Engineering Technology, 10(5): 4037 – 4045.

Bernice Efua Buabeng-Odoom, Karuna Singh and Monika Thakur. 2022, Process Standardization, Acceptability and Nutritional Evaluation of Value-Added Bread with Mushroom Powder, (2): 29 – 44, DOI: https://doi.org/10.22271/bs.book.48.

Debasmita Chatterjee, Dipankar halder and satadal das. 2021, Varieties of mushrooms and their nutraceutical importance: A systematic Review, Journal of Clinical and Diagnostic Research, 15(3): OE01 – OE06.DOI: 10.7860/JCDR/2021/47240.14660.

Fakhreddin Salehi. 2019, Characterization of different mushrooms powder and its application in bakery products: A review, International Journal of Food Properties, 22(1): 1375–1385.

Filipa Antunes, Sara Marçal, Oludemi Taofiq, Alcina M. M. B. Morais, Ana Cristina Freitas, Isabel C. F. R. Ferreira and Manuela Pintado. 2020, Valorisation of Mushroom By-Products as a Source of Value-Added Compounds and Potential Applications, MDPI – Molecules, 25, 2672; doi:10.3390/molecules25112672.

G C Wakchaure. 2011, Mushrooms – Value Added Products, Mushrooms: Cultivation, Marketing and consumption, Chapter – 27, 233 – 238.

GC Wakchaure, Mahantesh Shirur, K Manikandan and Lekhraj Rana. 2010, Development and evaluation of oyster mushroom value added products, Mushroom Research – An International Journal, 19(4): 40 – 44.

Hassan El-Ramady,Neama Abdalla, Khandsuren Badgar, Xhensila Llanaj, Gréta Tör ″os, Peter Hajdú, Yahya Eid and József Prokisch. 2022, Edible Mushrooms for Sustainable and Healthy Human Food: Nutritional and Medicinal Attributes, Sustainability, DOI:https://doi.org/10.3390/su14094941.

Javad Sharifi-Rad, Monica Butnariu, Shahira M. Ezzat, Charles Oluwaseun Adetunji, Muhammad Imran, Seyyed Reza Sobhani, Tabussam Tufail, Tahereh Hosseinabadi, Karina Ram´ırez-Alarco´ n, Miquel Martorell, Alfred Maroyi and Nata´ lia Martins. 2020, Mushrooms-Rich Preparations on Wound Healing: From Nutritional to Medicinal Attributes, Frontiers in Pharmacology, 11,doi:10.3389/fphar.2020.567518.

Judy Gopal, Iyyakkannu Sivanesan, Manikandan Muthu and Jae-Wook Oh. 2022, Scrutinizing the Nutritional Aspects of Asian Mushrooms, Its Commercialization and Scope for Value-Added Products, Nutrients, DOI: https://doi.org/10.3390/nu14183700.

Jyoti Singh,Sangeeta C. Sindhu, Anil Sindhu. 2016, Development and evaluation of value - added pickle from dehydrated shiitake (*Lentinus edodes*) Mushroom, International Journal of Food Science and Nutrition, 1(1): 24 – 26.

Jyoti Singh, Sindhu, S. C., Sindhu, A. and Yadav, A. 2016, Development and evaluation of value - added Biscuit from dehydrated shiitake (*Lentinus edodes*) Mushroom, International Journal of Current Research, 8(3): 27155-27159.

Karuna Singh and Monika Thakur. 2016, Formulation, organoleptic and nutritional evaluation of value - added baked product incorporating Oyster mushrooms (*Pleurotus ostearus*) powder, International Journal of Food Science and Nutrition, 1(6): 16 – 20.

M. A. M. Sheikh, A. Kumar, M. M. Islam and M. S. Mahomud. 2010, The effect of mushroom powder on the quantity of cake, Progress in Agricultural Engineering Sciences, 21(1 & 2): 205 – 214.

Mohd Zaffrie Mat Amin, Azahar Harun, Mohd Amirul Mukmin Abdul Wahab, Hairazi Rahim and Mohd Tarmizi Haimid. 2017. A study of consumer behaviour towards mushroom-based products in Malaysia, Economic and Technology Management Review, 12: 55 – 63.

Muhammad Usman, Ghulam Murtaza and Allah Ditta. 2021, Nutritional, Medicinal, and Cosmetic Value of Bioactive Compounds in Button Mushroom (Agaricus bisporus): A Review, Applied Sciences,*11*(13): 5943; https://doi.org/10.3390/app11135943.

Nagulwar MM, More DR and Mandhare LL. 2020, Nutritional properties and value addition of mushroom: A review, The Pharma Innovation Journal, 9(10): 395-398.

Nityamanjari Mishra and Arghya Mani. 2019, Post Harvest Management, Processing and Value Addition of Mushroom, 291 – 315.

Prasenjit Maity, Sunil K. Bhanja and Indranil Chakraborty. 2021, Nutritional and medicinal properties of edible mushrooms: A review, BIGYAN - an interdisciplinary Journal of Science.

Priyanka Devi and Dr Kamini Jain. 2022, Value added products and many health benefit of mushroom products, International Journal of Home Science, 8(2): 73-75.

Priyanka Priyadarsini and Abhinab Mishra. 2020, A storage study of value-added oyster mushroom products, International Journal of Chemical Studies,8(4): 266-270.

Raj D Rai and T. Arumuganathan. 2007, Value-Addition in Mushrooms, Mushroom Biology and Biotechnology, 265 – 291.

Shikha Markam. 2014, Value addition of mushroom, Journal of Pharmacognosy and Phytochemistry, 3(4): 253 – 256.

Shilpa Huchchannanavar, G. Ravishankar and V. Anand Kumar. 2020, Impact of Milky Mushroom Cultivation and Value Addition Trainings among the Unemployed Youth of Ballari District, India, International Journal of Current Microbiology and Applied Sciences, 9(1): 1853 – 1860.

S. T. R. K. Durga, Akanksha Priya and Vinay Kumar. 2022, Commercial Cultivation of Mushrooms and its Value Addition, Vigyan Vartha, 3(5):128 – 132, E-ISSN: 2582 – 9467.

Sudeepta PPattanayak and Siddartha Das. 2022, Value addition and Nutritional Enrichment of Wonder Food Mushroom, Journal of Mycopathological Research, 60(4): 507 – 514.

Sushila Rathee, Dharmender Rathee, Deepti Rathee, Vikash Kumar, Permender Rathee. 2011, Mushrooms as therapeutic agents, Brazilian Journal of Pharmacognosy, 22(2): 459-474.

Tanmay Kotasthane, 2021, Oyster mushroom and its value-added products, Research square, DOI: https://doi.org/10.21203/rs.3.rs-364351/v2.

Virginia Paul. 2021, Mushroom Products, 183 – 191.

Wan Adibah Wan Maharia, Wanxi Peng, Wai Lun Nam, Han Yang, Xie Yi Lee, Yik Kin Lee, Rock Keey Liew, Nyuk Ling Ma, Aqilah Mohammad, Christian Sonne, Quyet Van Le, Pau Loke Show, Wei-Hsin Chen, Su Shiung Lam. 2020. A review on valorization of oyster mushroom and waste generated in the mushroom cultivation industry, Journal of Hazardous Materials, 400:123 - 156.

Y. Prabhabati Devi 2018. Processing and Value addition of Underutilised Mushroom of Channdel district, Manipur, International Journal of Creative Research Thoughts, 6(2): 796 – 804.

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A Molecular Approach of Signaling Molecules, Its Receptors and Their Responses

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Introduction to signaling

Signaling molecules are produced by the cells that can bind to the target cell's receptor to transmit the information so as to produce the desired response. The information can be propagated as intracellular or intercellular signals. Intracellular signals are executed within the cell and intercellular signals are executed between the cells. Intercellular signaling is executed by extracellular signaling molecules which are produced and released by the signaling cell. These kinds of signaling regulate different aspects of physiological functions such as appetite, mood, blood pressure regulation, cell motility,immune system function, metabolism, water and salt homeostasis. The signaling molecules are called ligands whose functions are varied and diverse. These signaling molecules are required in very low concentration (typically $\leq 10^{-8}$ M), to execute their desired functions. The ligands are bound to the receptors of the target cell which propagate the signal downstream to execute the desired functions. This process is said to be signal transduction.

Types of Signaling

The signaling molecules can propagate the signal by

Autocrine signaling

In Autocrine signaling, the cell transduces the signal by synthesizing and releasing the extracellular mediators which interacts with the receptor present in the same cell thereby producing the desired result. A well knownillustration of the autocrine signaling is the production of Interleukin -1 (IL-1) by macrophages. IL-1 binds to the receptors present in the macrophages, stimulates these cells to further produce the additional cytokines including IL-1. These kinds of pure autocrine signaling are not common to normal physiology but very important in certain pathological conditions such as

cancer. In tumor cells the cell proliferations are executed by autocrine signaling(Thomas, 2007).

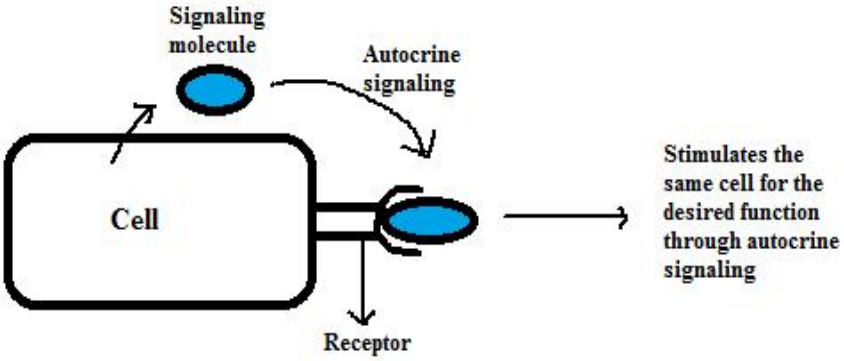


Figure 1- Autocrine Signaling

Paracrine signaling

In paracrine signaling the cells produces the ligand molecules which interactwith the receptor present in the neighboring target cell. One of the best examples of the paracrine signaling is the growth factors. The growth factor produced by one cell diffuses through a short distance and binds to the receptors of the neighboring target cell which stimulates the growth signal(Robert andAbbot, 2008).

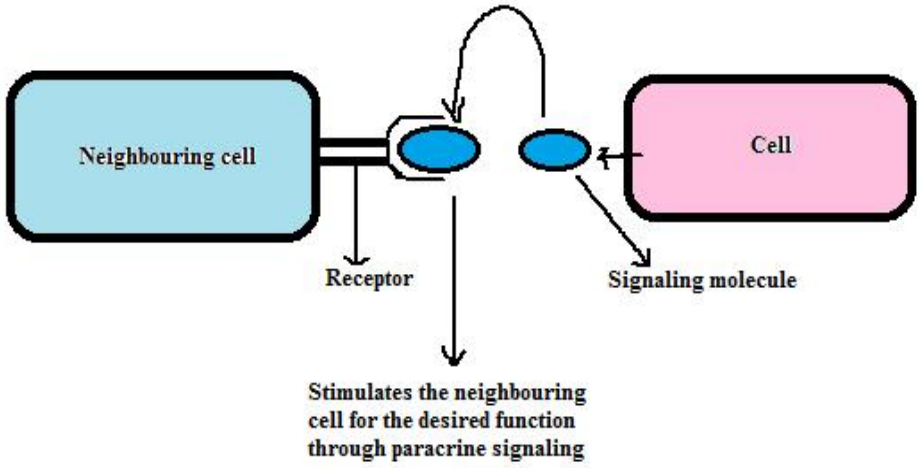


Figure 2-Paracrine Signaling

Endocrine signaling

In endocrine signaling the endocrine cell secrets the signaling molecule called hormones. These hormones travel through the blood and reach the sight of action, the target cell where it binds to the receptors present in the target cells. The function of this signaling is to regulate the activity of the other cell or organ to achieve the physiological or metabolic balance called homeostasis. This kind of signaling also regulates the functions of other endocrine organs and cells(Cooper, 2000).

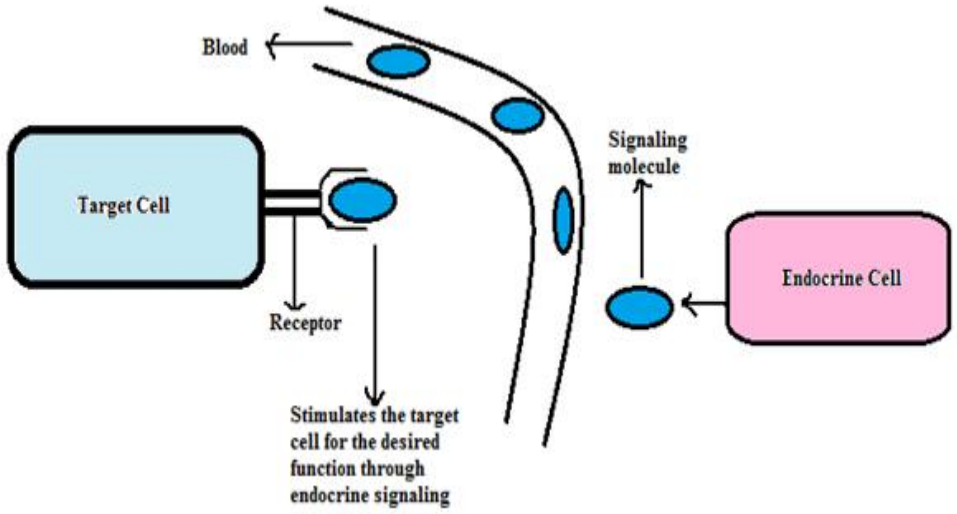


Figure 3- Endocrine Signaling

Direct signaling across gap junctions

Plasmodesmata in plants and gap junctions in animals are associations between the plasma membrane of the neighboring cells. These junctions are water filled which will enable the small molecules and ions such as Ca^{2+}, signaling molecules except large molecular weight protein and DNA to pass through the junction. These cells remain independent due to the specificity of the channels. These signals are propagated easily and quickly across the gap junctions. This signaling allows coordinating their response to a signal received by a single cell to a group of cells. Plasmodesmata in plants make a giant communication network of the entire plant (Batra, 2012).

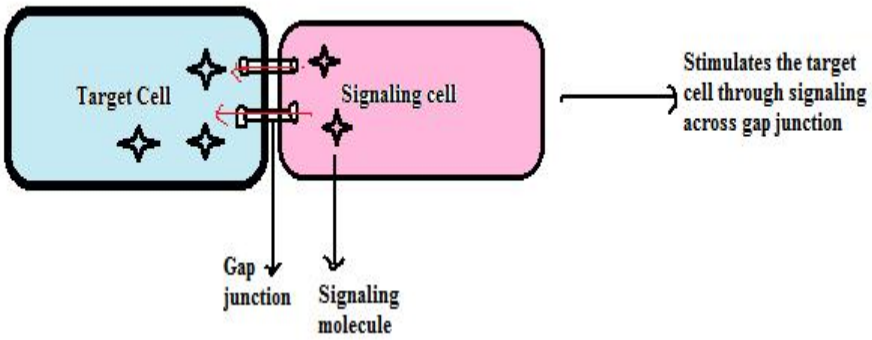


Figure 4-Direct Signaling Across Gap Junction

Classification of Signaling Molecules

Signaling molecules are produced by signaling cells and are called as ligands. There are different types of ligands based of the nature. These ligands are grouped into majorly Steroid hormones, Peptides and Growth factors, Gaseous form of signaling molecule - Nitric oxide and Carbon monoxide, Neurotransmitters and Eicosanoids(Cooper, 2000). The molecules that are structurally similar which can bind the receptor and execute the signaling in the same way as the original ligand are called as agonist. The molecules that are similar in structure and can bind the receptor but execute the opposite function of the original ligand are called as antagonist. These are the drug targets for the several clinical diseases and disorders(Raymon, 2013).

Peptides and Growth factors

Peptides are the widespread variety of signaling molecule ranging from few to hundred amino acids. These include peptide hormones, polypeptide growth factors and neuropeptides. Examples of peptide hormones include insulin, glucagon, growth hormone, prolactin, follicle stimulating hormone etc. The neuropeptides are secreted by neurons which include enkephalins and endorphins functions as neurohormones as well as neurotransmitters. The enkephalins and endorphins acts as natural analgesics which can reduce pain response in the central nervous system. Peptide hormones cannot pass through the plasma membrane and thus have its receptors in the cell surface of the target cells. Upon saturation of the cell surface receptor the extracellular signal will be converted to intracellular signal with the production of secondary messenger through effector molecule via G-protein. These cell surface

receptors are always coupled with their respective G-Proteins to elicit the desired function (Posner, 2010).

Steroid hormones

Steroid hormones such as thyroid hormone, vitamin D_3, retinoic acid, testosterone, estrogen, progesterone, the corticosteroids and ecdysoneare hydrophobic in nature. The steroid hormones vitamin D_3, testosterone, estrogen, progesterone, the corticosteroids and ecdysone are producedfrom cholesterol. Because of the hydrophobicity these steroid hormones can passthrough the plasma membrane. For these hormones the receptor are present in either the cytosol or inside the nucleus i.e., intracellular receptors. Once the hormones are bound to their respective intracellular receptors, the hormone receptor complex is formed which then get translocated to the nucleus. In the nucleus it binds to the Hormone Response Element (HRE) in the DNA thereby alter the target gene expression. The altered target gene expression performs the desired functions of the hormone(Gerald Litwack, 2018).

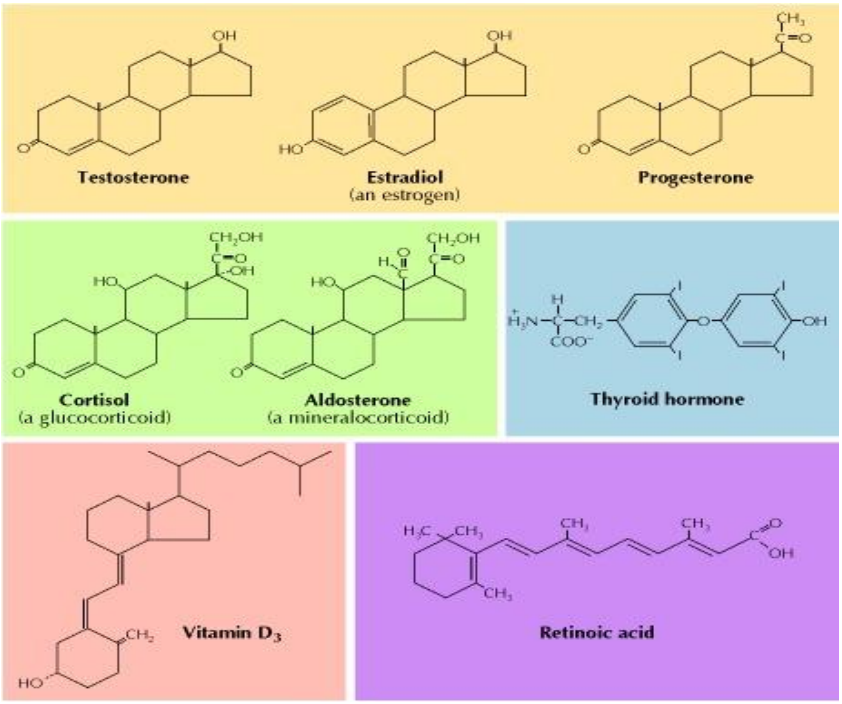


Figure 5- Structure of steroid hormones, thyroid hormone, vitamin D 3, and retinoic acid

Courtesy: https://www.ncbi.nlm.nih.gov/books/NBK9924/

Gaseous form of signaling molecule - Nitric oxide and Carbon monoxide

The simple nitric oxide (NO) gas is a main gaseous form of signaling molecule in the nervous, immune, and circulatory systems through paracrine signaling. Since NO is a gas it can easily diffuse across the plasma membrane of the target cells. The NO mechanism of actionis different from regular steroid hormone. Instead of binding to a receptor which regulates transcription and NO alters the activity of intracellular target enzymes.Nitric oxide synthase synthesizes the NO from the amino acid arginine. NO can affect the nearby cells locally since it is highly unstable and has a half-life of few seconds. The major function of NO is dilation of blood vessels. Carbon monoxide (CO) is similar to NO which acts as neurotransmitter and mediate blood vessel dilation. CO is synthesized by the brain cells and stimulates guanylatecyclase(Walewska, 2018).

Neurotransmitters as the signaling molecule

The neurotransmitters transmit the signals between the neurons and neurons and the target cells. These neurotransmittersare small hydrophilic molecules which including acetylcholine, dopamine, epinephrine (adrenaline), glutamate, histamine, serotonin, glycine, and γ-amino butyric acid (GABA). The neurotransmitters are released by the signal received from the action potential in the axon terminal. The neurotransmitters diffuse in the synaptic cleft region and bind to the receptors present on the dendrites of the next neuron cell, if the signaling is between neurons or on the receptors of the target cells, if the signaling is between the neuron and the target cell. Some of the neurotransmitters are acting as hormones also which includes epinephrine, norepinephrine, dopamine (Cooper, 2000).

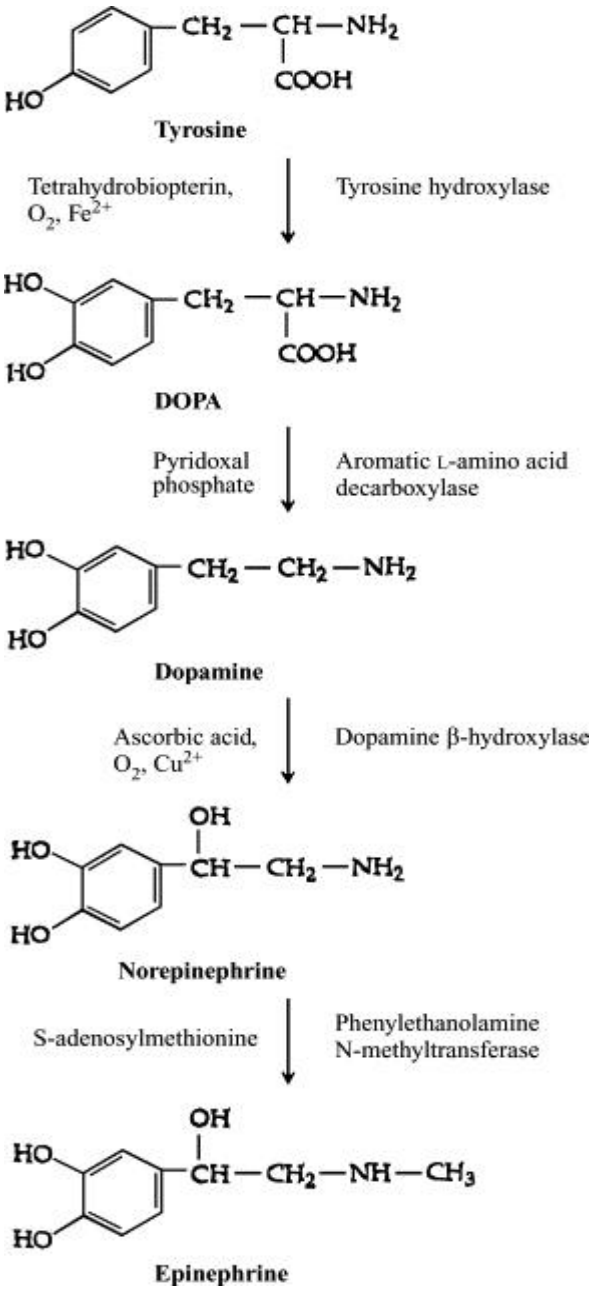


Figure 6- Structures of Neurotransmitters

Courtesy: https://www.sciencedirect.com/topics/medicine-and-dentistry/neurotransmitter

Eicosanoids

In contrast to steroids certain lipids are serving as signaling molecules by binding with the cell surface receptors. These signaling molecules fall into the class of lipid molecules called eicosanoids which comprises prostaglandins, prostacyclin, thromboxanes, and leukotrienes(figure-7). The eicosanoids act locally because they rapidly break down and signal in autocrine or paracrine signaling pathways. They excite a diverse response such as inflammation, smooth-muscle contraction andblood platelet aggregation. All the eicosanoids are derived from arachidonic acid, the first step in the synthesis uses cyclooxygenase enzyme which is the target for aspirin and non-steroidal anti-inflammatory drugs. Thus, aspirin inhibits cyclooxygenase enzyme and decreases pain and inflammation(Calder, 2020).

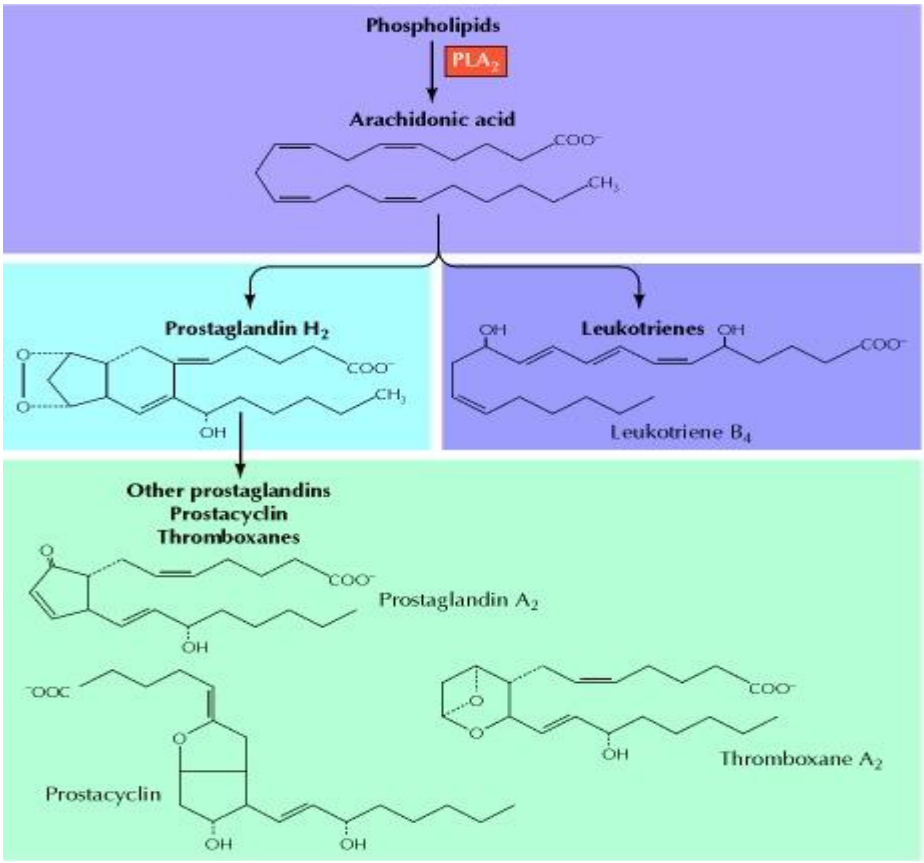


Figure 7-Structures of Eicosanoids

Courtesy: https://www.ncbi.nlm.nih.gov/books/NBK9924/

Receptors

The receptors are highly specific in nature. The ligands bind this receptor to elicit the desirable response. Based on the nature of the ligand the receptors may be present on the cell surface i.e., cell surface receptor or inside the cell i.e., internal receptors.

Cell surface receptors

The cell surface receptors are integral membrane protein present in the plasma membrane of each cell. The receptors mostly depending on the pathway coupled with the G protein. The receptor has extracellular ligand binding domain, transmembrane domain and cytosolic G protein binding domain. Upon binding of the ligand the conformation of the receptor changes in such a way that it triggers the G-Protein, which in turn activates the effector molecule which may be a channel or an enzyme. The effector molecule thus propagates the signal downstream. The binding of ligand with the receptor is by weak non-covalent interactions such as ionic bond, hydrogen bond, van der Waals interaction, hydrophobic interactions etc., and the effectiveness of the interaction also depends on the complementarity of the receptor and the ligand structure. These receptors are highly specific; it can distinguish the closely related ligands. The binding of ligand with receptor is a simple reversalreaction,

$$R + L \underset{K_{off}}{\overset{K_{on}}{\rightleftharpoons}} RL \quad \text{Equation 1}$$

where,

R is receptor

L is ligand

RL is receptor ligand complex

K_{on} is the rate constant for association of a ligand with its receptor

K_{off} is the rate constant for dissociation of a ligand from its receptor

The equation 1 can be written as,

$$K_d = (R)(L) / (RL) \quad \text{Equation 2}$$

where,

K_d, the dissociation constant of the receptor-ligand complex {K_d is K_{off}/K_{on}}, measures the affinity of the receptor for the ligand.

(R)is concentration of free receptor

(L)is concentration of free ligand

(RL) is the concentration of the receptor-ligand complex

The higher the K_d value, the lower is the affinity between the receptor for the ligand. The lower the K_d value, the higher the affinity between the receptor for the ligand. The value of K_d which is equal to the concentration of the ligand at which half of the receptors are saturated with the ligand(Gerald Karp, 2010).

G- Protein

The signal transducing G - Protein is trimeric G protein, which contains α, β and γ subunits. The Gα subunit has GTPase activity that makes the G-protein to be in switch on and off state. The β and γ subunits remain intact during the signal transduction while the Gα disassociates. The receptors that are coupled with the G-protein upon ligand binding get activated and thus activate the G-protein. The activated Gα subunit of the G-Protein detaches from the trimeric complex and associates with the effector molecule. There by activates the effector molecule. The effector molecule thus propagates the signal downstream. The G-protein is coupled always with the seven transmembrane domain containing receptor. These receptors are called as serpentine receptors.

There are different types of G-protein which involves in signal transduction. Depending on the pathway the specific type of G-protein is involved in the signal transduction. Gsα is a stimulatory G-protein, Giα is an inhibitory G-protein and other G-proteins are involved in signal transduction of specific cell types. The Gsα G – protein upon activation propagates the stimulatory signal. The Giα G – protein upon activation propagates the inhibitory signal. Both the inhibitory and stimulatory signal is equally important for the biological functions(Lodish, 2003).

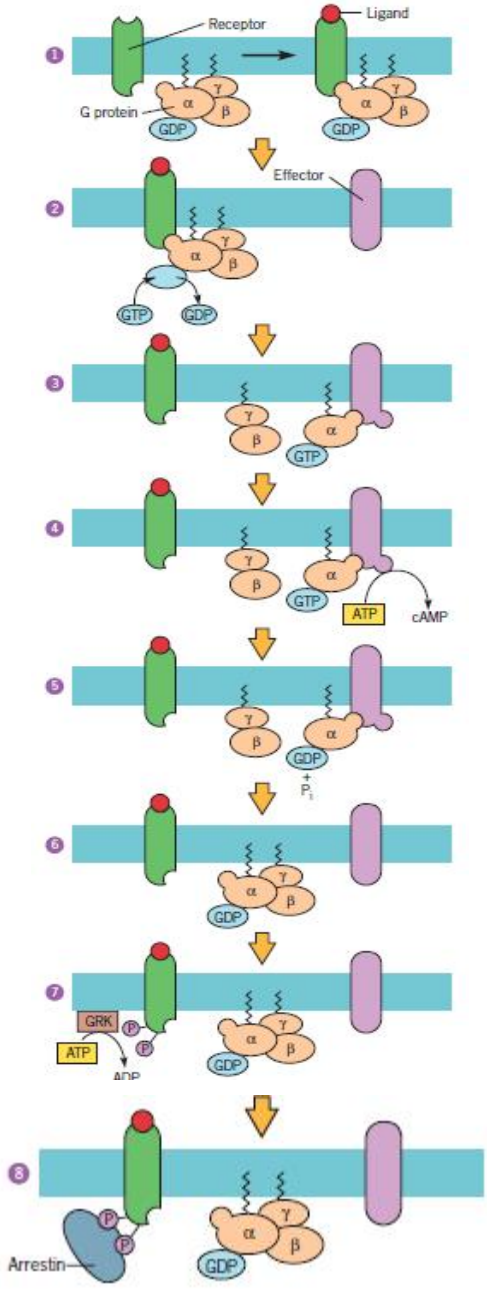


Figure 8-Signal Transduction by G Protein

Courtesy: Cell and Molecular Biology by Gerald Karp, 6th edition, Wiley Plus, 2010

G_α Class	Associated Effector	2nd Messenger	Receptor Examples
$G_{s\alpha}$	Adenylyl cyclase	cAMP (increased)	β-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
$G_{i\alpha}$	Adenylyl cyclase K^+ channel ($G_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_1-Adrenergic receptor Muscarinic acetylcholine receptor
$G_{olf\alpha}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
$G_{q\alpha}$	Phospholipase C	IP_3, DAG (increased)	α_2-Adrenergic receptor
$G_{o\alpha}$	Phospholipase C	IP_3, DAG (increased)	Acetylcholine receptor in endothelial cells
$G_{t\alpha}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G_α subclass may be associated with more than one effector protein. To date, only one major $G_{s\alpha}$ has been identified, but multiple $G_{q\alpha}$ and $G_{i\alpha}$ proteins have been described. Effector proteins commonly are regulated by G_α but in some cases by $G_{\beta\gamma}$ or the combined action of G_α and $G_{\beta\gamma}$. IP_3 = inositol 1,4,5-trisphosphate. DAG = 1,2-diacylglycerol.

SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al. 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

Table 1: Major classes of G Protein and its Effectors

Courtesy: Molecular Cell Biology by Lodish H, 5th edition. Freeman publication, 2003

Effectors

The effectors are integral membrane proteins and get activated by interaction of Gα subunit of the G Protein. The effectors are usually the enzymes which upon activation produce secondary messengers or it can be ion channels upon activation opens or closes the particular ion channel. Both the events propagate the signal downstream. The enzymes include adenylyl cyclase, guanylylcyclase, phospholipase C, cGMPphosphodiesterase. These enzymes produce secondary messengers or it destroys the available secondary messengers, both the events lead to the dissemination of the desired signal. The ion channel effectors include K^+ ion channel, chloride ion channel and sodium ion channels, lead to either opening or closing of the ion channels upon activation by Gα subunit of the G Protein. Either opening or closing of these ion channelsleads to dissemination of the desired signal. The adenylyl cyclase produces cAMP as secondary messenger, guanylylcyclase produces cGMP as the secondary messenger, phospholipase C produces IP_3 and Ca^{2+} as the

secondary messenger whereas the cGMP phosphodiesterase cleaves cGMP to GMP thereby reduces the concentration of cGMP (Radchenko, 2011).

Secondary messengers

The extracellular signaling is prorogated by ligand interaction with the receptor. This signal propagates inside the cell in an amplified way through secondary messengers. The secondary messengers execute the cell signaling through the activation of different protein kinases specific to the particular pathway. The various secondary messengers include, 3',5'-cyclic AMP (cAMP), 3',5'- cyclic GMP (cGMP), 1,2-diacylglycerol (DAG), and inositol1,4,5-trisphosphate (IP_3) represented in the figure-10.

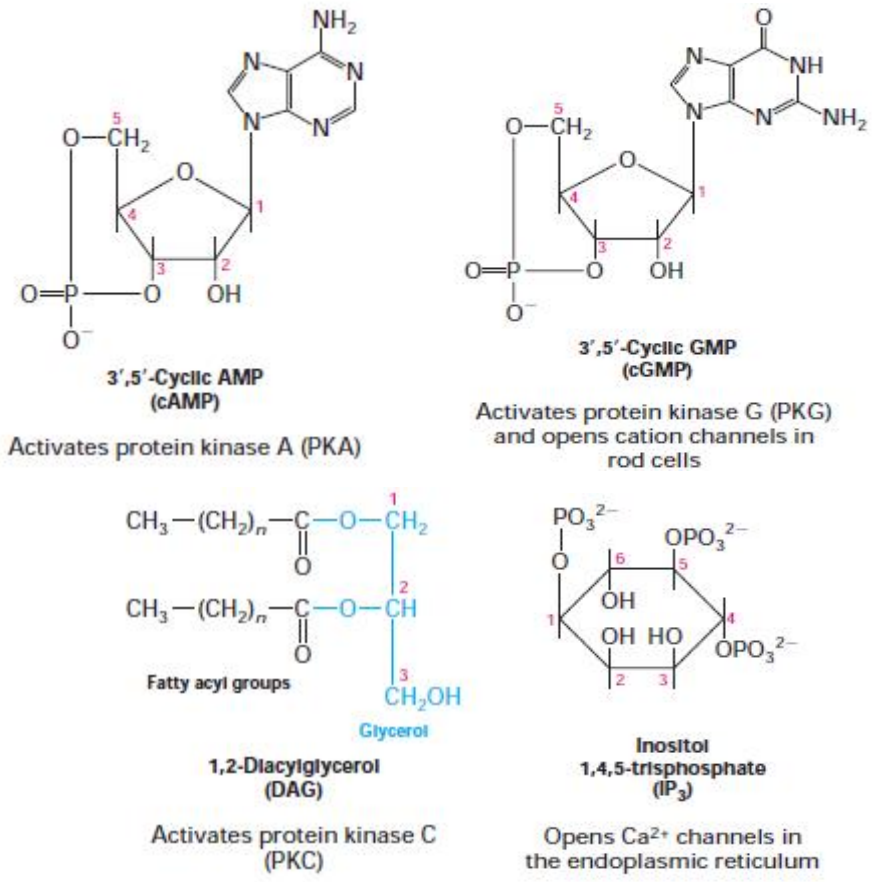


Figure 9- Structures of Secondary Messengers

Courtesy: Molecular Cell Biology by Lodish H, 5th edition. Freeman publication, 2003

These secondary messengers specifically activate the protein kinases which are pertaining to a particular pathway. The cAMP activates protein kinase A, cGMP activates protein kinase G and opens cation channel in rod cells, DAG activates protein kinase C and IP_3 opens calcium ion channel in endoplasmic reticulum. The protein kinase A is aheterotetramer have two types of subunits, two regulatory subunits {R} and two catalytic subunits {C}. To the regulatory subunit cAMP binds and makes the catalytic subunit to dissociate from it. The catalytic subunit becomes fully active and phosphorylates the specific proteins there by the effect of the ligand is achieved. The phosphorylation happens in serine or threonine residues. The catalytic subunit forms dimer and enter into the nucleus. In the nucleus, the dimer of catalytic subunits binds to cAMPresponse element and alter the expression of the target gene. The expressed protein will perform the function of the ligand(Lodish, 2003).

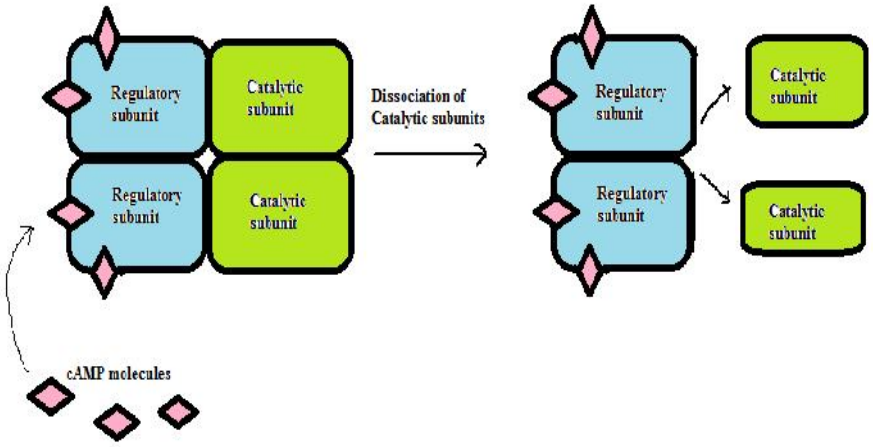


Figure 10- Activation of Protein Kinase

Internal receptors

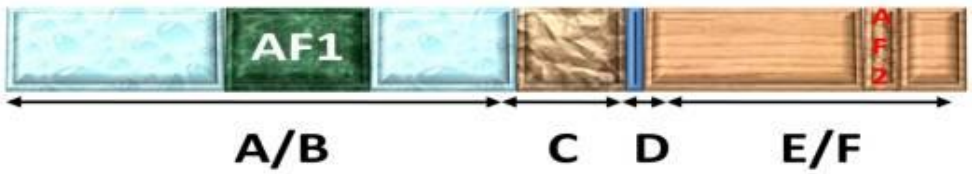
Internal receptors are present inside the cell. These receptors can be present in the cytoplasm which is called as cytoplasmic receptor or inside the nucleus which are called as nuclear receptor. Cytoplasmic receptors are classified under type I receptors and the nuclear receptors are classified under type II receptors.

Types of internal receptors

- **Type I:**these receptors are present in the cytoplasm of the cell upon activation by the ligand interaction; the receptor ligand complex will be trans located to the nucleus to alter the target gene expression. Examples of such receptors include,androgen (AR), glucocorticoid (GRa), mineralocorticoid (MR), and progesterone receptors (PR).
- **Type II:**these receptors are present in the nucleus, the ligand moves to the nucleus and interact with the receptor. Upon activation the receptor ligand complex alters the gene expression. Examples of such receptor includes,peroxisome proliferator (PPAR a,ß,g, sigma), retinoic acid (RAR a,ß,g), thyroid receptors (TR a,ß).
- Some of the nuclear receptors do not have known ligand at present; those receptors are called as **orphan receptors**.

General structure of the receptors

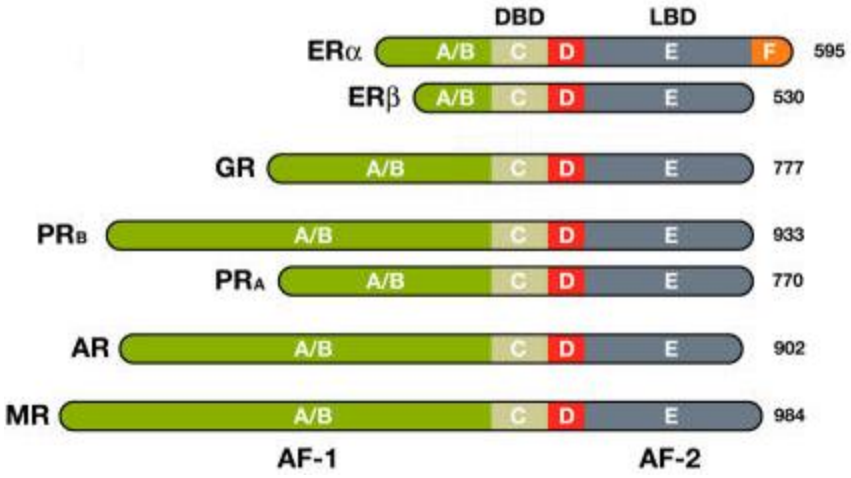
- The receptors are *monomeric proteins*.
- *N-terminal Domain* – this domain has Activation Function 1 (AF1) site binds other transcription factor which can alter the activity of the receptor itself. For a given cell these transcription factors are highly specific.
- *Core Domain* – this domain is made of two zinc finger motifs with cysteine/histidine rich loops held by zinc ions.This domain also has DNA bonding site. The receptor also has hinge region of the flexibility of the receptor during dimerization and DNA binding.
- *C-terminal Domain* – this domain is ligand binding domain where the respective ligand interacts with the receptor(Kumar, 2009).



A/B = N-terminal domain (NTD); C = DNA-binding domain (DBD); D = hinge region (in some cases also known as Tau2); E/F = ligand binding domain (LBD)

Figure 11- General Structure of the Internal Receptor

Courtesy: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3074935/



ER: estrogen receptor; **G**R:glucocorticoid receptor; **PR:**progesterone receptor; **AR:**androgen receptor; **MR:**mineralocorticoid receptor

Figure 12- Structure of specific receptors

Courtesy:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1853070/

The ligands for the internal receptors are hydrophobic in nature. These hydrophobic ligands cross the plasma membrane and enter the cytoplasm, if the receptor is in the nucleus ligand enters into nucleus. The cytoplasmic receptors are associated with the Heat Shock Protein 90 (HSP 90) proteins which makes the receptor inactive conformation. Upon interaction of receptor with ligand the HSP 90 will dissociate from the receptor and makes it active. The ligand receptor complex forms a dimer and enters into the nucleus where it searches for the specific response element and bind their respective response element. Thus alters the target gene expression(Griekspoor, 2007).

Hormone Response Element:

Hormone response elements (HREs) for thyroid, steroid, sterol, and retinoid hormones have been identified, as has a cAMP response. The HRE of these hormones all have the same consensus sequence which will mediate the effect of these hormones. Subtle sequence differences may occur in specific HREs, ligand availability may be limiting, receptor concentrations may be limiting in different cell types, or HREs might not exist as isolated elements but in fact interact with other cis/trans elements to mediate their effect. Such complex hormone responsehas been the topic of numerouscurrent publications.

The protein which interacts with the cAMP Response Element (CRE) is cAMP Response Element Binding (CREB) Protein. This protein helps in the interaction of ligand receptor complex with the respective response element(Vincent Laudet, 2002).

Conclusion

Signaling molecules have very diverse physiological functions such as appetite, mood, blood pressure regulation, cell motility, immune system function, metabolism, water and salt homeostasis. These molecules are required invery low concentrations (typically $\leq 10^{-8}$ M) to exert its desired physiological functions. The signaling includes both extracellular signaling as well as intracellular signaling. The extracellular signaling transmits into intracellular signaling via different signaling intermediates. The signaling has both early and delayed late response in order to have the physiological response. The early response includes the alteration in the activation and inactivation of the existing enzymes in the target cells which governs the desired function of the ligand. The delayed late response will alter the expression of the target gene in the specific cells which execute the desired function of the ligand. Both the responses execute the desired complete physiological functions.

References

Calder P. C. (2020). Eicosanoids. *Essays in biochemistry*, *64*(3), 423–441. https://doi.org/10.1042/EBC20190083

Batra, N., Kar, R., & Jiang, J. X. (2012). Gap junctions and hemichannels in signal transmission, function and development of bone. *Biochimicaetbiophysicaacta*, *1818*(8), 1909–1918. https://doi.org/10.1016/j.bbamem.2011.09.018

Cooper GM. The Cell: A Molecular Approach. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. Signaling Molecules and Their Receptors. Available from: https://www.ncbi.nlm.nih.gov/books/NBK9924/

Gerald Karp, Cell and Molecular Biology, 2010, 6th edition, Wiley Plus.Pages 621 - 624, 655 - 656, ISBN 978-0470483374.

Gerald Litwack, Chapter 16 - Steroid Hormones, Editor(s): Gerald Litwack, Human Biochemistry, Academic Press, 2018, Pages 467-506, ISBN 9780123838643, https://doi.org/10.1016/B978-0-12-383864-3.00016-8.

Griekspoor, A., Zwart, W., Neefjes, J., &Michalides, R. (2007). Visualizing the action of steroid hormone receptors in living cells. *Nuclear receptor signaling*, *5*, e003. https://doi.org/10.1621/nrs.05003

Griekspoor, A., Zwart, W., Neefjes, J., &Michalides, R. (2007). Visualizing the action of steroid hormone receptors in living cells.Nuclear receptor signaling, 5, e003. https://doi.org/10.1621/nrs.05003

Kumar, R., & Litwack, G. (2009).Structural and functional relationships of the steroid hormone receptors' N-terminal transactivation domain. *Steroids*, *74*(12), 877–883. https://doi.org/10.1016/j.steroids.2009.07.012

Lodish H, Molecular Cell Biology, 2003, 5th edition.Freeman publication.Pages 553 - 570, ISBN 978-0-71674366-8.

Motta, V., Soares, F., Sun, T., &Philpott, D. J. (2015). NOD-like receptors: versatile cytosolic sentinels. *Physiological reviews*, *95*(1), 149–178. https://doi.org/10.1152/physrev.00009.2014

Posner, B. I., &Laporte, S. A. (2010). Cellular signalling: Peptide hormones and growth factors. *Progress in brain research*, *181*, 1–16. https://doi.org/10.1016/S0079-6123(08)81001-1

Radchenko, M., & Merrick, M. (2011).The role of effector molecules in signal transduction by PII proteins. *Biochemical Society transactions*, *39*(1), 189–194. https://doi.org/10.1042/BST0390189

Ray S. (2014). The Cell: A Molecular Approach. *The Yale Journal of Biology and Medicine*, *87*(4), 603–604.

Raymon L.P., Pharmacology and Mechanism of Action of Drugs, Editor(s): Jay A. Siegel, Pekka J. Saukko, Max M. Houck, Encyclopedia of Forensic Sciences (Second Edition), Academic Press, 2013, Pages 210-217, ISBN 9780123821669, https://doi.org/10.1016/B978-0-12-382165-2.00309-3.

Robert J. Wordinger, Abbot F. Clark (2008). Chapter 5 - Growth Factors and Neurotrophic Factors as Targets. Editor(s): Thomas Yorio, Abbot F. Clark, Martin B. Wax, Ocular Therapeutics, Academic Press, Pages 87-116, ISBN 9780123705853, https://doi.org/10.1016/B978-012370585-3.50007-8.

Thomas C. King (2007). 3 - Tissue Homeostasis, Damage, and Repair. Editor(s): Thomas C. King, Elsevier's Integrated Pathology, Mosby,

Pages 59-88, ISBN 9780323043281, https://doi.org/10.1016/B978-0-323-04328-1.50009-7.

Vincent Laudet, HinrichGronemeyer, 2 - DNA recognition by nuclear receptors, Editor(s): Vincent Laudet, HinrichGronemeyer, In Factsbook, The Nuclear Receptor FactsBook, Academic Press, 2002, Pages 22-36, ISBN 9780124377356, https://doi.org/10.1016/B978-012437735-6/50004-7.

Walewska, A., Szewczyk, A., &Koprowski, P. (2018).Gas Signaling Molecules and Mitochondrial Potassium Channels. *International journal of molecular sciences*, *19*(10), 3227. https://doi.org/10.3390/ijms19103227

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Bioplastics – A boon or curse

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Introduction

Plastic, despite its widespread use and convenience, poses significant threats to the environment, human health, and ecosystems. The following are some of the key threats associated with plastic:

Pollution and Environmental Degradation

Plastic Waste: Improper disposal and inadequate waste management systems result in plastic waste accumulating in landfills, oceans, rivers, and other natural environments. This waste takes hundreds of years to decompose, leading to long-term pollution.

Micro plastics: Plastics break down into tiny particles called micro plastics, which are less than 5mm in size. Micro plastics are found in various ecosystems, including marine environments, soil, and even the air, posing a threat to wildlife and potentially entering the food chain.

Habitat Destruction: Plastic debris can harm and destroy habitats, particularly in aquatic environments. It can entangle marine animals, damage coral reefs, and disrupt ecosystems, leading to the loss of biodiversity.

Wildlife and Marine Life

Ingestion and Entanglement: Marine animals, birds, and other wildlife often mistake plastic debris for food or become entangled in it. Ingestion of plastics can cause internal injuries, starvation, and even death.

Disruption of Ecosystems: Plastic pollution can disturb marine ecosystems by affecting the natural balance and interdependence of species. This disruption has far-reaching consequences on marine biodiversity and ecological functions.

Health Risks

Chemical Leaching: Plastics can release harmful chemicals, such as biphenyl A (BPA) and phthalates, which are known to be endocrine disruptors. These chemicals can leach into food, water, and the environment, posing health risks to humans and wildlife.

Contamination of Food Chain: Plastics, especially micro plastics, can contaminate the food chain as they are consumed by marine organisms. This contamination can ultimately affect human health when consuming seafood.

Climate Change and Energy Consumption

Fossil Fuel Dependency: Plastics are primarily made from fossil fuels, contributing to carbon emissions and exacerbating climate change.

Manufacturing Process: The production of plastics requires significant energy consumption, which contribute to greenhouse gas emissions and environmental degradation.

Economic Impact

Clean up Costs: The management and clean-up of plastic waste incur substantial economic burdens on governments, communities, and industries.

Tourism and Aesthetic Damage: Plastic pollution diminishes the aesthetic appeal of natural landscapes and coastal areas, negatively impacting tourism and related industries.

It is crucial to raise awareness, implement stricter regulations, and foster behavioral changes to mitigate the threats of plastic and safeguard our environment and health. Addressing these threats requires a multi- faceted approach that includes reducing plastic production and consumption, improving waste management infrastructure, promoting recycling and circular economy initiatives, and encouraging sustainable alternatives to plastic like bio-plastics.

Bio-plastics – An alternative to plastics

Bioplastics, also known as biodegradable or compostable plastics, have emerged as a potential alternative to traditional petroleum-based plastics. These plastics are derived from renewable resources such as plants, corn starch, or vegetable oil, that are typically rich in polymers such as starch or cellulose, which can be processed to create plastic-like materials. This can be used to reduce the problem of plastic waste that is suffocating the planet polluting the

environment and are touted as being more environmentally friendly in nature. (Alfred Rudin et al,2013)

Bio-plastics are made by converting the sugar present in plants like corn, sugar cane, sugar beets, wheat, potatoes or microbe such as yeast into plastic. This makes bioplastics renewable and better for the environment than conventional plastics. Bioplastics are made wholly or in part from renewable biomass sources.

Types of Bio-plastics

There are two main categories of bio plastics based on their raw materials

1 Bio-based bio-plastics

2. Biodegradable bio-plastics

Bio-based bio-plastics: These are derived from renewable resources and have a similar chemical structure to traditional plastics. They can be partially or fully bio-based, depending on the composition. Bio-based bioplastics reduce reliance on fossil fuels and have a lower carbon footprint compared to petroleum-based plastics. (Syed Ali Ashter,2016).

Bio based bio-plastics are of 3 types

Starch-Based Bio-plastics: Simple bio-plastic derived from corn starch. They are often mixed with biodegradable polyesters. Starch blends are used in packaging, disposable items, and agricultural films.

Example: Green Dot Bioplastics has successfully developed cell phone cases from compostable, starch- based plastics. Additional opportunities are expected in compostable yard and kitchen bags, food service disposables and various types of packaging. (Renee Cho, 2017, Monika Metro 2021).

Cellulose-Based Bio-plastics: Produced using cellulose esters and cellulose derivatives.

Example: Major applications for cellulose plastics include thermoplastics, extruded films, eyeglass frames, electronics, sheets, rods, etc.

Molding materials is the most dominant application segment for cellulose plastics and the trend is expected to continue. Plastic is produced mainly using non-renewable resources such as crude oil and its several derivatives owing to which, the carbon footprint is high in the production of cellulose plastics. (Renee Cho, 2017, Monika Metro 2021).

3.Protein-Based bioplastics: These are produced using protein sources such as wheat gluten, casein and milk.

Example: Biopolymers that are protein-based have become a leading alternative for food packaging. There have been major advances in protein-based films and coatings for food packaging made from plant and animal proteins. (Renee Cho, 2017, Monika Metro 2021).

Biodegradable bio-plastics: These are designed to break down naturally in the environment through biological processes, such as microbial action or composting. However, it's important to note that not all biodegradable plastics are bio-based, and not all bio-based plastics are biodegradable. (The Basics of Bioplastics ,2018)

Here are some common types of bio degradable bio-plastics:

Polylactic Acid (PLA)

PLA is one of the most widely used bioplastics. It is derived from fermented plant sugars, usually obtained from corn, sugarcane, or other starch-rich crops. PLA is known for its transparency, high strength, and versatility. It is commonly used in packaging, disposable cutlery, food containers, and 3D printing. (Monika Metro 2021).

Polyhydroxyalkanoates (PHA)

PHA is a family of biopolymers produced by bacteria through fermentation of plant sugars or plant oils. It is a biodegradable and compostable plastic that can be tailored to various applications. PHA exhibits a wide range of properties, including flexibility, durability, and resistance to heat and chemicals. PHA is used in packaging, agricultural films, medical products, and disposable items. (Renee Cho, 2017, Monika Metro 2021).

Polybutylene Succinate (PBS)

PBS is a biodegradable polyester derived from succinic acid, which can be obtained from plant-based feed stocks or petrochemicals. It has good mechanical strength, biodegradability, and thermal stability. PBS is commonly used in packaging films, disposable cutlery, and agricultural applications. (Renee Cho, 2017, Monika Metro 2021).

Polyethylene Furanoate (PEF)

PEF is a bio-based polyester that can replace traditional petroleum-based polyethylene terephthalate (PET). It is produced from renewable sources;

such as plant sugars or cellulose. PEF offers excellent barrier properties for packaging applications, including improved gas and moisture resistance. PEF has the potential to reduce greenhouse gas emissions and improve recycling capabilities. (Renee Cho, 2017, Monika Metro 2021).

Polyhydroxyurethanes (PHUs)

PHUs are a newer class of biodegradable plastics that can be derived from plant-based oils or waste materials. They have excellent mechanical strength, biocompatibility, and biodegradability. PHUs show promise in medical applications, such as sutures, implants, and drug delivery systems. (Renee Cho, 2017, Monika Metro 2021).

Environmental Benefits

Bioplastics offer several advantages over traditional petroleum-based plastics, making them an attractive option for various applications. Bioplastics have the potential to reduce greenhouse gas emissions and decrease reliance on fossil fuels. Since they are derived from renewable resources, their production generally requires less energy compared to traditional plastics. Biodegradable bioplastics can also help reduce accumulation of waste by breaking down into natural components over time.

Key advantages of bioplastics:

Renewable Resource: Bioplastics are derived from renewable resources, such as plants, crops, or biomass. Unlike fossil fuels used in traditional plastics, these resources can be replenished, reducing dependency on finite resources and contributing to a more sustainable and circular economy.

Reduced Carbon Footprint: The production of bioplastics generates fewer greenhouse gas emissions compared to conventional plastics. Renewable feed stocks used in bioplastics absorb CO2 during their growth, helping to mitigate climate change. The overall carbon footprint of bioplastics can be significantly lower, especially when coupled with efficient manufacturing processes and renewable energy sources.

Biodegradability and Compostability: Many bioplastics have the ability to biodegrade, breaking down into natural elements through enzymatic processes. Some bioplastics are also compostable, meaning they can be converted into compost under specific conditions. Biodegradable and compostable bioplastics offer the advantage of reducing long-term pollution and litter, particularly in soil and marine environments.

Versatility and Performance: Bioplastics can exhibit a wide range of properties, allowing them to be tailored to specific applications. They can be transparent, flexible, rigid, or heat-resistant, depending on the desired characteristics. Bioplastics have shown good performance in packaging, consumer products, agriculture, textiles, and medical applications.

Potential for Recycling: Bioplastics have the potential to be recycled alongside traditional plastics, depending on their specific composition. This can help in diverting waste from landfills and reducing the demand for new plastic production. However, it's importance to note that not all bioplastics are compatible with existing recycling infrastructure, and proper sorting and labelling are necessary for effective recycling.

Innovation and Research: The development of bioplastics encourages research and innovation in sustainable materials. Ongoing advancements aim to improve the performance, functionality, and end-of-life options of bioplastics, leading to more environmentally friendly alternatives.

Consumer Demand and Perception: With growing awareness of plastic pollution and environmental concerns, consumers are increasingly demanding eco-friendly alternatives. Bioplastics offer a more sustainable choice, aligning with consumer values and corporate sustainability initiatives.

Challenges and Considerations

It is important to note that while bioplastics have numerous advantages, they also come with certain challenges and considerations, such as land use conflicts, limited infrastructure, and the need for proper disposal and education. Nonetheless, with continued advancements and responsible use, bioplastics can contribute to reducing the environmental impact of plastics and promoting a more sustainable future. (Jim Robbins, 2020)

Here are some of the key disadvantages of bioplastics:

Land Use and Resource Competition: The production of bioplastics often requires significant amounts of agricultural land, which may compete with food production and contribute to deforestation. It is crucial to ensure that the feed stocks used for bioplastics do not compromise food security or lead to environmental degradation as bioplastics rely on agricultural crops as feedstock, there is a concern about competition with food production and land use. Balancing the demand for both food and bioplastic feedstock is crucial. (Renee Cho, 2017).

Limited Biodegradability and Compostability: Not all bioplastics are easily biodegradable in natural environments. Some bioplastics require specific industrial composting facilities with controlled conditions for proper breakdown. If disposed of improperly, bioplastics that do not readily biodegrade can still contribute to pollution and litter. Biodegradable bioplastics typically require specific conditions, such as high temperatures or industrial composting facilities, to break down efficiently. If they end up in the natural environment or conventional landfill, they may not biodegrade as intended.

Recycling Challenges: While some bioplastics can be recycled alongside traditional plastics, the compatibility and recyclability of bioplastics vary depending on their composition and local recycling capabilities. The lack of separate collection and recycling infrastructure for bioplastics can limit their recycling potential and hinder their effective management. Bioplastics often require different processing techniques and recycling systems compared to traditional plastics. Mixing bioplastics with traditional plastics can contaminate the recycling stream and hinder the recycling process.

Energy Intensive Production: The production of bioplastics can require significant energy inputs, particularly in the extraction and processing of plant-based feed stocks. Depending on the energy sources used, the overall environmental impact and carbon footprint of bioplastics may not always be significantly lower than that of traditional plastics.

Chemicals and Additives: Bioplastics may still require additives, such as plasticizers or fillers, to achieve desired properties. Some of these additives can be derived from fossil fuels or have potential health and environmental impacts. It is essential to carefully assess the composition and safety of bioplastic formulations.

Cost Considerations: Bioplastics are often more expensive to produce than conventional plastics due to the costs associated with sourcing, processing, and refining renewable feed stocks. This cost disparity can limit their widespread adoption, especially in price-sensitive markets.

Misinterpretation and Confusion: The labelling and marketing of bioplastics can sometimes be misleading or misunderstood by consumers. Claims of "biodegradability" or "Compostability" without proper context or understanding can lead to improper disposal and environmental harm.

Applications

Bioplastics can be used in a wide range of applications, include packaging materials, disposable cutlery, agricultural films, textile fibers, and more. However, the suitability of bioplastics for specific applications depends on factors such as durability, heat resistance, and barrier properties.

It's worth noting that while bioplastics offer potential benefits, they are not a silver bullet for solving the plastic waste problem. A comprehensive approach that includes reducing plastic consumption, improving waste management systems, and promoting recycling and reuse is necessary to address the broader plastic pollution issue. The development of bioplastics provides businesses with eco-friendly alternatives for packaging and products, especially single-use items that contribute a lot of waste. The bioplastic industry is still in its early stages but steadily growing and will begin replacing many disposable items made from petroleum-based plastic, such as cups, cutlery, packaging, containers and straws (Jan-Georg Rosenboom et al ,2022).

It's important to note that the properties, biodegradability, and Compostability of bioplastics can vary depending on their composition, manufacturing processes, and environmental conditions. Therefore, understanding the specific characteristics and proper disposal methods of each type of bioplastic is crucial for ensuring their sustainable use.

To maximize the benefits of bio-plastics and mitigate their disadvantages, it is important to address these challenges through sustainable sourcing practices, improved waste management infrastructure, proper labelling and education for consumers, and continued research and development to enhance the performance and end-of-life options of bio-plastics. A comprehensive approach is necessary to ensure that bio-plastics are used responsibly and contribute to a more sustainable future (Fridovich-Keil et al ,2020).

Boon or curse??

The debate related to overall impact and sustainability of bioplastics continues. In this Chapter I tried to explore both the benefits and challenges associated with bioplastics, aiming to provide a balanced perspective on whether they are a boon or curse.

Environmental Benefits:

Bioplastics offer several environmental advantages that make them appealing in the context of plastic pollution and climate change.

Reduced Carbon Footprint: Bioplastics have a lower carbon footprint compared to conventional plastics, as they are derived from renewable resources instead of fossil fuels. This reduces greenhouse gas emissions during production.

Biodegradability: Unlike traditional plastics, bioplastics are designed to break down more rapidly in natural environments, reducing the risk of long-term pollution and harm to wildlife.

Renewable Resources: Bioplastics can be made from plant-based sources, such as corn or sugarcane, which can be grown sustainably and serve as a renewable feedstock.

Challenges and Considerations:

Despite the environmental benefits, there are several challenges and considerations that need to be addressed when assessing the overall impact of bioplastics.

Land Use and Food Security: The production of bioplastics requires agricultural land, which may compete with food production. If not managed carefully, it could lead to deforestation, increased water usage, and potential food shortages.

Limited Infrastructure: Bioplastics often require specific conditions for proper composting and recycling. The lack of adequate infrastructure may hinder their effective disposal and lead to unintended environmental consequences.

Misinterpretation of Biodegradability: Bioplastics may be labelled as "biodegradable," but not all biodegradable plastics break down easily in natural environments. Some may require industrial composting facilities, leading to confusion and improper disposal.

Balancing the Pros and Cons:

To ensure that bioplastics are a boon rather than a curse, several measures can be taken:

Sustainable Sourcing: Bioplastics should be produced from non-food, sustainable biomass sources, minimizing the impact on food security and land use.

Infrastructure Development: Investment in composting and recycling facilities should be prioritized to support the proper disposal and management of bioplastics.

Consumer Education: Clear labelling and public awareness campaigns are necessary to educate consumers about the proper disposal and limitations of bioplastics.

Technological Innovation: Continued research and development should focus on improving the production processes, performance, and end-of-life options for bio-plastics.

Replacing plastic and paper materials with bio plastics in the workplace

Replacing plastic and paper materials with bio plastics in the workplace is a great initiative for reducing environmental impact. These are some steps to implement this transition:

Assess Current Usage: Begin by assessing the types and quantities of plastic and paper materials currently used in your workplace. Identify the areas where they can be replaced with bioplastics.

Research Bioplastic Alternatives: Explore different types of bioplastics available in the market. Consider factors such as their composition, durability, cost, and compatibility with your specific applications. some common bioplastics include polylactic acid (PLA), polyhydroxy alkanoates (PHA), and starch-based bioplastics.

Set Goals and Prioritize: Set realistic goals for phasing out plastic and paper materials and prioritize the areas where their replacement will have the most significant impact. Start with items that are commonly used or have readily available bioplastic alternatives.

Engage Suppliers: Contact your current suppliers and inquire about their bio plastic offerings. If they don't provide bio plastics, explore alternative suppliers who specialize in eco-friendly packaging and materials.

Educate Employees: Inform your employees about the transition to bio plastics, highlighting the environmental benefits and explaining the proper usage and disposal methods. Provide training on identifying and sorting bio plastics for recycling or composting.

Test and Implement: Start by conducting small-scale trials to evaluate the performance and suitability of bioplastics for your workplace. Gather

feedback from employees and make any necessary adjustments before implementing the switch on a larger scale.

Update Procurement Policies: Modify your procurement policies to prioritize bioplastics. Include guidelines for purchasing bioplastic alternatives whenever possible and ensure that all new supplies meet your sustainability criteria.

Recycling and Disposal: Determine the proper recycling or composting methods for the bioplastics used in your workplace. Communicate these guidelines to employees and provide clearly labeled recycling and composting bins.

Monitor Progress: Continuously track and measure your progress toward reducing plastic and paper materials in the workplace. Evaluate the environmental impact and cost savings achieved through the adoption of bio plastics.

Share Successes: Celebrate your achievements and share your success stories with employees, clients, and the wider community. Inspire others to adopt sustainable practices in their workplaces as well.

Conclusion

The use of bioplastics presents a complex and multifaceted issue. While they offer several potential advantages such as reduced reliance on fossil fuels, lower carbon emissions, and improved waste management, there are also significant challenges and drawbacks associated with their production and disposal.

Bioplastics can be a boon when it comes to mitigating the environmental impact of traditional plastics. They can help to reduce greenhouse gas emissions, conserve non-renewable resources, and decrease dependence on fossil fuels. Additionally, their potential to be biodegradable or compostable offers the promise of reducing plastic waste in landfills and oceans.

However, there are also concerns surrounding the production of bioplastics. The cultivation of crops for bioplastic feed stocks may lead to deforestation, habitat destruction, and competition for land with food crops. Additionally, the manufacturing process can consume significant amounts of energy and water, potentially offsetting some of the environmental benefits.

The disposal of bioplastics is another critical aspect to consider. While some bioplastics are designed to be biodegradable or compostable, proper infrastructure for their disposal is often lacking. Inadequate waste management systems and mixed disposal streams can lead to the contamination of recycling streams and hinder the biodegradation process, resulting in bioplastics persisting in the environment much like traditional plastics.

Furthermore, the term "bioplastics" encompasses a wide range of materials with varying properties and environmental impacts. It is essential to differentiate between biodegradable and non-biodegradable bioplastics, as well as assess their life cycle impacts to make informed decisions about their usage.

Bioplastics offer the potential for reducing plastic pollution and lowering carbon emissions, presenting a positive alternative to conventional plastics. However, challenges such as land use conflicts, limited infrastructure, and the misinterpretation of biodegradability must be addressed. With proper measures in place, including sustainable sourcing, infrastructure development, consumer education, and technological advancements, bioplastics can indeed become a boon by mitigating the environmental impacts associated with traditional plastics. A comprehensive and balanced approach is essential to harness the benefits of bioplastics while minimizing the potential downsides, ensuring a sustainable future for both our planet and society.

In conclusion, bioplastics have the potential to be a beneficial alternative to traditional plastics, but their full environmental impact depends on various factors such as feedstock sourcing, manufacturing processes, and end-of-life management. To fully realize their benefits, it is crucial to invest in sustainable practices, support technological advancements, and establish effective waste management systems. Ultimately, a holistic approach that considers the entire life cycle of bioplastics is necessary to determine whether they are a true boon or curse for our planet.

References

Alfred Rudin, Phillip Choi (2013). Chapter 13 – Biopolymers. The Elements of Polymer Science & Engineering (Third Edition)., Pages 521-535.

Syed Ali Ashter, (2016). Introduction to Bioplastics Engineering. ISBN: 978-0-323-39396-6. Pages 81-151.

Renee Cho, (2017). The Truth About Bioplastics, News from the Columbia Climate School (climate earth and society) .

The Basics of Bioplastics (2018). www.urthpact.com

Jim Robbins, (2020) Why Bioplastics will not solve the worlds plastics problem. YALE school of Environment.

Fridovich-Keil, Judith L (2020). Bioplastic. Encyclopedia Britannica. https://www.britannica.com/technology/bioplastic.

Jan-Georg Rosenboom, Robert Langer, Giovanni Traverso (2022). Bioplastics for a Circular economy. Nature Reviews Materials, Vol 7, Pages 117-137

Advantages of Bioplastic Packaging for the Food and Drinks Industry (2022). Takeaway Packaging.

Monika Metro (2021).5 types of Bioplastics : Starch, Cellulose, Protein , Organic ,Aliphatic Polyesters .www.greenbusinessbureau.com

Why Bioplastics? www.biopasstic.com.sg

What are Bioplastics. Sustainability for all.www.activesustainability.com

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Precision Agriculture

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Introduction

Precision Agriculture (PA) technology is an approach to farming that employs data sensors, connected devices, remote control tools and other advanced technologies to give farmers more control over the field. It is an approach to farm management that uses it to ensure that crops and soil receive exactly what they need for optimum health and productivity. It is also known as satellite agriculture. To be more precise, it is a site -specific crop management with modern farming practices that make production more efficient. It is considered as the vibrant agricultural system of 21stcentury, as it symbolizes a better balance between reliance om traditional knowledge as well as information and management intensive technique.PA can also be called as Precision farming, satellite agriculture.

Pierre Robert invented precision agriculture. He is referred as father of precision farming because of his active promotion of the idea and organization of the first workshop, “Soil Specific Crop Management,” during 1990s.

It is generally defined as, information and technology-based farm management system to identify, analyze and manage spatial and temporal variability within fields for optimum productivity, profitability, sustainability and protection of the land resource by minimizing the use of chemicals.

Precision Agriculture increases efficiency and productivity by enabling farmers to target their inputs such as seeds, fertilizers, and pesticides to specific areas of the field that need them the most, rather than applying them uniformly across the entire field. It plays an important role in sustainable agriculture. The primary goal of PA is to enhance sustainable soil and crop management of the farm by utilizing resources to increase food production and long-term profitability while reducing variable costs and environmental contamination. It basically aims to optimize field -level management with regard to crop science, by matching farming practices more closely to crop needs and environmental protection by reducing environmental risks and footprint of farming. With

regards to economics, it boosts the competitiveness through more efficient practices. The significance of PA is, it prevents soil degradation by reduction of chemical application in crop production, efficient use of water resources, dissemination of modern farm practices to improve quality, quantity and reduced cost of production (Reyns etal,2002).

The 5Rs of PA include, applyingthe right input, at the right amount, to the right place, at the right time and in right manner.

Components of precision farming

The major components of precision farming are: Information, Technology and Management. All these components working together in Precision agriculture leads to a sustainable agriculture. Belowlisted, will briefly explain the components of Precision farming.

1.Geographical information system (GIS)

2.Geographical positioning system (GPS)

3.Remote sensing

4.Variable rate technology (VRT)

5.Normalized Difference Vegetation Index (NDVI)

6.Nutrient Expert System

7.Site specific nutrient management (SSNM)

8.Biointensive farming

9.Real time Nitrogen management

10.Diagnosis and Recommendation Integrated System (DRIS approach)

11.Soil testing and yield monitoring

Geographical information system (GIS)

Geographical information system (GIS) is a technology that provides the means to collect and use geographic data to assist in the development of Agriculture.GIS is a tool that lets users create multilayered interactive maps that can be used for the visualization of complex data and for spatial analysis. This enables farmers to map field data, organize and analyze it and monitor their crops remotely. Precision Agriculture relies heavily on GIS to collect and interpret massive field data for informed decision making. Robotics, GPS,drone and satellite monitoring that contributes to farm automation

underpin collecting GIS data. By visualizing data,GIS helps farmers spot trends and patterns,implement change detection and quickly address the issues.GIS software provides detailed vegetation and productivity maps,including crop informationfor making reasonable decisions. Agricultures GIS tools can identify vegetation levels in the field and surrounding areas.Agriculture machinery can then use this information to adjust seed,nutrients,herbicides and fertilizer amounts for each plot (Sergieieva,2022).

Components of GIS:

GIS has five key components

Hardware, software, data, people and methods. Hardware is the computer on which a GIS operates. The GIS software runs on a wide range of hardware types, from centralized computer servers to desktop computers used in stand-alone or networked configurations.

Hardware, software and information all come together in GIS technology.A simple lap top or desk top computer to a more complex, like a satellite or a Drone,can serve as the hardware.GIS software utilizes maps to display spatial data.Images are created using variety of GIS technologies and then linked to relevant maps and data that is hidden from the view.These maps not only show the location and overall health of the crop but also accounts other relevant factors such as terrain,soil type and fertilization.(EOS DATA ANALYTICS).

The two major types of GIS file formats are raster and vector. Raster formats are grids of cells or pixels. Raster formats are useful for storing GIS data that vary, such as elevation or satellite imagery. Vector formats are polygons that use points that are called nodes and lines. The main data sources for GIS are hard copy maps, aerialphotographs, remotely-sensed imagery, point data, samples from surveys and existing digital files. The main systems of GIS which are interactive are, an input subsystem for converting into digital form maps and other spatial data, storage and retrieval subsystem, an analysis subsystem and an output subsystem for producing maps, tables and answers to geographical queries. This GIS approach is based on four stages that is, input data selection,spatial decomposition, mapsunification, and the generation of the informed topologic graph.(Paris et al., 2009).

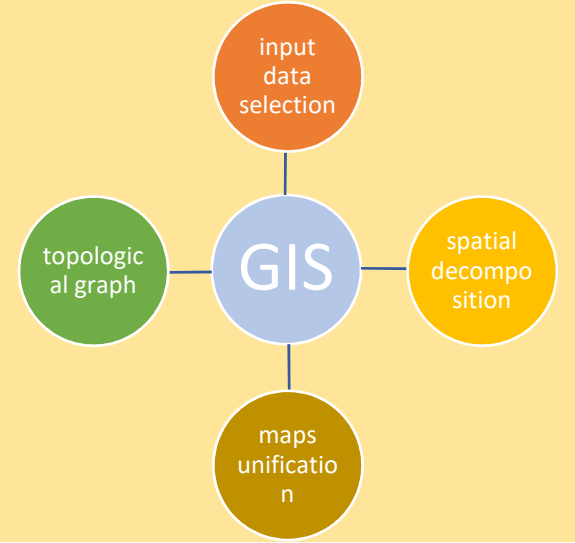

Fig 1: ***Four stages of GIS approach***

The most popular current applications of GIS in agriculture are:

Precision Agriculture, Agriculture mapping, Crop health monitoring, Livestock monitoring,Insect and pest control,Irrigation control,Nutrient distribution,crop yield prediction.

Geographical Positioning System (GPS)

GeographicalPositioning System (GPS) is a network of satellites and receiving devices used to determine the location of something on earth.Some GPS receivers are so accurate that they can establish their location within 1 centimeter. It is a technology useful for determining the exact location in an agricultural field with considerate accuracy. In precision agriculture, GPS allows farmers to accurately navigate to specific locations in field,year afteryear,to collect soil samples or monitor crop conditions.Location information is collected by GPS receiversfor mapping field boundaries, roads,irrigation systems and problem areas of crops such as weeds or disease.

GPS has improved accuracy in terms of recording coordinates regarding position,time and direction with reliability (Shanwad et.al.2002).

Uses of GPS

The uses of precision farming are, farm planning,identification of areas suitable for cultivation and classification of areas for cultivation based on various characteristics and land usage in the locality and contour mapping,

plantation and water bodies mapping, soil mapping and sampling,river's mapping,weed location,accurate planting and determination of planting ratios. In addition, tractor guidance,machinery location and direction, crop scouting,correlation of production techniques with crop yields, variablerate applications, assessment of the availability of water in area,identification of irrigated crops,identification of swamps and other water-loggedareas and irrigation systems mapping such as dams and canals. Yield mapping and harvesting and environmental control benefits a lot to agriculture. GPS allows farmers to work during low visibility field conditions such as rain,dust,fog and darkness, use of meteorological mapping such as climatic patterns and personnel mapping adds a complete substance to this farming practice**.**

Components

A typical configuration for on farm agricultural applications includes a GPS receiver and antenna, a differential corrections receiver and antenna and cables to interface differentially-corrected GPS data from receiver to other electronic equipment such as yield monitor or a variable rate controller.

Henceforth three basic components:

1.An antenna which receives the signal and, in some cases, has anti-jamming capabilities

2. A receiver processor unit, which converts radio signal to useable navigation solution

3. A control/display unit, which displays the positioning information

Benefits of GPS in Precision Agriculture

- Precision soil sampling,data collecting and data analysis,this helps in the application of localized chemicals and the adjustment of plant density.
- Accurate field navigation reduces the number of redundant.
- Productivity is increased by the ability to work in low visibility like rain,dust,fog and darkness.
- Accurately monitored yield data enables future site-specific field preparation.
- Elimination of human flaggers improves spray efficiency.

One of the most significant functions of GPS in agriculture is soil sampling. It is critical to understand the type of soil available on given

farmland because this will help determine the type of crop to be planted on that farm (Pragati, 2022).

Remote Sensing

Remote sensing is the use of satellite images that take photos of a field over time so that, itcan analyze conditions based on the data and take action that will have a positive influence on crop yield. The main principle of remote sensing is, the source of this sensing data is the electromagnetic radiations which are emitted or reflected by theobject, which then helps in their identification and classification. Remote sensing technologies and tools enable farmers to characterize spatial variability in fertilizers among farms and crop fields. They also estimate the number of fertilizers, herbicides and insecticides to be used in the farm (Brown and Molly2015).

Components of remote sensing are

1.Platform: It is a carrier for remote sensing sensors

2.Sensors: It is a device that receives electromagnetic radiations and converts it into a signal that can be recorded and displayed as either numerical data or an image

Remote sensing Systems used for Precision Agriculture are classified based on:

1. Sensor platform
2. Type of sensor

Since the 1970s, satellite products have been extensively used for PA.Recently aerial platforms which include aircraft and unmanned aerial vehicles (UAVs) are also used in PA.

Ground -based platforms used for PA can be grouped into three categories:

1.Hand held

2.free standing in the field

3.mounted in tractor or farm machinery

The Ground based systems are also referred to as proximal remote sensing systems because they are located inclose proximity to the target surface as compared to aerial or satellite -based systems.

Sensors used for remote sensing differ based on:

The spatial, spectral, radiometric and temporal resolution they offer.

Types of remote sensing systems are,Visual remote sensing system such as human visual system,optical,infrared,microwave, radar, satellite, airborne, acoustic and near -acoustic remote sensing.

Basic process in remote sensing

Data acquisition (energypropagation, platform)

Processing (conversion of energy pattern to images)

Analysis (quantitative and qualitative)

Accuracy assessment (radiometric and geometric correction)

Information distribution to users

Land mapping,mapping of soil properties,classification of crop species,crop monitoring,irrigation management,nutrient management,estimation of soil moisture,yield prediction,detection of crop water stress,mapping of vegetation indices,weed management,disease and pest management,mapping of crop yield,verifying the effectiveness of variable-rate fertilizer,control of agriculture inputs,evaporation estimation ,weather forecasting,environmental study,study of natural hazard and resource exploration(Quattrochi,etal;1998).

Working of remote sensing in agriculture

The basic working of this technology with UAVs, satellites and other platforms is almost the same. The energy in the form of light will travel from sun to earth. The energy emitted from the sun is known as electromagnetic energy and is a part of the electromagnetic spectrum. Light travel in the form of waves, the distance between the peak of one wave to the next wave peak is the wavelength of light. The wavelengths that are used for agriculture applications cover a small amount of electromagnetic spectrum. When the electromagnetic energy hits the plants during hyperspectral remote sensing, the energy will be reflected, absorbed, and transmitted. This energy that is reflected, absorbed, and transmitted can be detected by remote sensing technology. The relationship between the three occurrences determines the spectral signature of the plants. This signature is unique to different plant species. Remote sensing farming helps identify stressed areas by determining

the spectral signatures of plants that are healthy(Dragonfly aerospace,blog,2023).

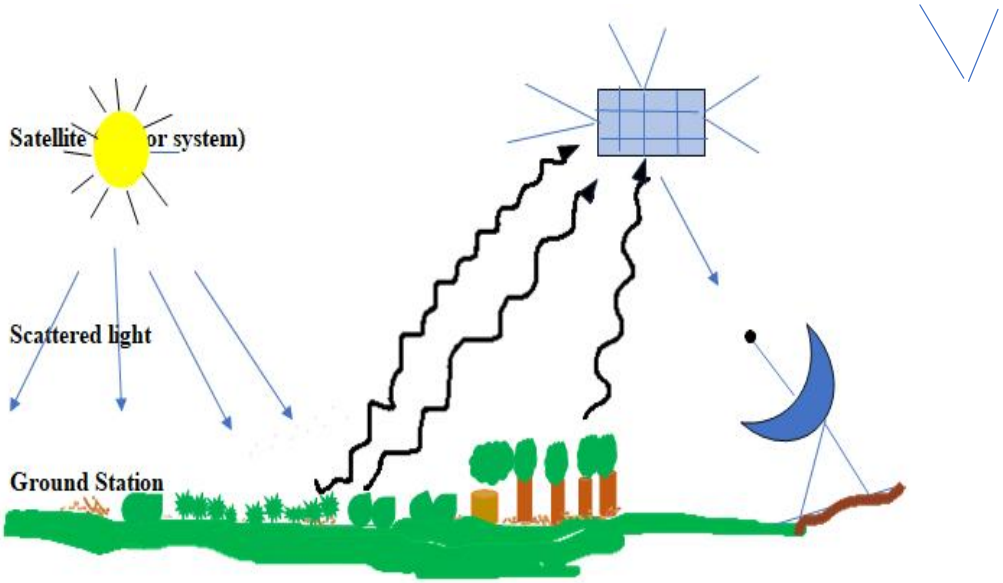

Fig2.Howremote sensing works in agriculture?
[Source:https://image.slidesharecdn.com/remotesensingagriculture, recreated]

Variable rate technology (VRT)

Variable rate technology uses data and automation to apply fertilizer, crop protection products, seeds and even irrigation water at different rates in different locations without a grower having to change application rates or make multiple passes manually. Here it enables the farmers save on fertilizers and improve crop yields and quality, by applying fertilizers at different rates across the field, depending on the soil nutrient levels and crop requirements in each area. To be more precise, the application of the material, such that the rate of application is based on the precise location, or qualities of the area that the material is applied to. This is different from uniform application, and can be used to save money by using less product and lessen the environmental impact (Swayer,1994 and Khan and Sarwa,2012).

Variable rate application

1.Map based: The applications are based on variable rate prescription maps that an agronomist prepares based on data sources. These maps can be created using electromagnetic induction, which is cost effective and non - destructive.

2.Sensor based: The application is calculated real-time, based on sensors that are local to the variable rate applicator.

Applications

It is used for seeding, weed control and fertilizer application(Robert,G,et.al,2011).

Practical implementation of Variable Rate Technologies (Sarauskis, E. et.al.2022):

It is a cyclical process that involves: 1. Data collection 2. Interpretation of data collected 3. Implementation of appropriate management response 4. Monitoring of results in a continuous learning process (Patil and Shanwad, 2009)

Data collection strongly depends on the resolution and the accuracy provided by the sensing technology. This data can be collected in various ways:1. Soil mapping 2. Satellite images 3. Drone images 4. Yield mapping or Hand-held Devices. (Lorenz and Munchoff,2018).

Normalized Difference Vegetation Index(NDVI):

NDVI is widely used in agriculture, forestry and ecology to monitor the growth and health of vegetation and to identify areas of stress or damage. It is a simple graphical indicator that is often used to analyze RS measurements and assess whether the target being observed contains green health vegetation or not. NDVI values can also be used to map and classify vegetation types, and to detect changes in vegetation cover over time. In simple terms, NDVI measures the greenness and the density of vegetation captured in a satellite image. Healthy vegetation has a very characteristic spectral reflectance curve which we can benefit from by calculating the difference between two bands that is, visible and near -infrared. NDVI is that difference expressed as a number ranging from -1 to 1. This index range of -1to 1., where negative values are mainly formed from clouds, water and snow, and values close to zero are formed from rocks and bare soil. Moderate valuesie., 0.2 to 0.3, represent

shrubs and meadows while large values from 0.6 to 0.8 indicate temperate and tropical forest.

NDVI calculation:

It is derived from satellite imagery and calculated in accordance with the formula:

$$NDVI=\frac{NIR-RED}{NIR+RED}$$

where:

NIR-light reflected in the near -infrared spectrum

Red-light reflected in the red range of spectrum

Here in precision agriculture, Chlorophyll, a health indicator pigment strongly absorbs visible light, and the cellular structure of the leaves strongly reflects near infrared light. When the plant becomes dehydrated, sick, afflicted with disease., the spongy layer deteriorates and the plant absorb more of the near infrared. Thus, observing how NIR changes compared to red light provides an accurate indication of the presence of chlorophyll, which correlates with plant health(EOS Data Analytics).

Application of NDVI:

- To monitor
- Climate change
- Agriculture production
- Desertification
- Fire prediction
- Land cover change
- Vegetation heath

Nutrient management in Precision Agriculture

Nutrient Expert System

Nutrient expert system is an easy to use, interactive and computer -based decision support tool that can rapidly provide nutrient recommendations for an individual farmer field in the presence or absence of soil testing. It is based on principle of site-specific nutrient management. Integrated plant nutrient management developed the Nutrient Expert fertilizer decision support tool. The precision nutrient management is the science of applying advanced,

innovative, cutting-edge, site-specific technology to regulate spatial and temporal variability in natural nutrient supply from soil in order to improve agricultural production systems, productivity efficiency and profitability.

Site specific nutrient management (SSNM)

Site specific nutrient management provides a field specific approach for dynamically applying nutrients to crops as and when needed. This approach advocates optimal use of indigenous nutrients originating from soil, plantresidues, manures and irrigation water(Pateletal,2022).

Biointensive farming

Biointensive farming is an organic system of agriculture, where farmers focus on achieving the maximum yields, from existing limited pieces of land, while at the same time increase the biodiversity as well as maintaining sustainability and fertility of the soil.

When the importance and benefits are considered, as we are time bound or rather have a limited time, biointensive farming can help combat dwindling crop production due to global warming and climate change, by maintaining the soil fertility, it gives more profit to the farmers. All said and done, it is better than the conventional method. Saves more land for other use. Mainprinciples of biointensive farming are, deep soil preparation, choosing plant spacing, composting, carbonfarming, calorie farming for increasing the fertility of the soil, companionfarming, growing open pollinated seeds and a whole system method which is a progressive farming technology. The main source of inspiration in this farming is great biodiversity and harmony between plants in nature, wherein the quorum quenching and sensing leads to a consortium of interaction between all the elements of nature, benefitting not only the farmers but the environment too.

Real time Nitrogen management

Real time nitrogen management helps to estimate nitrogen status of plant and is used to improve N management by estimating the need of the crop for fertilizer before sowing and by distributing the fertilizer during the cropping season based on the crop need.Wherein precision nutrient management is an advanced nutrient management as well as precision agriculture for solving problems in food and environment security for sustainable agriculture and social development. This helps us to enhance the production of the farms and ensure site specific nutrient application. As the

farming community is now aware that, continuous application of the fertilizer has resulted in detrimental impacts on soil quality and the overall environment.

Diagnosis and Recommendation Integrated System (DRIS approach)

DRIS is a new approach to interpreting leaf or plant analysis which was developed by “Beaufils” (1973) named as Diagnosis and Recommendation Integrated System. A system which identifies all the nutritional factors limiting and increases the chances of obtaining high yield by improving fertilizer recommendations.

The very concept gives results of plant nutritional diagnosis through indices, which represent in a continuous numeric scale, the effect of each nutrient in the nutritional balance of the plant. DRIS mainly uses the nutritional balancing concept in the detection of nutritional deficiency or excess in plant.

DRIS is a mathematical technique to apply plant analysis information (nutrient concentration) for diagnosing the most limiting nutrient in a production system. The evaluation is made by comparing relative balance of nutrient content with norms established for that crop under high yield conditions. Usually, DRIS norms employ the average value of each element obtained from the concentration of leaf tissues of the reference population as reference value, thus establishing the sufficiency average.

For interpretation of tissue analysis DRIS method uses nutrient ratios. The nutrient ratios are helpful to obtain special indexes which are called “Nutrient Index” or “Beaufills nutrient Indexes” (BNI). There will be a positive and negative values for the nutrient index. The nutrient with positive indexes appears to be in excess and nutrient with negative index are deficient in plants (Filho ,2004).

Soil testing and yield monitoring

Yield monitoring or mapping refers to the process of georeferenced data collection with the aid of farm equipmentsuch as drones, tractors or harvester along with the information including grain yield, moisture levels and soil properties, among other during crop harvesting. A significant facet of precision agriculture that assists the farmers in making educated decisions by providing more information about their fields. The sensors in the yield monitoring system usually incorporates data from yield sensor, a moisture sensor, a groundspeed sensor, a cut width sensor,a clean grain elevator speed sensor,and a differential Global Positioning System (DGPS) receiver to relate sensed grain flow to yield as a function of location (Redhu,N.S,et.al.2022).

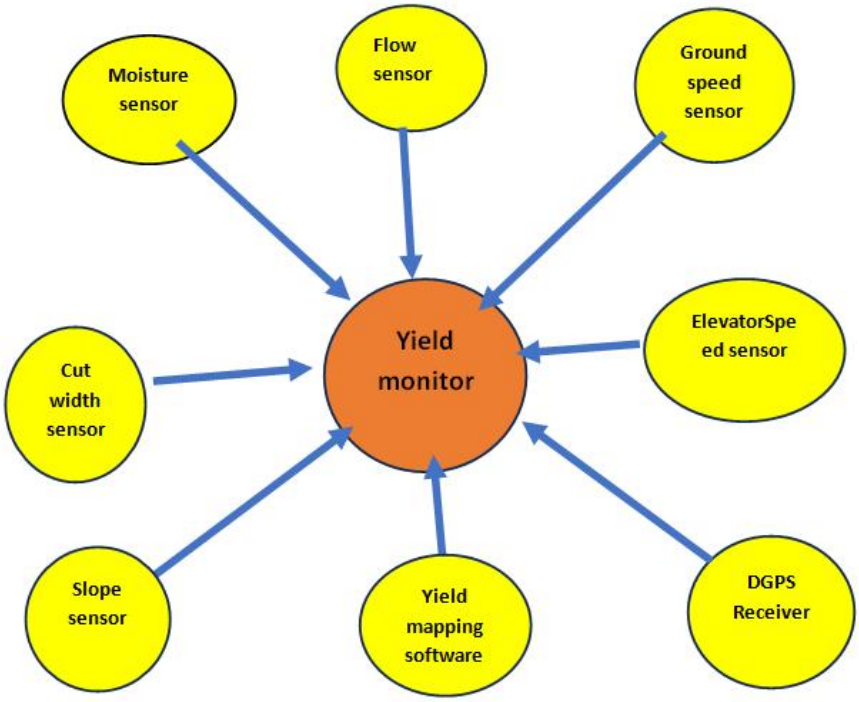

Figure – 3: Schematic representation of the sensors that transmit data to yield monitor (Selcuk Arslan, and Thomas S.Colvin,2002).

The process of Yield Monitoring involves feeding the harvested grain into the elevator for sensinggrain moisture and a transfer of these grains to a holding tank for sensing grain yield and then displayed on the screen. The information obtained is then georeferenced to the field and the associated field data can further help the farmers in assessing, when to sow, fertilizeor harvest, the effects of weather etc., (MagalhaesandCerri,2007)

As for its application, it is used in precision agriculture because it helps identify measure and describe the intra field variability within a cropping system which is exactly what forms the basis of the concept of precision agriculture. It provides variable data within a single field.

Advantages of Precision Agriculture

The advantages of Precision Agriculture is its sustenance in agriculturedevelopment, reducesexcessive chemical usage in crop production, dissemination of information about agricultural practices to improve quality, quantity and reduce cost of production in agricultural crops and water resources will be utilized efficiently under precision farming by optimizing irrigation practices. It has a beneficial impact on environment with respect to leaching and ground water contamination by means of the optimization of agro-chemical products. GPS allows agricultural fields to be surveyed with ease. As the soil and yield characteristics can be mapped. The non-uniform fields can be subdivided into smaller plots based on their unique requirements, hence better resource management and hence reduce the wastage of resource. It implements a refined set of cultivation practices and choice of crops based on the sustainability of the land. Elimination of volatility and risk and optimized use of chemical fertilizer.

Disadvantages of Precision Agriculture

As Precision Agriculture is technology based its high capital cost initially for maintenance due to hardware, software and infrastructure needed is the main disadvantage. Moreover, techniques are still under development and therefore requires expert in these techniques before actual implementation.Actual collection of the data may take several years to fully implementand analysisare for now, extremely difficult.Data availability and quality can vary depending on location, season and crop.

Challenges

- Lack of technical expertise knowledge and technology.
- Deploying and using the technologies, interpreting the captured data require high level of awareness and skills
- Not viable for small land holdings
- Requires high investment.

Future Perspectives

The future aspiration in precision agriculture is, to test and evaluate the latest methodologies and advanced technology in practices on farms and make an assessment of the soil, plant and environmental parameters, and if required, changes in the proposed methods (Sarauskis, et.al.,2022). Thus, it can give the best solution and the most optimal methods for farmers in time of crisis. This

area gives an opportunity for advanced scientific research studies and also opens the doors to many entrepreneurs.

References

Arslan, S and Colvin, T. S. (2002, June). Grain Yield Mapping: Yield Sensing, Yield Reconstruction, and Errors. *Precision Agriculture* 3(2):135-154.

Brown and Molly (2015).Satellite Remote Sensing in Agriculture &Food Security Assessment.29:307.10.1016/j.proenv.2015.07.278.

Dubey,P. (2022,June15). Smart farming

EOS Data Analytics.(2022). Google scholar.https://eos.com>make an analysis

Filho,F.D.A.A.M.(2004,October). DRIS: Concepts and applications on nutritional diagnosis in fruit crops,a review.*Sci.agric*.61(5)-SciELO, Google search,https://www.scielo.br>.

https://doi.org/10.1590/SO103-90162004000500015

GIS for Agriculture:Solution,Application ,Benefits-EOS Data Analytics.http://eos.com

Khan,Fahad, S and Dalhousie University.Department of Engineering,(2012).Mapping Soil Properties and Water Table Depths Using Electromagnetic Induction Methods .

Lorenz,F and Munchoff,K.(2018).Google scholar.

Magalhaes,P.S.G and Cerri, D.G.P (2007).Yield Monitoring of Sugarcane.*Biosystems Engineering*.96(1).pp-1-6.Google scholar

Paris, S, Gerdelan,A and Sullivan, C.O.(2009,November 21-24).CA-LOD: Collision Avoidance level of detail for Scalable, Controllable Crowds.Conference:Motion in Games , Second International Workshop, MIG 2009,Netherlands,Proceedings.

Patil,V.C and U.K.Shanwad.U.K.(2009,December). Relevance of precision farming to Indian Agriculture, Second National Conference on Agro-informatics and Precision farming.

Patel,V.K, Ghosh, S and Kumar,S. (2022,April). Precision Nutrient Management. Just Agriculture. 8(2).

Quattrochi, Dale & Luvall, Jeffery (1998). Thermal Infrared Remote Sensing for Analysis of Landscape Ecological Processes: Methods and Applications. *Landscape Ecology*.14.10.1023/A:1008168910634

Redhu, N.S, Thakur, Z, Yashveer, S and Mor,P. (2022).Next Generation Sequencing Era,Artificial intelligence :a way forward for agriculture. *Bioinformatics in Agriculture* Chapter 37,641-668

Reyns, P.,Missotten,B,Ramon,H. (2002). *Precision agriculture* .3:169.

Robert, G & Alley, Marcus & Thomason, Wade & Holshouser, D &Roberson,O.T..(2011).Precision farming tools: Variable-rate application. Precision Geospatial,& Sensor Technologies.442-505.

Santosh,K.M,Sundaresan,J., Roggem,R., Deri,A.Singh,R.P.(2014).Geospatial technologies and Climate change.Springer International Publishing:Dordrecht,The Netherlands(Google Scholar)

Sarauskis, E, Kazlauskas, M, Naujoklene,V, Bruclene, I, Steponavicius, D, Romaneckas, K Jasninskas, A.(2022). Variable Rate Seeding in Precision Agriculture, A Review: Recent Advances and Future Perspectives. Agriculture .12.305, 1-24.

Sawyer,J.E.(1994).Concepts of Variable Rate Technology with Considerations for Fertilizer Application. Journal of Production Agriculture.7:195-201.

Shanwad,U.K, V.C.Patil, V.C, Dasog, G.S, Mansur,C.P and Shashidhar,C.K.(2002).*AsianGPS proceedings*

Sergieieva,K,(2022, December,15). Geographical Information System in Agriculture: Best practices for AgriTech Leaders, 2022. Earth Observing System.

Wikipedia, www.ispag.Retrieved 20 December 2021.

Applications of remote sensing in precision agriculture. Grind GIS, blog

The Global Positioning System for Geosciences: Summary and Proceedings of a Workshop on improving the GPS Reference Station Infrastructure for earth Oceanic and Atmospheric Science Applications(1997).*National Academic Press* ,Washington DC

Thirty uses or applications of Geographic Positioning System in agriculture.(2018 January 5).Blog,grindgis.com

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Phytochemicals and their Functional Aspects

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Introduction

Plants are very diverse in nature. They are extensively distributed over plains, mountains, hills, and marine environments. In this world, there are about 4 lakh species (Pitman, N. C. A. et al, 2002). But only a small fraction of about thirty-five thousand to seventy thousand plant species are used to study their medicinal properties (Veeresham et al, 2012). Phytochemicals exhibit their medicinal abilities from primitive plants to higher plants. As stated by famous authors D. S. Fabricant and N. R.Farnsworth (Fabricant et al, 2001), drugs extracted from around 80% of 122 plants are associated with their traditional uses. In the beginning of21st century, approximately 27 medicines were extracted from flowering plants (Veeresham et al, 2012, Chukwuebuka egbuna et al, 2018).

Phytochemicals are chemicals originated from plants. Phytochemical is a Greek word, where phyton means plant. It helps in resisting bacterial, fungal, and viral infections and even used for consumption by animals and insects. They are produced as primary and secondary metabolites and play an important role in defence mechanism(Molyneux et al, 2007,Harborne et al, 1999). Few phytochemicals have been used as toxic substances while others are utilized in traditional medicine. These metabolites aresynthesized to use as an effective remedy against pests, insects, herbivores, and other pathogens (Molyneux et al, 1999). Phytochemicals are different from essential nutrients like proteins, carbohydrates, fats, vitamins, and minerals which are required for plant survival. Their role differs as antioxidants, phytonutrients, anti-nutrients, phytotoxins, nutraceuticals, etc. At present, around 50,000 (Afendi et al, 2012) to 1,30,000 (Rutz et al, 2022)phytochemicals have been discovered. There is a sequence followed in the study of phytochemicals

Step - 1: Extraction

Step - 2: Isolation

Step - 3: Lab testing

Step -4: Application in medicines

The most difficult task is the isolation and finding the chemical structure (Molyneux et al, 2007). A diverse group of phytochemicals possesses health-supporting medium (phenolic compounds, carotenoids, tocotrienols, non-digestible carbohydrates, organosulfur compounds, phytosterols, and phytosterols). Theyare resistant to high blood pressure, coronary heart disease, diabetes, parasitic infections, microbial and viral infections, ulcers, psychotic diseases, inflammation, etc. Due to the medical properties of phytochemicals, its use isincreasing in functional foods and nutraceuticals. Despite that, there is a need to keep a check on the safety and permissible limit of ingredients used in food (Simran Arora et al, 2020). Medicinal plants are the most important source providing medicine for the majority of the population. Plants play the role of therapeutic agents to relieve the disease in humans. This discovery helped in defencemechanisms, remedies, and durability in relieving pain, and discomfort, and acts as an immediate natural resource against various ailments. Hence the interest renewed in traditional medicine and there is a growing demand for more drugs from plant sources. Also, green medicine is safe, economical and has no side effects compared to manmade drugs (Amin Mir et al, 2018).

Medicinal plants are a boon to mankind as it provides various health benefits. These medicinal plants are in use for centuries in India due to their medicinal properties, which makes them still to be used in India at present. Ayurveda, Unani, Siddha, etc. are a variety of traditional medical practices or systems in India. Due to the introduction of Western culture, the knowledge of traditional medicine has slowly vanished. But now it has been reappearing again as their prominence has been realized and lack of side effects, which is also an important aspect of traditional medicine. Based ona report by World Health Organization (WHO), around 80% of people belonging to developing countries have been relying on traditional medicine which is extracted from medicinal plants, to treat their primary health needs. To prepare modern medicines, the use of traditional medicines is compulsory. Hence, ‘Phytomedicines’ serve as a bridge between modern medicine and traditional medicine. Medicinal plants are vital in health care to treat a community or an individual in most developing countries. Primary and secondary metabolites

are the two main classifications of phytochemicals. Primary metabolites are in charge of fundamental growth or development in plants which comprise, sugars, proteins, amino acids, chlorophyll, nucleic acids, etc. Secondary metabolites are necessary for plant survival in harsh environments. The secondary metabolites are responsible for forming the colour, taste, and smell of the plant. Tannins, flavonoids, alkaloids, saponins, phytosterols, and steroids are commercially used as flavouring agents, colouring agents, pesticides, insecticides, and antifungal and anti-bacterial products. They are also used in protecting humans from cardiovascular diseases, cancer, diabetes, arthritis, aging, etc. (Vishnu Balamurugan et al, 2019).

Classification of phytochemicals (Chukwuebuka egbuna et al, 2018)

In present times, a huge number of phytochemicals are being discovered rapidly yet there is no continuity in their classification, hence the phytochemicals are divided into three major classes based on different functional groups present in them.So far, there is no constantsortingof phytochemicals due to its huge number plus increasing speed of discovery of newer ones.

There are 5 main types of Phytochemicals. They are

1. Phenolic phytochemicals They are subdivided into Polyphenols and Aromatic acids. **Polyphenols** are micronutrients that occur in many food sources. They are again divided as Flavonoids, isoflavonoids, chalconoids, lignans, stilbenoids (e.g., resveratrol), curcuminoids, tannins (e.g., protocatechuic and chlorogenic acids).

Aromatic acids are divided as Phenolic acids (e.g., gallic acid, tannic acid, vanillin, ellagic acid), hydroxycinnamic acids (e.g., coumarin).

Polyphenols are favourable phytochemical molecules for preventing much human pathological disorders like neurodegenerative diseases. However, like any other drug, they might exhibit parallel adverse effects or toxicity, particularly due to the accumulation of high levels in the organism (Figure- 1).

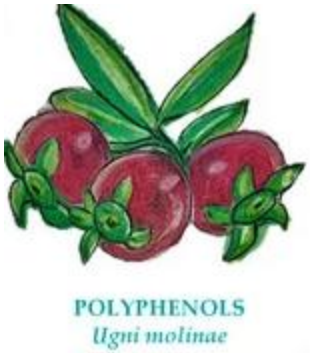

Figure 1: Polyphenols

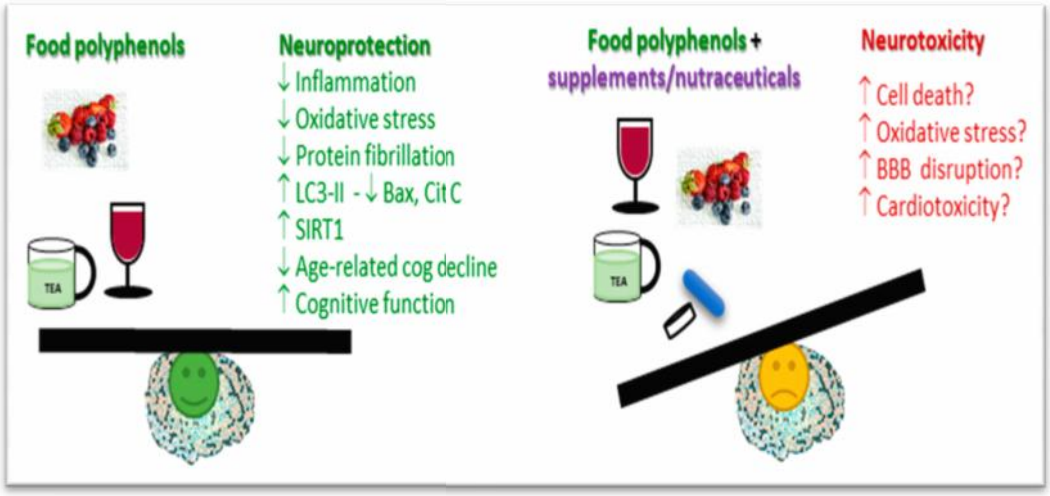

Figure 2:Summary of Polyphenol neuroprotection and effects of polyphenol overconsumption (Rui Silva etal, 2020).

2. a) **Terpene** This class of phytochemicals are divided into Mono Terpenes (Ex. Geraniol, limonene, pyrethroids, myrcene), Sesquiterpenes (Costunolides), Diterpenes (Abietic acid, cafestol, gibberellins), Triterpenes (Azadirachtin, phytoecdysones) and Polyterpenes(Tetraterpenes)

b) **Terpenoid** class of phytochemicals are divided into Carotenoids (β-carotene, lycopene, phytoene), Xanthophylls (Lutein, zeaxanthin), Triterpenoid (Saponins, ursolic acid), Steroids (β-sitosterol, campesterol)

Terpenes and Terpenoids are the bioactive compounds found in essential oils which are extracted from different parts of the plants. They have a several

biological activities like anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic. They also have food preservative properties that are considered as a real potential application in food industry. (Ayu Masyita etal, 2022).

3. Nitrogen containing phytochemicals are divided into Alkaloids (Nicotine, morphine, caffeine, theobromine), Cyanogenic glucosides, Nonprotein amino acids

Figure 3: Alkaloids (*Aristotelia chilensis*)

(Image Courtesy: https://tse2.mm.bing.net/th?id=OIP.V1yumJ2tbd_7C8aReX5xLgHaHa&pid=Api&P=0&h=220)

4. Sulphur containing phytochemicals are divided into Allicin, alliin, piperine Glutathione, phytoalexins

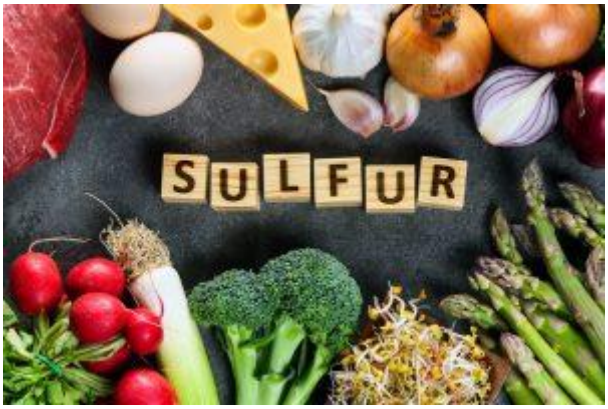

Figure 4: Sulphur containing phytochemicals

(Image Courtesy; https://optimsm.com/wp-content/uploads/sulfur-300x200.jpg)

Sources

They are present in herbs, shrubs and trees.They get stored up in different concentrations in parts of the plant such as stem, bark, leaves, roots, fruit, flowers, and seeds. Living organisms like fungi can also synthesize some phytochemicals, method of synthesis might differ. Moreover, foods too contain phytochemicals.It is possible to get more phytochemicals by consuming a minimum of 5 to 9 servings of different kinds of fruits and vegetables.

Some of the phytochemicals found in food are listed below

- **Allicin** is phytochemical initially found in garlic and onions, which expels certain toxicants from viruses and bacteria.
- Blue and red fruits like blueberries and raspberries and vegetables contain **Anthocyanins.** They prevent blood clots, protect against heart disease and tumours, help to decrease the aging process and oppose allergies and inflammation.
- Citrus fruitscontain**Bioflavonoids.**
- **Carotenoids** are presentin green, yellow, and orange-coloured fruits and vegetables
- Green tea, wine, kale, onions, beans, and apples contain **Flavonoids.**

- **Indoles** are found in kale, cabbage, broccoli, turnips, and Brussels sprouts. Indoles containsulphur and activate agents that act against destroying chemicals that cause cancer.
- Soybean and its products contain **Isoflavones**
- **Lignins** are present in whole grains and also in flax seeds.
- Greenvegetables also contain **Lutein.** It inhibits cataracts and macular degeneration. It also decreases the possibility of breast cancer and heart-related disorders.
- Tomatoes contain **Lycopene,** hence consuming it, will decrease heart attacks, cancer, etc.
- **Phenolic compounds** are present in fruits, oilseeds, and cereals. Itis highly powerful and is examined for different health benefits like protecting against heart disease and tumours, slowing the aging process, allergies, blood clots, and fighting inflammation.

Phytochemicals are also present in brightly coloured fruits like berries. They shield plants against fungi, bacteria, and viruses.

Foods having a high content of phytochemicals

Phytochemicals are found only in food and are not present in supplements. The following food contains a high number of phytochemicals.

Berries, Turnips, Soynuts, Carrots, Olives, Spinach, Tomatoes, Cantaloupe, Lentils, Apricots, Garlic, Cabbage, Onions, Apples, Kale, Bok choy, Apples, Brussels sprouts, Soybeans, Green tea, red wine, and Seeds.

Role of Phytochemicals in Living Organisms

Phytochemicals include compounds classified as essential nutrients in their category, having important biological roles.Phytochemicals are often considered non-digestible dietary complexes. As per the literature available, it has been found that they have a lot of benefits in combating deadly diseases like cancer and heart problems (Halliwell et al, 2007).Phytochemicals perform numerous roles in living organisms but the mechanism has not been fully understood. However, some of the other functional aspects of phytochemicals include their role as follows.

1. Phytochemicals prevent oxidative destruction of significant biomolecules such as proteins, nucleic acids, lipids, etc. Thus, they play the role of antioxidants.

2. Phytochemicals also act as antifungal, antibacterial, and other antimicrobial agents.

3. They help in stimulation of the immune system

4. They modify the detoxifying enzymes.

5. They have a functional role as anti-inflammatory agents.

6. They reduce Platelet aggregation.

7. They play an important role in autoimmune stimulation

8. They have an important role as Anti-cancer agents

Role of Phytochemicals as Anti-CancerAgents

(https://stanfordhealthcare.org/medical-clinics/cancer-nutrition-services/reducing-cancer risk/phytochemicals.html)

Consuming large amounts of brightly coloured fruits and vegetables (yellow, orange, red, green, white, blue, purple), whole grains, cereals and beans containing phytochemicals may reduce the risk of developing certain cancers. The action of phytochemicals varies by colour and type of food. They may act as antioxidants or nutrient protectors, or prevent the formation of carcinogens (cancer-causing agents).

Available literature, shows that few of the Phytochemicals inhibit the growth of cancerous cells. According to some scientists, cancer can be reduced by consuming plant food containing specific phytochemicals. Researchers have proved that few phytochemicals may inhibit the growth of carcinogens. But in-depth research is required to find exactly which phytochemicals play the role of anticancer agents and also their sources must be explored.

Few Scientists have predicted that the phytochemicals such as beta carotene and other carotenoids, found in fruits and vegetables, isothiocyanates (found in the cabbage family), polyphenols in tea, resveratrol in red wine are beneficial phytochemicals.The above-mentioned phytochemicals are present in our daily diet.Ex: More than 100 phytochemicals have been found in carrot. Approximately 4000 phytochemicals have been discovered, but a study of only 150 phytochemicals has beendone hence, an exhaustivestudy has to be done to identify the cancer-preventing phytochemicals.

Flavonoids are an important type of phytochemicals that have been found in large amounts in fruits, vegetables, and grains. Some of the flavonoids found in Soybeans and chickpeas may act like the hormone estrogen, that

might affect breast cancer. Such flavonoids are called phytoestrogens, though they have mild estrogen-like activity. When such a mild estrogen-like phytochemical substitutes natural estrogen, then it acts as an antiestrogen and henceworks against breast cancer.

Phytochemicals also play the role of antioxidants. They protect our body cells from free radicals formed during normal metabolic reactions. The sources of free radicals are pollution, cigarette smoke, radiation, herbicides, etc. Free radicals affect the genes leading to abnormal growth of cancerous cells, thus finally leading to cancer.

Phytochemicals play the role of anthocyanins which give a dark colour to fruits like grapes. It has been found from the research data, that these anthocyanins have antitumor and anti-inflammatory properties.

Phytochemicals in the form of sulphides enhance immunity. Other functions include anthelminthic, antimalarial, antidiarrheal, antiatherosclerosis, and antihypertension. They are also used in curingcold, cough, tooth pain, sore throat, stomach upset, etc.(Chukwuebuka egbuna et al, 2018).

	Phytochemical present	**Source**	**Application in various types of Cancer**
1.	5-Fluorouracil	*Withania somnifera*	Human cervical cancer cell (Yadav etal, 2010)
2.	Vindesine	*Catharanthus roseus*	Leukemias, testicular, breast and lung cancer (Cragg etal, 2005)
3.	Vincristine	*Catharanthus roseus*	Lymphocytic leukemia (Cragg etal, 2005)
4.	Vinblastine	*Catharanthus roseus*	Lymphocytic leukemia (Cragg etal, 2005)
5.	Colchicine	Colchicum autumnale	Multiple solid tumors (Atkinson etal, 2010)
6.	Larotaxel	*Taxus baccata*	Breast, bladder, and pancreatic cancer (Dieras et al, 2008)
7.	Cabazitaxel	*Taxus baccata*	Prostate cancer (De Bono etal, 2010)
8.	Paclitaxel	*Taxus brevifolia*	Breast and ovarian cancer (Cragg etal, 2005)
9.	Bullatacin	*Annona*	Liver cancer (V. Biba etal, 2013)

		squamosa	
10	Bryophyllin A	*Bryophyllum pinnatum*	Cervical cancer (S. Mahata etal, 2012)
11	Harmine	*Peganum harmala*	Breast cancer (I.Ayoob etal, 2017)
12	Artemisinin	*Artemisia annua*	Liver, breast, and pancreatic cancer (T. Efferth etal, 2017)
13	Tannins	*Debregeasia saeneb*	Internal tumors (A. Tariq etal, 2017)
14	(Theabrownin	*Camellia sinensis*	Lung cancer (F. Wu etal, 2016)
15	Solamargine	*Solanum nigrum*	Breast, liver, lung, and skin cancer (S. S. Al Sinanietal, 2016)
16	Psoralidin	*Psoralea corylifolia*	Stomach and prostate cancer (P. Pahari, etal, 2016)
17	Xanthatin	*Xanthium strumarium*	Lymphocytic leukaemia and liver cancer (S. Sharad etal, 2016)
18	(Thymoquinone	*Nigella sativa*	Colon, prostate, breast, and pancreas cancer (L.-Y. Tu etal, 2016)
19	Kaempferol galactoside	*galactoside Bauhinia variegata*	Breast, lung, and liver cancer (L.-Y. Tu etal, 2016)
20	Withaferin A, D	*Withania somnifera*	Breast, cervix, prostate, and colon cancer (I.-C. Lee etal, 2016)
21	Ginger	*Zingiber officinale*	Ovary, cervix, colon, liver, and urinary caner 9 N. Rastogi etal, 2015)
22	Silibinin	*Sylibum marianum*	Lung, liver, skin, colon, and prostate cancer (C.-C. Tsai etal, 2015)
23	Luteolin	*Capsicum annuum*	Colorectal cancer (N. H. A. Osman etal , 2015)
24	Colchicine	*Colchicum autumnale*	Hodgkin's lymphoma, chronic granulocytic leukemia (X. Lin etal, 2015)
25	Skimmianine	*Aegle marmelos*	Liver cancer (M. Mukhija, 2015)

26	Boswellic acid	*Boswellia serrata*	Prostate cancer (P. Garg etal, 2015)
27	Silymarin	*Sylibum marianum*	Colorectal cancer and colon cancer (K. Ramasamy etal, 2008)
28	Curcumin	*Curcuma longa*	Colon adenocarcinoma (N. G. Vallianou etal, 2015)
29	Podophyllotoxin	*Podophyllum peltatum*	Non-small-cell lung carcinoma (J. Y. Shin etal, 2015)
30	Andrographolide	*Andrographis paniculata*	Colon cancer (N. H. A. Osman etal, 2015)
31	Podophyllotoxin	*Podophyllum hexandrum*	Breast, ovary, lung, liver, bladder, and testis cancer (Y.-Q. Liu etal, 2015)
32	Betulinic acid	*Betula utilis*	Melanomas (S. K. Kr´ol etal, 2015)
33	Panaxadiol Panax	*Ginseng*	Human colon cancer (C.-Z. Wang etal, 2015)
34	Gossypol	*Gossypium hirsutum*	Colorectal cancer (L. Lan etal, 2015)
35	Chrysin Passiflora	*Caerulea*	Colorectal cancer (I. E. Leon etal, 2015)
36	Plumbagin	*Plumbago zeylanica*	Liver, fibrosarcoma, leukaemia, and breast cancer (C.H. Yanetal etal, 2015)
37	6-Shogaol	*Zingiber officinale*	Ovary cancer (A. Ghasemzadeh etal, 2015)
38	Curcumin	*Curcuma longa*	Breast, lung, colon, prostate esophagus, liver, and skin cancer (D. Perrone etal, 2015)
39	Ursolic acid	*Oldenlandia diffusa*	Lungs, ovary, uterus, stomach, liver, colon, rectum, and brain cancer (L. Wozniak etal, 2015)
40	Isoliquiritigenin	*Glycyrrhiza uralensis*	Human lung cancer (S. K. Jung etal,2014)
41	Punarnavine	*Boerrhavia diffusa*	Malignant melanoma cancer (S. Mishraetal, 2014)
42	Procyanidins	*Vitis vinifera*	Human colon cancer (K. Y. Cheah etal, 2014)
43	Resveratrol	*Polygonum*	Colorectal, skin, and liver cancer (I.

		cuspidatum	Ali etal, 2014)
44	Damnacanthal	*Morinda citrifolia*	Lung cancer, sarcomas (M. Y. A. Bhat etal, 2014)
45	Gossypol	*Gossypium hirsutum*	Breast, stomach, liver, prostate, and bladder cancer (Y. Zhan etal, 2009)
46	Niazinine A	*Moringa oliefera*	Blood cancer (M. M. Khalafalla etal, 2011)
47	Amooranin	*Amoora rohituka*	Lymphocytic leukemia (L. L. Chan etal, 2011)
48	Betulinic acid	*Ziziphus rugosa*	Cytotoxicity against human melanoma cells (U. Shahetal, 2013)
49	Asiatic acid	*Centella asiatica*	Melanoma, glioblastoma, breast cancer (M. Heidari etal, 2012)
50	Gallic acid	*Leea indica*	Ehrlich ascites carcinoma (M. Raihan etal, 2012)
51	Combretastatins	*Combretum caffrum*	Colon, leukemia, and lung cancer (C. Lauritano etal, 2016)
52	Lycopene	*Solanum lycopersicum*	Prostate and colon cancer (E.-R. Hahm etal, 2011)
53	Plumbagin	*Plumbago zeylanica*	Blood and skin cancer (R. Checker etal, 2010)
54	Cannabinoid	*Cannabis sativa*	Lung, pancreas, breast, prostate, and colorectal cancer (G. Appendino etal, 2011)
55	Silymarin	*Sylibum marianum*	Colorectal cancer (V. Colombo etal, 2011)
56	Tylophorine	*Tylophora indica*	Breast cancer (C. Lauritano etal, 2016)
57	Saffron	*Saffron crocus*	Liver, lung cancer and pancreatic cancer (F. Ververidis etal, 2007)
58	nab-paclitaxel	*Taxus brevifolia*	Ovarian and breast cancer (M. Caruso etal, 2000)
59	Cyanidin	*Vitis vinifera*	Colon cancer 9 D. Y. Lim etal, 2009)
60	Actein	*Actaea racemosa*	Liver and breast cancer (L. S. Einbond etal, 2009)
61	Betulinic acid	*Betula Sp.*	Human melanoma xenografts and leukemia (Cragg etal, 2005)

62	Allin	*Allium sativum*	Carcinoma of human mammary gland (M. Sabnis etal, 2006)
63	Neferine	*Nelumbo nucifera*	Liver cancer (J. S. Yoon etal, 2013)
64	Calcaelin	*Calvatia caelata*	Breast and spleen cancer cells (T. B. Ng etal, 2003)
65	Lentinan	*Lentinus edodes*	Sarcoma-180 in mice (P. H. K. Ngai etal, 2004)
66	Schizophyllan	*Schizophyllum commune*	Head and neck cancer (J. Smith etal, 2002)
67	Apigenin	*Matricaria chamomilla*	Colorectal cancer (J. K. Srivastava etal, 2007)
68	Vitex	*Vitex agnus-castu*	Human uterine, ovarian, cervical, and breast cancer (M. Imai etal, 2009)

Table 1 -Novel phytochemicals, their source and application in different types of cancer(https://doi.org/10.1155/2020/8602879)

Influence of Food processing on Phytochemicals.

There are several food processing techniques. The simplest one is cooking. By this technique, the phytochemicals present in fresh plant foods are destroyed due to heat. They undergo thermal decomposition (Palermo et al, 2014).Some mechanical food processing techniques release carotenoids and other phytochemicals from the plant food, thereby enhancing dietary intake. A reverse phenomenon is observed in the case of a carotenoid called Lycopene found in tomatoes. In this case,in the cooking process, the amount of lycopene is increased (Dewanto et al 2002, Hotz et al, 2007).Sometimes food processing is done to remove toxins.Example unprocessed Cassava has toxic ingredient. Hence, Cassava is processed by different techniques like soaking, cooking, or fermenting.Cassava flour when processed by traditional methods resulted in higher moisture and higher hydrogen cyanide content, and poorer quality as compared to analtered laboratory process (Inyang, C.U. etal, 2006). Processed potato products were found to contain high fat and sodium and also a significant source of carbohydrate, in the form of starch (Amber etal, 2018)

Different traditional food processing techniques have special effect on natural antioxidants present in fruits and vegetables. Thermal processing techniques such as roasting, boiling, blanching, drying, and pasteurization maydamage bioactive ingredients. Harmful effects of heat are more noticeable

in coloured foods which are prepared using colouredfruits or vegetables that contain antioxidant pigments such as Anthocyanins. But some research studiesdepicted that thermal damage to bioactive ingredientswill not decrease the overall antioxidant property of food products. Some of the nonthermal techniques (Ultra Violet,pulsed electric field, High-pressure (HP) processing, irradiation and combined non thermal methods) do not significantly deteriorate important phytochemicals and in some cases may improve their activity. Moreover, these techniques provide best option to thermal processing, which is commonly used for microbial and enzyme inactivation in foods despite of its largely negative effects on bioactive compounds and undesirable changes to the nutritional characteristics of foods. Hence, optimal processing techniques must be selected to safeguard the antioxidant property of fresh fruits and vegetables and their products in order to achieve not only the desired purposes of a food processing technique but also preserve the action and quality of natural health-promoting constituents(Al-juhaimi, etal, 2018).

Phytochemicals as antimicrobial agents

There is a huge data confirming that a number of phytochemicals have possibility to become useful antimicrobial agents that could be used in preventative or treatment therapies against microbial and viral diseases. Though, there are some promising effects IN VIVO to inhibit growth of pathogenic microorganisms without affecting useful bacteria in the gastrointestinal tracts, yet some in depth studies are required for the safety and efficiency of these phytochemicals to find whether they could offer therapeutic benefits over traditional treatments. Apart from this, it has been found from the literature that the coupling of some antimicrobial drugs with phytochemicals may act as better antimicrobial agents than antimicrobial drugs alone.Nowadays it is noted that most of the bacterial and microbial strains are unaffected by several drugs and antibiotics hence there is an increasing demand for revival of research interest in new antimicrobial agents from natural sources for therapeutic and preventive purposes against microbial diseases.

	Phytochemical type	Functional aspect
1.	Phenolic compounds	Antimicrobial agent (Patra, A. K., 2012).
2.	Phenols and phenolic acids Ex. Synaptic acid, vanillic acid, and caffeic acid	Antibacterial, antiviral and antifungal (Patra, A. K., 2012). Antimicrobial agent (Ganan et al. 2009, Patra, A. K., 2012).
3.	Flavonoids	antiviral activity against a wide range of viruses such as HSV, HIV, coxsackie B virus, coronavirus, cytomegalovirus, poliomyelitis virus, rhinovirus, rotavirus, poliovirus, sindbis virus, and rabies virus. (De Bruyne et al. 1999; Evers et al. 2005; Nowakowska 2007, (Patra, A. K., 2012).
4.	Polyphenols Ex. Polyphenols from berry extracts	antibacterial, antiviral and antifungal activities (Patra, A. K., 2012). Antimicrobial agent (Rauha et al. ,2000, Patra, A. K., 2012).
5.	Naphthoquinones Ex. Lapachol, plumbagone, juglone and lawsone	antimicrobial effects against various pathogenic bacteria and fungi (Patra, A. K., 2012).
6.	Alkaloids (STRYCHNOS POTATORUM L.f. (Loganiaceae) seeds)	Antimicrobial properties against some pathogenic Gram-positive, Gram-negative and acid-fast bacteria and fungi (Mallikharjuna and Seetharam 2009, Patra, A. K., 2012).
7.	Allicin (Garlic and onion)	Antimicrobial agents (Ankri and Mirelman 1999, Patra, A. K., 2012).
8.	Glucosinolates (sulphur-containing phytochemicals)	Antibacterial and antifungal properties (Fahey et al. 2001, Patra, A. K., 2012).
9.	Iridoids	Antiviral Geng et al. 2009a, b, 2011, Patra, A. K., 2012).
10.	Secoiridoids	Antiviral Geng et al. 2009a, b, 2011, Patra, A. K., 2012).
11	saponins	Antimicrobial activities (Sen et al. 1998; Avato et al. 2006, , Patra, A. K., 2012).
12	limonoid compounds Ex.	Antimicrobial activities (Siddiqui et

	mahmoodin, azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin and 17-hydroxyazadiradione (Extracted from AZADIRACHTA INDICA)	al. 1992; Govindachari et al. 2000; Atawodi and Atawodi 2009, , Patra, A. K., 2012).
13	Anthranoids	Antimicrobial (Babu et al. 2003, Patra, A. K., 2012).
14	Anthranoids (rhizome of RHEUM EMODI)	Antibacterial and antifungal (Babu et al. 2003, Patra, A. K., 2012).

Table 2 – Phytochemicals and their functional aspect
https://link.springer.com/chapter/10.1007/978-94-007-3926-0_1#citeas

Conclusion

Recent studies reveal that some phytochemicals even have anticancer, antimicrobial and antiviral properties or help in the reduction of cancer risks.Theyalso highlight that, though phytochemicals help in reducing cancer risk but are inefficient in complete eradication of cancer. Hence efforts must be taken in the direction of inventing novel phytochemicals to eradicate the deadly disease like cancer. Food processing techniques must depend on the type of food processed, as in some cases, processing is required to remove phytotoxins whereas in some cases it increases the number of phytochemicals. In certain foods it destroys the phytochemicals.The vigilant selection of suitable food-handling techniques from farm to consumer for every product can ensure that the health-related benefits of specific phytochemicals are exploited.By knowingand experiencing the significance of Phytochemicals in our day today life,more awareness and research is required in this field.

References

Afendi, F.M., Okada, T., Yamazaki, M., Hirai-Morita A., Nakamura, Y., Nakamura, K., Ikeda, S., Takahashi,H., Altaf-Ul-Amin M., Darusman, L.K., Saito, K, and Kanaya S. 2012. KNApSAcK family databases: integrated metabolite-plant species databases for multifaceted plant research. Plant Cell Physiol. 53(2):e1. doi: 10.1093/pcp/pcr165. Epub 2011 Nov 28. PMID: 22123792.

Amin, M. Mir., Anuj, Kumar., and Abhishek, Goel., 2018, Phytochemical Analysis and Antioxidant Properties of the Various Extracts of *Catharanthus roseus*, Journal of Chemical and Pharmaceutical Research, 10(10): 22-31.

Al Sinani, S. S., Eltayeb, E. A., Coomber, B. L,and Adham, S. A., 2016. Solamargine triggers cellular necrosis BioMed Research International 5 selectively in different types of human melanoma cancer cells through extrinsic lysosomal mitochondrial death pathway," Cancer Cell International, 16, (11).

Al-juhaimi, F., Ghafoor, K., Ozcan, M. M., Jaharul M. H., Elfadil E., Babiker, Jinap S., Sahena F., Sharifudin, M.S., 2018. Effect of various food processing and handling methods on preservation of natural oxidants in fruits and vegetables. Journal of Food Science and Technology, 55: 3872-3880. http://doi.org/10.1007/s13197-018-3370-0

Ali I., and Braun, D. P., 2014. "Resveratrol enhances mitomycin C-mediated suppression of human colorectal cancer cell proliferation by up-regulation of p21WAF1/CIP1," Anticancer Research, 34 (34): 5439–5446.

Amber, N., Furrer, Mohd., Cheginin, Mario., Ferruzzi, G., 2018. Impact of potato processing on nutrients, phytochemicals, and human health, 58(1):146-168, doi:10.1080/10408398.2016.1139542. Epub 2017 Jul 11.

Antifungal activity of some tetranortriterpenoids. Fitoterapia. 71:317–320.

Angapazham, R. L., Sharad, S., and Maheshwari, R. K., 2016. "Phytochemicals in wound healing," Advances in Wound Care, 5, (5): 230–241.

Ankri, S., Mirelman, D.,1999. Antimicrobial properties of allicin from garlic. Microbes and Infection, 1:125–129.

Appendino, G., Chianese, G., Taglialatela-Scafati, O.,2011. Cannabinoids: occurrence and medicinal chemistry. Current Medicinal Chemistry, 18(7):1085-99. doi: 10.2174/092986711794940888. PMID: 21254969.

Atawodi, S., Atawodi, J., 2009. AZADIRACHTA INDICA *(*neem): a plant of multiple biological and pharmacological activities. Phytochemistry Reviews, 8: 601–620.

Atkinson, J. M., Falconer, R A., Edwards D. R., Pennington, C.J., Catherine, S.S, Steven, D. Shnyder., Michael, C. Bibby, Laurence, H. Patterson., Paul, M., Loadman, and Jason, H. Gill.,2010. Development of a novel tumor-targeted vascular disrupting agent activated by membrane-type matrix metalloproteinases," Cancer Research, 70 (17): 6902– 6912.

Avato, P., Bucci, R., Tava, A., Vitali, C., Rosato, A., Bialy, Z., Jurzysta, M., 2006 Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. Phytotherapy Research,20: 454–457.

Ayu, Masyita., Reka, Mustika., Sari, Ayun Dwi Astuti., Budiman, Yasir., Nur Rahma, Rumata .,Talha Bin, Emran., Firzan, Nainu., Jesus Simal, Gandara., 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives, Food Chemistry: X, 13 :100217, https://doi.org/10.1016/j.fochx.2022.100217.

Ayoob, I., Hazari, Y. M., Lone S. H., Mohammad, Shakeel-u-Rehman., Khuroo, A., Fazili, Khalid M., and Bhat, Khursheed A., 2017. Phytochemical and cytotoxic evaluation of *Peganum harmala*: structure activity relationship studies of harmine," ChemistrySelect, 2, (10): 2965–2968.

Babu, K.S., Srinivas, P.V., Praveen, B., Kishore, K.S., Murty, U.S. and Rao, J.M., 2003. Antimicrobial constituents from the rhizomes of RHEUM EMODI, Photochemistry. 62: 203–207.

Bhat, M. Y. A., Omar, A. R., Subramani T., 2014. Damnacanthal is a potent inducer of apoptosis with anticancer activity by stimulating p53 and p21 genes in MCF-7 breast cancer cells, Oncology Letters, 7 (5); 1479–1484.

Biba, V., Jeba, M. P. W., and Remani, P., 2013. Differential effects of *Annona squamosa* seed extracts: antioxidant, antibacterial, cytotoxic and apoptotic study, International Journal of Pharma and Bio Sciences, 4: 899–907.

Caruso, M.,Colombo. A., Fedeli L., Angelo, P., 2000. Isolation of endophytic fungi and actinomycetes taxane producers, Annals of Microbiology, 50: 3–14.

Chan, L. L., George, S., Ahmad I., Saujanya L.G.,Atiya, A., Briyan, T. C., and Kenneth, l. W., 2011. Cytotoxicity effects of amoora rohituka and chittagonga on breast and pancreatic cancer cells, Evidence-Based Complementary and Alternative Medicine, Article ID 860605: 1-8.

Cheah, K. Y., Howarth, G. S., Bindon, K. A., Kennedy, J. A., and Bastian, S. E., 2014. Low molecular weight procyanidins from grape seeds enhance the impact of 5-Fluorouracil chemotherapy on Caco-2 human colon cancer cells,PLoS One, 9.

Checker, R., Sharma, D., Sandur S. K., Subramanyam, G., Krishnan, S., Poduval T. B., and Sainis, K. B., 2010. Plumbagin inhibits proliferative and inflammatory responses of T cells independent of ROS generation but by modulating intracellular thiols, Journal of Cellular Biochemistry, 110 (5): 1082–1093.

Chukwuebuka egbuna, Jonathan. C., Ifemeje Toske, Kryeziu., Minakshi, Mukherjee., Hameed, shah., Narasimha rao, G.M., Aurence, john., Francis, j., gido and Habibu, tijjani., 2018. Introduction to Phytochemistry, DOI: 10.13140/RG.2.2.18812.23687.

Cragg, G. M., and Newman, D. J., 2005. Plants as a source of anticancer agents, Journal of Ethnopharmacology, 100 (1-2): 72–79.

Dewanto, V., Wu, X., Adom, K.K., and Liu, R.H., 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry. 50 (10): 3010-3040. Doi:10.1021/jf0115589. PMID 11982434.

Di´eras, V., Limentani, S., Romieu G., Hulin, M. T., Lortholary, A., Kaufman, P., Girre, V., and Besenval, M. Valero, V., 2008. Phase II multicenter study of larotaxel (XRP9881), a novel taxoid, in patients with metastatic breast cancer who previously received taxane-based therapy, Annals of Oncology, 19 (7):1255–1260.

De Bono,J. S., Oudard, S., Ozguroglu M., Hansen S., Macheils, J. P., Kocak, I., and Gravis, G., Bodrogi, I., 2010. Prednisone plus cabazitaxel or mitoxantrone for metastatic castrationresistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial,; e Lancet, 376, (9747): 1147–1154.

De Bruyne, T., Pieters, L., Witvrouw, M., De Clercq, E., Vanden Berghe, D., Vlietinck A.J. 1999. Biological evaluation of proanthocyanidin dimers and related polyphenols. Journal ofNatural Products, 62: 954–958.

Efferth, T.,2017. From ancient herb to modern drug: artemisia annua and artemisinin for cancer therapy, Seminars in Cancer Biology, 46: 65–83.

Einbond, L. S., Soffritti, M., and Esposti, D. D., 2009. Actein activates stress- and statin-associated responses and is bioavailable in Sprague-Dawley rats, Fundamental & Clinical Pharmacology, 23 (3): 311–321.

Evers, D. L., Chao C.F., Wang, X., Zhang, Z., Huong, S.M., and Huang, E.S., 2005. Human cytomegalovirus-inhibitory flavonoids: studies on antiviral activity and mechanism of action. Antiviral Research, 68: 124–134.

Fabricant, D. S., and Farnsworth, N. R., 2001. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environmental Health Perspectives, 109: 69–75.

Fahey, J.W., Zalcmann, A.T., and Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, 56: 5–51.

Ganan, M., Martínez-Rodríguez A.J., Carrascos,a A.V., 2009. Antimicrobial activity of phenolic compounds of wine against *Campylobacter jejuni*. Food Control. 20: 739–742.

Geng, C.A., Wang, L.J., Zhang, X.M., Ma, Y.B., Huang, X.Y., Luo, J., Guo, R.H., Zhou, J., Shen, Y., Zuo, A.X., Jiang, Z.Y., and Chen, J.J., 2011 Anti-hepatitis B virus active lactones from the traditional Chinese herb: SWERTIA MILEENSIS. Chemistry 17: 3893–3903.

Ghasemzadeh, A., Jaafar, H. Z., and Rahmat, A., 2015. Optimization protocol for the extraction of 6-gingerol and 6-shogaol from *Zingiber officinale* var. rubrum (eilade and improving antioxidant and anticancer activity using response surface methodology, BMC Complementary and Alternative Medicine, 15: 258.

Govindachari, T.R.,Suresh,G., Gopalakrishnan, G., Masilamani, S., Banumathi, B., 2000.

Garg P., and Deep, A., 2015. Anti-cancer potential of boswellic acid: a mini, Journal for Drugs and Medicines, 7: 18–27.

Hahm, E.R., Moura, M. B.,Kelley, E. E., Van Houten, B., Shiva, S., and Singh, S. V., 2011. Withaferin A-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species,PloS One, 6.

Halliwell, B., 2007. Dietary polyphenols: Good, bad, or indifferent for your health?. Cardiovascular Research. 73 (2): 341–7. doi: 10.1016/j.cardiores.2006.10.004. PMID 17141749.

Harborne Jeffrey, B., Baxter Herbert, and Moss., Gerard P. eds.,1999. General Introduction. Phytochemical dictionary a handbook of bioactive compounds from plants (2nd ed.). London. Taylor & Francis. p. vii. ISBN 9780203483756

Heidari, M., Heidari-Vala, H., Sadeghi, M. R., and Akhondi, M. M., 2012. Inductive effects of *Centella asiatica* on rat spermatogenic cell apoptosis in vivo, Journal of Natural Medicines, 66 (2): 271–278.

Hotz, C., and Gibson, R. S., 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. The Journal of Nutrition. 137 (4):1097–100. doi:10.1093/jn/137.4.1097. PMID 17374686

Imai, M., Kikuchi, H., Denda, T., Ohyama, K., Hirobe, C., and Toyoda, H., 2009. Cytotoxic effects of flavonoids against a human colon cancer derived cell line, COLO 201: a potential natural anti-cancer substance,Cancer Letters, 276 (1): 74–80.

Inyang, C.U., Tsav-Wua, J.A., and Akpapunam, M.A., 2006. Impact of traditional processing methods on some physico chemical and sensory qualities of fermented casava flour "Kpor umilin, African Journal of Biotechnology 5 (20): 1985-1988, http://www.academicjournals.org/AJB

Jung, S. K., Lee, M.H., Limentani, S., Romieu G., Hulin, M. T., Girre, V., 2014.Isoliquiritigenin Induces apoptosis and inhibits xenograft tumor growth of human lung cancer cells by targeting both wild type and L858R/T790M mutant EGFR, Journal of Biological Chemistry, 289(52): 35839–35848.

Khalafalla, M. M., Dafalla, H. M., Nassrallah, A., AboulEnein, K. M., El-Shemy, H. A., and Abdellatef, E., 2011. Dedifferentiation of leaf explants and antileukemia activity of an ethanolic extract of cell cultures of *Moringa oleifera*, African Journal of Biotechnology, 10: 2746–2750.

Kr´ol, S. K., Kiełbus, M., Rivero-M¨uller, A., and Stepulak, A., 2015. Comprehensive review on betulin as a potent anticancer agent,BioMed Research International, 1- 11.

Lan, L., Appelman, C. Smith A. R., Larsen S., Marquez R. T., Liu, H., 2015. Natural product (-)-gossypol inhibits colon cancer cell growth by targeting RNA-binding protein Musashi-1, Molecular Oncology, 9 (7): 1406–1420.

Lauritano, C., Andersen, J. H., Hansen, E.,Laura, E., Francesco E., 2016. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities, Frontiers in Marine Science, 3: 68.

Lee I. C., and Choi, B., Withaferin- A., 2016. A natural anticancer agent with pleitropic mechanisms of action, International Journal of Molecular Sciences, 17 (3):290.

Lin, X., Peng, Z., and Su, C., 2015. Potential anti-cancer activities and mechanisms of costunolide and dehydrocostuslactone, International Journal of Molecular Sciences, 16 (12): 10888–10906.

Lim,D. Y.,andPark, J. H. Y., 2009. Induction of p53 contributes to apoptosis of HCT-116 human colon cancer cells induced by the dietary compound fisetin, American Journal of Physiology-Gastrointestinal and Liver Physiology, 296 (5): G1060–G1068.

Leon, I. E., Cadavid-Vargas, J. F.,Tiscornia I., 2015. Oxi- ´ dovanadium (IV) complexes with chrysin and silibinin: anticancer activity and mechanisms of action in a human colon adenocarcinoma model, Journal of Biological Inorganic Chemistry, 20 (7): 1175–1191.

Liu, Y.Q.,Tian, J., Qian K., Keduo, Q., Xiao Bo. Z., Xuan T., Lee K. H., 2015. Recent progress on C-4- modified podophyllotoxin analogs as potent antitumor agents, Medicinal Research Reviews, 35 (1): 1–62.

Mahata, S., Maru, S., Shukla S., Pandey, A., Mugesh, G., and Das, B. C., 2012. Anticancer property of *Bryophyllum pinnata* (Lam.) Oken. leaf on human cervical cancer cells, BMC Complementary and Alternative Medicine, 12, (15).

Mallikharjuna, P.B., and Seetharam, Y.N., 2009. In vitro antimicrobial screening of alkaloid fractions from STRYCHNOS POTATORUM, European Journal of Advanced Chemistry Research, 6:1200–1204.

Mishra, S., Aeri, V., Gaur, P. K., and Jachak, S. M., 2014. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa*Linn," BioMed Research International,1-19.

Molyneux, R.J., Lee, S.T., Gardner, D.R., Panter, K.E., James, L.F., 2007. Phytochemicals: the good, the bad and the ugly?. Phytochemistry,68 (22–24): 2973–2985.

Mukhija, M., Singh. M. P., Dhar, K. L., and Kalia, A. N., 2015. Cytotoxic and antioxidant activity of stem bark and its flavonoid constituents, Journal of Pharmacognosy and Phytochemistry, 4: 86.

Ng, T. B., Lam, Y. W., and Wang, H., 2003. "Calcaelin, a new protein with translation-inhibiting, antiproliferative and antimitogenic activities from the mosaic puffball Mushroom*Calvatia caelata*," Planta Medica, 69 (3):212–217.

Ngai P. H. K., and Ng T. B., 2004. "A ribonuclease with antimicrobial, antimitogenic and antiproliferative activities from the edible mushroom *Pleurotus sajor-caju*," Peptides, 25 (1): 11–17.

Osman, N. H. A., Said, U. Z., El-Waseef, A. M., and Ahmed, E. S. A., 2015. Luteolin supplementation adjacent to aspirin treatment reduced dimethylhydrazine-induced experimental colon carcinogenesis in rats,Tumor Biology, 36 (2): 1179–1190.

Patra, A. K., 2012. An Overview of Antimicrobial Properties of Different Classes of Phytochemicals. In: Patra, A. (eds) Dietary Phytochemicals and Microbes. Springer, Dordrecht: 1–32, http://doi: 10.1007/978-94-007-3926-0_1. PMCID: PMC7121617.

Pahari, P. U. P., Saikia, Das, T. P., Damodaran, C., and Rohr, J., 2016. "Synthesis of psoralidin derivatives and their anticancer activity: first synthesis of Lespeflorin I1," Tetrahedron, 72, (23): 3324 –3334.

Palermo, M., Pellegrini, N. Fogliano, V., 2014. The effect of cooking on the phytochemical content of vegetables. Journal of the Science of Food and Agriculture. 94 (6):1057–1070. doi:10.1002/jsfa.6478. HDL: 11381/2677278. PMID 24227349

Perrone, D., Ardito, F., Giannatempo G., Mario Dioguardi Giuseppe Troiano, Lucio Lo Russo , Alfredo De Lillo , Luigi Laino and Lorenzo Lo Muzio., 2015. Biological and therapeutic activities, and anticancer

properties of curcumin," Experimental and; erapeutic Medicine, 10 (5) :1615–1623.

Pitman, N. C. A., Jorgensen, P. M., 2002. Estimating the Size of the World's Threatened Flora. Brevia, 298: 989.

Ramasamy K., and Agarwal, R., 2008. Multitargeted therapy of cancer by silymarin,Cancer Letters, 269(2): 352–362.

Raihan, M., Tareq, S. M., Brishti, A., Alam, M., Haque, A., and Ali, M., 2012. Evaluation of antitumor activity of (Burm. F.) merr. extract against ehrlich ascites carcinoma (EAC) bearing mice, American Journal of Biomedical Sciences, 4.

Rastogi, N., Duggal, S., Singh S. K., Porwal, K., Srivatsava, V. K., Maurya, R., Bhatt, M. L. B., Mishra, D. P., 2015. Proteasome inhibition mediates p53 reactivation and anti-cancer activity of 6-gingerol in cervical cancer cells, Oncotarget, 6: 43310.

Rauha, J.P., Remes, S., Heinonen, M., Hopia, A.,Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., and Vuorela, P., 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology, 56: 3–12.

Rui, F. M., Silva. Lea, Pogacnik., 2020. Polyphenols from Food and Natural Products: Neuroprotection and Safety, Antioxidants, 9: 61,https://doi.org/10.3390/antiox9010061

Rutz, Adriano., Sorokina, Maria., Galgonek, Jakub., et al. 2022. The LOTUS initiative for open knowledge management in natural products research. eLife. 11: e70780. doi:10.7554/eLife.70780. PMC 9135406. PMID 35616633

Sabnis, M., 2006. Chemistry and Pharmacology of Ayurvedic Medicinal Plants, Chaukhambha Amarabharati Prakashan, Varanasi, India

Shah, U., Shah, R., Acharya, S., and Acharya, N., 2013. Novel anticancer agents from plant sources," Chinese Journal of Natural Medicines, 11 (1): 16–23.

Sen, S., Makkar, H.P.S., Muetzel, S., Becker, K., 1998. Effect of *Quillaja saponins* and *Yucca schidigera* plant extract on growth of *Escherichia coli*. Letters in Applied Microbiology, 27: 35–38.

Shin, J. Y., Hong, W. G., Cho J. H., et al. 2015. Podophyllotoxin acetate triggers anticancer effects against non-small cell lung cancer cells by promoting cell death via cell cycle arrest, ER stress and autophagy,International Journal of Oncology, 47 (4): 1257–1265.

Siddiqui, S., Faizi, S., Siddiqui, B.S., and Ghiasuddin, S.M., 1992. Constituents of *Azadirachta indica*: isolation and structure elucidation of a new antibacterial tetranortriterpenoid, mahmoodin, and a new protolimonoid, naheedin. Journal of Natural Products. 55: 303–310.

Simran, Arora., and Suvartan, Ranvir., 2020. Phytochemicals: Benefits, Concerns and Challenges, Book on Advancement in Functional Food Ingredients: chapter 11: 205–227.

Smith, J., Rowan, N., and Sullivan, R., 2002. Medicinal Mushrooms: therapeutic Properties and Current Medical Usage with Special Emphasis on Cancer Treatments, Cancer Research, London, UK.

Srivastava J. K., and Gupta, S., 2007. Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells,Journal of Agricultural and Food Chemistry, 55 (23): 9470–9478.

Tariq, A., Sadia,Sehrish Sadia, Kaiwen Pan, Ihteram Ullah, SakinaMussarat, Feng Sun, Olatunji Olusanya Abiodun, Altanzagas Batbaatar, Zilong Li, Dagang Song, Qinli Xiong, Riaz Ullah, Suliman Khan, Buddha Bahadur Basnet, Brawin Kumar, Rabiul Islam, Muhammad Adnan, 2017. A systematic review on ethnomedicines of anti-cancer plants, Phytotherapy Research, 31 (2): 202–264.

Tsai, C.C., Chuang, T.W., Chen L.J.,Niu H.S., Chung, K. M., Cheng, J. T., 2015. Increase in apoptosis by combination of metformin with silibinin in human colorectal cancer cells,World Journal of Gastroenterology, 21(14): 4169.

Tu, L.-Y., Pi, J., Jin, H., Cai, J.-Y., and Deng, S.P., 2016. Synthesis, characterization and anticancer activity of kaempferol-zinc (II) complex,Bioorganic and Medicinal Chemistry Letters, 26 (11): 2730–2734.

Ubezio, P., 2011. Chemotherapeutic activity of silymarin combined with doxorubicin or paclitaxel in sensitive and multidrug-resistant colon cancer cells,Cancer Chemotherapy and Pharmacology, 67 (2):369–379.

Vallianou, N. G., Evangelopoulos, A., Schizas, N., and Kazazis, C., 2015. Potential anticancer properties and mechanisms of action of curcumin, Anticancer Research, 35 (35): 645–651.

Veeresham, C., 2012. Natural Products Derived from Plants as a Source of Drugs. Journal of Advanced Pharmaceutical Technology and Research, 3(4): 200–201. DOI: 10.4103/2231–4040.104709

Ververidis, F., Trantas, E., Douglas, C., Vollmer, G., Kretzschmar, G., and Panopoulos, N., 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: chemical diversity, impacts on plant biology and human health, Biotechnology Journal, 2 (10): 1214–1234.

Vishnu, Balamurugan., Fathima, M. A., Sheerin, Sreenithi Velurajan., 2019. A guide to phytochemical analysis, International Journal of Advance Research and Innovative Ideas in Education, 5(1): 236-245.

Wang, C.Z., Zhang, Z., Wan J.Y., et al., 2015. Protopanaxadiol, an active ginseng metabolite, significantly enhances the effects of fluorouracil on colon cancer, Nutrients, 7, (2): 799–814.

Wozniak, L., Skapska, S., and Marszałek, K., 2015. Ursolic acid-a ´ pentacyclic triterpenoid with a wide spectrum of pharmacological activities, Molecules, 20: 20614–20641.

Wu, F., Zhou, L., Jin W., et al., 2016. Anti-proliferative and apoptosis-inducing effect of theabrownin against non-small cell lung adenocarcinoma A549 cells, Frontiers in Pharmacology, 7, 465.

Yadav, B., Bajaj, A., Saxena, M., 2010. In vitro anticancer activity of the root, stem and leaves of *Withania somnifera* against various human cancer cell lines, Indian Journal of Pharmaceutical Sciences, 72, (5): 659.

Yan, C. H., Li, F., and Ma, Y.C., 2015. Plumbagin shows anticancer activity in human osteosarcoma (MG-63) cells via the inhibition of S-Phase checkpoints and down-regulation of c-myc, International Journal of Clinical and Experimental Medicine, 8: 14432.

Yoon, J. S., Kim, H.M.,Yadunandam, A. K., Kim, N. H., Jung, H. A., Choi, J. S., Kim, C.Y., and Kim, G. D., 2013. Neferine isolated from *Nelumbo nucifera* enhances anti-cancer activities in Hep3B cells: molecular mechanisms of cell cycle arrest, ER stress induced apoptosis and anti-angiogenic response, Phytomedicine, 20 (11):1013–1022.

Zhan, Y., Jia, G., Wu, D., Xu, Y., and Xu, L., 2009. Design and synthesis of a gossypol derivative with improved antitumor activities,Archive der Pharmazie, 342 (4): 223– 229.

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Nano bio-pesticides in sustainable agriculture

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Introduction

Agriculture is practice or science of cultivating the crops, farming and textile for human needs. In the process of agriculture, we tend to find a better, easy and effective ways to get more yield. To avoid the crop destruction such as major abiotic conditions (droughts, floods, salinity and temperature variation) humans found different ways like rain water harvesting, irrigation, small dams and to maintain temperature, green house set is used. Biotic factors such as pests, animals, birds, insects and disease-causing pathogens affects more than abiotic factors. The controlling of these biotic factors is a hectic work for farmers. To avoid these pestsfarmers, use pesticides, insecticides etc. to avoid disease and to produce high yield crops genetically modified crops are now available. Using chemical fertilizers, insecticides and pesticides reduces the soil fertility and make soil chemically adulterated. To reduce the cause of damage to the nature people follow sustainable agriculture.

Sustainable agriculture is eco-friendly. It reduces the use of chemical fertilizers, pesticides and insecticides. There are many types in sustainable agriculture like organic farming, biodynamic farming, urban agriculture, hydroponics, agrology, cover crop etc. Sustainable agriculture contributes to development of rural areas and fighting starvation and poverty, by allowing lives in rural that are safe secured, economically viable and healthy(Matysiak et al,2017).

Nano bio pesticides are pesticides that are formulated using nanotechnology. This means that they contain active ingredients that have been engineered at the nanoscale, typically between 1 and 100 nano meters in size. Nanoparticles have unique properties that can make them more effective and less harmful than traditional pesticides.

Sustainable agriculture

Sustainable agriculture refers to a holistic approach to food production that aims to address the challenges of increasing food demands and to meet the current and future needs of humanity while minimizing negative environmental impacts, conserving natural resources, and ensuring social and economic equity. It recognizes the interdependence between agricultural practices, ecosystems, and the well-being of communities. One area of significant progress in sustainable agriculture is the development of Nano bio pesticides. These innovative solutions combine the power of nanotechnology and biological agents to revolutionize pest management in a sustainable and environmentally friendly manner. This essay explores the potential of Nano bio pesticides and their contributions to sustainable agriculture (Matysiak et al,2017).

Principles of Sustainable Agriculture (Kookanaet al, 2014)

1. **Environmental Stewardship**: Sustainable agriculture seeks to minimize the negative impacts of agricultural activities on the environment. It promotes the responsible use of natural resources such as soil, water, and air, and aims to protect biodiversity and ecosystems. Practices such as organic farming, agroforestry, and conservation tillage are employed to reduce soil erosion, water pollution, and greenhouse gas emissions.

2. **Soil Health and Fertility**: Sustainable agriculture focuses on maintaining and improving soil health. It emphasizes practices that enhance soil fertility, such as crop rotation, cover cropping, and composting. By nurturing the soil's natural processes, sustainable agriculture promotes long-term productivity and resilience, reduces the need for synthetic inputs, and mitigates soil degradation.

3. **Water Conservation**: Efficient water management is crucial in sustainable agriculture. Techniques like drip irrigation, rainwater harvesting, and precision farming help conserve water resources and reduce water waste. Sustainable agriculture also seeks to protect water quality by minimizing runoff and the leaching of agrochemicals into water bodies.

4. **Biodiversity Conservation**: Sustainable agriculture recognizes the importance of biodiversity for ecosystem stability and resilience. It encourages the preservation of natural habitats, promotes the use of native plant species, and integrates biodiversity-friendly practices such as agro ecology and integrated pest management. By fostering biodiversity, sustainable agriculture supports natural pest control, pollination, and nutrient cycling.

5. **Social Responsibility**: Sustainable agriculture acknowledges the social aspects of farming and aims to promote equitable and fair agricultural systems. It advocates for the rights and welfare of farmers, farm workers, and rural communities. Sustainable agriculture also emphasizes local and community-based food systems, promoting access to nutritious and culturally appropriate food for all.

6. **Economic Viability**: Sustainable agriculture seeks to ensure the economic viability and resilience of agricultural systems. It emphasizes diversification, value addition, and fair market access for farmers. By adopting sustainable practices, farmers can reduce input costs, increase productivity, and improve long-term profitability. Moreover, sustainable agriculture encourages the development of local and regional markets, reducing dependence on long-distance transportation and supporting local economies.

Benefits of Sustainable Agriculture

1. **Environmental Conservation**: Sustainable agriculture helps protect and restore ecosystems, conserves water and soil resources, and reduces pollution and greenhouse gas emissions. It promotes a more harmonious relationship between agriculture and the environment, ensuring the long-term sustainability of food production.

2. **Food Security**: By promoting resilient farming systems, sustainable agriculture enhances food security. Diverse crop rotations, agro ecological practices, and the preservation of genetic diversity contribute to increased crop productivity and reduced vulnerability to climate change and pests.

3. Human Health: Sustainable agriculture aims to produce safe and nutritious food while minimizing exposure to harmful chemicals. By reducing the use of synthetic pesticides and fertilizers, sustainable practices contribute to healthier diets and a reduced risk of pesticide-related health issues.

4. **Rural Development**: Sustainable agriculture supports vibrant rural communities by promoting fair trade, local economic development, and social well-being. It encourages the empowerment of farmers, the preservation of cultural heritage, and the strengthening of rural livelihoods.

5. **Climate Change Mitigation**: Sustainable agriculture plays a vital role in mitigating climate change. Carbon sequestration in agricultural soils, the use of renewable energy sources, and the reduction of emissions from agricultural practices contribute to greenhouse gas reduction and climate resilience.

Sustainable agriculture is a comprehensive approach that recognizes the intricate connections between agriculture, the environment, and society. By adopting practices that prioritize environmental stewardship, soil health, biodiversity conservation, social responsibility

Nano Bio pesticides

Nano biopesticides are innovative agricultural products that combine nanotechnology and biological agents to manage pests and diseases in a more targeted and environmentally friendly manner. Nano biopesticides are a new generation of plant protection products that utilize nanoparticles as delivery systems for biological agents such as biocontrol agents (e.g., beneficial microorganisms), plant extracts, or natural compounds to the pests or pathogens and enhancing their efficacy while minimizing the negative impacts on the environment and human health. These nanoparticles, typically in the nanometre scale, enhance the stability, solubility, and targeted delivery of the active ingredients against the pests or pathogens. (Hazafa, A,2022)

Key Features and Benefits of Nano biopesticides:(Opender Koul,2019)

Enhanced Efficacy and Targeted Delivery

Nano biopesticides offer significant advantages over conventional pesticides. The nanoparticles allow controlled release of the active ingredients, ensuring prolonged efficacy with reduced application frequency. Moreover, these nanoparticles can be engineered to target specific pests or pathogens while minimizing their impact on beneficial organisms, including pollinators and natural predators. Nanotechnology enables the encapsulation of active ingredients in nanoparticles, which improves their stability and provides controlled release. This controlled release ensures prolonged efficacy and reduces the need for frequent applications. Nano biopesticides have shown enhanced pest control capabilities compared to traditional formulations.

Targeted Delivery: The small size of nanoparticles allows for targeted delivery of the active ingredients. They can be designed to specifically interact with the pests or pathogens while minimizing their impact on beneficial organisms, such as pollinators and natural predators. This targeted approach increases the efficiency of pest control and reduces collateral damage.

Reduced Environmental Impact

Traditional chemical pesticides pose significant risks to the environment, including soil and water pollution, harmful effects on non-target organisms, and the development of pesticide resistance. Nano biopesticides, on the other hand, offer a greener alternative. By utilizing biological agents, they have reduced toxicity and minimal residual effects. Additionally, the targeted delivery systems minimize off-target effects, reducing environmental contamination. Nano biopesticides have the potential to reduce environmental contamination associated with conventional pesticides. Their targeted delivery systems minimize off-target effects, reducing the exposure of non-target organisms and minimizing environmental pollution. Furthermore, Nano biopesticides are often based on biological agents, which have lower toxicity and degrade more rapidly in the environment.

Increased Safety for Farmers and Consumers

Farmers, who are at the frontline of pesticide exposure, often face health risks due to the handling and application of conventional pesticides. Nano biopesticides, with their reduced toxicity, offer improved safety for farmers. Furthermore, these products leave minimal residues on crops, resulting in safer food for consumers. The development of Nano biopesticides aligns with sustainable agriculture's overarching goal of protecting both human health and the environment.(Bibin Lade, 2019).

Preservation of Biodiversity

Biodiversity is crucial for the long-term health and resilience of agricultural ecosystems. Conventional pesticides can harm non-target organisms, leading to disruptions in ecological balance. Nano biopesticides, with their targeted delivery systems and reduced toxicity, have the potential to preserve beneficial insects, microorganisms, and other components of biodiversity. This preservation can contribute to the natural regulation of pests and reduce the reliance on chemical interventions.

Mitigation of Pesticide Resistance

Pesticide resistance is a growing concern in agriculture, requiring the constant development of new products. Nano biopesticides can play a role in managing resistance. By using multiple modes of action and targeted delivery, they can reduce the selection pressure for resistance development in pests and pathogens. This can prolong the effectiveness of pest management strategies and reduce the need for frequent pesticide rotations. Pesticide resistance is a

significant challenge in agriculture. Nano biopesticides can play a role in managing resistance by employing multiple modes of action and targeted delivery. By reducing the selective pressure on pests and pathogens, they can help prolong the effectiveness of pest control strategies and reduce the development of resistance.

Lower Input Requirements

Nano biopesticides can offer more efficient pest management with lower application rates. The controlled release and enhanced efficacy of the active ingredients allow for reduced quantities of pesticides to be used, resulting in cost savings for farmers and reduced chemical load in agricultural systems.

Compatibility with Integrated Pest Management (IPM)

Integrated Pest Management is a holistic approach that aims to combine various pest control methods while minimizing reliance on chemical pesticides. Nano biopesticides align well with IPM principles as they are compatible with other pest management strategies, including cultural practices, biological control, and physical barriers. They can be used in combination with other control measures to create effective and sustainable pest management programs. (Vimala Devi, P.S et al ,2019)

Potential for Sustainable Agriculture

The use of Nano biopesticides contributes to the principles of sustainable agriculture. By reducing the reliance on conventional chemical pesticides, they help protect ecosystems, promote biodiversity, and support the long-term health of agricultural systems. They also reduce the risks associated with pesticide exposure for farmers and consumers, leading to safer and healthier food production.(Ayilara Modupe, et al 2023)

Potential risks associated with Nano Bio pesticide(Xiaohong Pan et al 2023)

Nano biopesticides offer several advantages over traditional pesticides. However, like any emerging technology, they also come with certain risks and limitations. Here are some potential risks and limitations associated with Nano biopesticides

Environmental impact

Nano biopesticides may have unintended effects on non-target organisms and ecosystems. The nanoparticles used in these formulations can persist in the environment and potentially accumulate in soil or water, leading

to long-term impacts on ecological systems. The potential ecological risks associated with these nanoparticles need to be thoroughly evaluated.

Human health concerns

While biopesticides are generally considered safer for human health compared to chemical pesticides, the introduction of nanoparticles raises additional concerns. Nanoparticles can potentially penetrate biological barriers such as skin, lung tissues, or the digestive system, leading to potential health risks. Nanoparticles can be more toxic to humans and other non-target organisms than traditional pesticides Further research is required to assess the toxicity and long-term effects of Nano biopesticides on human health.

Regulatory challenges

The regulatory frameworks for Nano biopesticides are still evolving and vary across different regions. Establishing appropriate guidelines and standards for the development, use, and disposal of these products can be challenging. Adequate testing methods and risk assessment protocols specific to Nano biopesticides need to be developed and implemented.

Resistance development

As with any pest control strategy, the risk of resistance development exists. Pests may evolve and develop resistance mechanisms against the biological agents used in Nano biopesticides, rendering them less effective over time. Proper management strategies, such as rotating or combining different biopesticides, should be employed to minimize the risk of resistance development.

Manufacturing challenges

The production of Nano biopesticides on a large scale can be technically challenging and expensive. Ensuring consistent quality and stability of nanoparticle formulations is essential for their effectiveness. Developing cost-effective and scalable manufacturing processes is necessary to make these products commercially viable.

Public acceptance

The acceptance and adoption of Nano biopesticides by farmers, consumers, and the general public may face challenges. There may be concerns about the safety and potential risks associated with these novel technologies. Effective communication, education, and transparency regarding the benefits

and limitations of Nano biopesticides are important for gaining public trust and acceptance.

It is worth noting that ongoing research and development efforts aim to address these risks and limitations associated with Nano biopesticides. However, careful evaluation, regulation, and monitoring of these products are necessary to ensure their safe and sustainable use.

Future research scope(Josef Jampilek et al,2019)

Nano bio-pesticides were an emerging area of research and development in the field of agriculture and pest management. These novel formulations combine the benefits of nanotechnology with biological agents, such as natural pesticides or biocontrol agents, to enhance their effectiveness, specificity, and environmental safety. Since the field is rapidly evolving, the future research scope of Nano bio pesticides is likely to focus on the following aspects:

Developing new methods for manufacturing Nano bio pesticides bio-pesticides: Future research will explore new and advanced nanomaterials to optimize the formulation of Nano biopesticides. Scientists will aim to design nanoparticles that can effectively encapsulate and deliver biological agents, ensuring stability, controlled release, and increased bioavailability to the target pests.

Enhanced Targeting and Specificity: Researchers will work on developing Nano bio pesticides that exhibit enhanced targeting capabilities, focusing on specific pests while minimizing any adverse effects on beneficial organisms, such as pollinators and other non-target species. Specificity is a crucial factor in reducing environmental impact and ensuring sustainable pest management.

Studying the efficacy and safety of Nano-biopesticides

Future studies aim to improve the overall efficacy and performance of nanobiopesticides, ensuring that they can compete with conventional chemical pesticides in terms of effectiveness. Research will focus on understanding the mechanisms of action and factors that influence the performance of Nano bio pesticides under different environmental conditions.

Long-Term Environmental Impact Studies: As Nano bio pesticides are relatively new, there will be a need for comprehensive and long-term studies to evaluate their environmental impact. Researchers will investigate factors such as nanoparticle persistence in soil and water, potential accumulation in ecosystems, and any unexpected ecological consequences.

Regulatory Approvals and Safety Assessments/ Developing regulations for Nano bio pesticides

The development and commercialization of Nano bio pesticides will require rigorous safety assessments and regulatory approvals. Future research will focus on conducting standardized toxicity studies and risk assessments to ensure the safe use of these novel formulations.

Developing innovative Application Methods and Technology: Researchers will explore innovative application methods for nanobiopesticides, including techniques that enhance delivery to plant surfaces or pest habitats. Advancements in application technology can significantly impact the efficiency and cost-effectiveness of these products.

Integration with Integrated Pest Management (IPM): Integrating Nano bio pesticides into broader Integrated Pest Management strategies will be an essential area of research. Understanding how Nano bio pesticides can complement other pest control methods and reduce reliance on conventional chemical pesticides will be crucial for sustainable pest management practices.

Commercialization and Scaling-up: Research efforts will focus on scaling up the production of Nano bio pesticides to make them economically viable and readily available to farmers. Identifying cost-effective and scalable production methods will be a key challenge.

Stakeholder Acceptance and Awareness: As with any new technology, building awareness and gaining stakeholder acceptance are vital for the successful adoption of nanobiopesticides. Future research may explore ways to communicate the benefits, risks, and proper use of these products to farmers, policymakers, and consumers.

Beyond Pesticidal Activity: Expanding the potential applications of Nano bio pesticides beyond their conventional pesticidal activity could be an exciting avenue for future research. For example, these nanoparticles could be designed to carry other beneficial agricultural agents like plant growth-promoting substances or micronutrients, thereby providing additional agricultural benefits.

It's important to note that the future research scope of Nano bio pesticides is subject to developments in nanotechnology, biology, and the agricultural sector as a whole. Researchers and stakeholders will continuously adapt their focus to address emerging challenges and opportunities in the field.

Conclusion

Nano biopesticides represent a promising innovation in pest management within the agricultural sector. By harnessing the power of nanotechnology and biological agents, these formulations offer enhanced efficacy, targeted delivery, reduced environmental impact, and compatibility with sustainable agricultural practices. Additionally, the incorporation of biological agents, such as beneficial microbes, further promotes sustainable agriculture by harnessing natural processes. As research and development in this field progress, Nano biopesticides have the potential to contribute significantly to sustainable agriculture by providing effective and environmentally friendly solutions for pest and disease control. Nano biopesticides represent a significant advancement in sustainable agriculture, providing effective pest control while minimizing environmental impacts. With their enhanced efficacy, targeted delivery, reduced toxicity, and potential to preserve biodiversity, these innovative solutions offer promising alternatives to conventional pesticides. The development and adoption of Nano biopesticides can contribute to a more sustainable and resilient agricultural system, ensuring food security for present and future generations while safeguarding our planet's health.

Nano-bio pesticides offer several advantages over conventional chemical pesticides. They exhibit increased selectivity, specifically targeting pests while sparing beneficial organisms, thus minimizing ecological disruption and preserving biodiversity. Furthermore, the reduced dosage requirements and targeted delivery systems contribute to decreased chemical residues in the environment and food products. This not only enhances consumer safety but also addresses concerns regarding pesticide accumulation and resistance development in pests.

Moreover, Nano-bio pesticides have the potential to mitigate the negative impacts associated with climate change and food security challenges. By reducing pest-related losses, they can enhance crop yields and contribute to global food production. Additionally, their eco-friendly nature aligns with sustainable agriculture practices, supporting long-term environmental sustainability and reducing the reliance on harmful chemical pesticides.

However, despite the promising potential, the development and commercialization of nano-bio pesticides face certain challenges. Safety concerns regarding the potential toxicity and long-term effects of nanomaterials on human health and the environment need to be thoroughly

addressed through rigorous testing and regulation. Furthermore, the high costs associated with research, development, and production hinder widespread adoption, particularly in developing countries.

In conclusion, Nano-bio pesticides represent an innovative solution to address the challenges of pest control in agriculture. They offer targeted and efficient pest management, reduced environmental impact, and potential contributions to food security. However, further research, safety assessments, and cost-effective production methods are necessary to fully harness the benefits of this technology and ensure its sustainable implementation in agriculture.

References

Ayilara Modupe S., Adeleke Bartholomew S., Akinola Saheed A., Fayose Chris A., Adeyemi Uswat T., GbadegesinLanre A., Omole Richard K., Johnson Remilekun M., Uthman Qudus O., Babalola Olubukola O. 2023.Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and Nano bio pesticides. Frontiers in Microbiology. 14 https://www.frontiersin.org/articles/10.3389/fmicb.2023.1040901. DOI=10.3389/fmicb.2023.1040901.

Bibin Lade, 2019. CHAPTER 7: Nano bio pesticide formulations: Application strategies today and future perspectives.Nano-Biopesticides Today and Future Perspectives, Pages -179 -206, ISBN: 978-0-12-815829-6

Hazafa, A., Murad, M., Umer Masood, M., Bilal, S., Nasir Khan, M., Farooq, Q., Muhammad Omer Iqbal, MuhammadShakir, Muhammad Naeem,2022. Nano-Biopesticides as an Emerging Technology for Pest Management. IntechOpen. Doi: 10.5772/intechopen.101285.

Josef Jampilek, KatarnaKralova, 2019,Chapter 17 - Nano bio pesticides in agriculture: State of the art and future opportunities,Editor(s): OpenderKoul, Nano-Biopesticides Today and Future Perspectives, Academic Press, 397-447,ISBN 9780128158296, https://doi.org/10.1016/B978-0-12-815829-6.00018-8.

Kookana RS, Boxall AB, Reeves PT, Ashauer R, Beulke S, Chaudhry Q, Cornelis G, Fernandes TF, Gan J, Kah M, Lynch I, Ranville J, Sinclair C, Spurgeon D, Tiede K, Van den Brink PJ., 2014.Nanopesticides: guiding principles for regulatory evaluation of environmental risks. Journal of Agricultural and Food Chemistry,62(19):4227-4240.

Matysiak M, Kruszewski M, Kapka-Skrzypczak L.,2017. Nanopestycydy – jasnaczyciemnastronamocy? [Nanopesticides - Light or dark side of the force?]. Medycyna pracy.; 68(3):423-432.

Modupe S Ayilara , Bartholomew S Adeleke , Saheed A Akinola , Chris A Fayose , Uswat T Adeyemi , Lanre A Gbadegesin , Richard K Omole , Remilekun M Johnson , Qudus O Uthman, Olubukola O Babalola,2023. Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and Nano bio pesticides, Front Microbiology, 16;14:1040901. doi: 10.3389/fmicb.2023.1040901. e-Collection 2023.

Opender Koul, 2019. Nano Biopesticides, Today and Future perspectives. Academic Press ISBN: 978-0-12-815829-6. DOI:https://doi.org/10.1016/C2017-0-03028-8.

Vimala Devi, P.S., Duraimurugan, P., Chandrika, K.S.V.P., Gayatri, B., Prasad, R.D., 2019. Nano bio pesticides for Crop Protection. In: Abd-Elsalam, K., Prasad, R. (eds) Nanobiotechnology Applications in Plant Protection. Nanotechnology in the Life Sciences. Springer, Cham. https://doi.org/10.1007/978-3-030-13296-5_8.

Xiaohong Pan, Xueping Guo,Tianyun Zhai,Dingyang Zhang,Wenhua Rao,Fang Cao and Xiong Guan,2023. Nano bio pesticides in sustainable agriculture: developments, challenges, and perspectives, Environmental Science: Nano ,**10**, 41-61.

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Molecular docking studies on the interaction of phytocompounds exploring the antifungal potency against *Candida* spp.

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Introduction

Given the convergence of prevalent ecological, socioeconomic, and demographic conditions, India unaccountably suffers from the burden of infectious diseases. It is critical to explore for new medications because the prevalence of multidrug resistant infections has significantly decreased the efficacy of current antibiotics and the future of antimicrobial treatments is still unclear. Opportunistic fungal infections are spreading more quickly worldwide. Despite the availability of a wide variety of medications for their treatment, invasive fungal infections are of great concern for people since they are linked to a high mortality rate that frequently exceeds 50%. Due to this, there is an ongoing and urgent need to find new antimicrobial agents with unique chemical structures and modes of action. Globally, the clinical *Candida* species that causes candidemia continues to be the most important source of opportunistic mycoses. The invasive fungal infections (IFI) with the highest prevalence are invasive candidiasis and candidemia. Despite the fact that *Candida albicans* predominates among all Candida species, new findings indicate that non-albican species, such as *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis* are increasingly producing invasive infections, particularly in immune-compromised individuals. Among the non-albican species of *Candida* that are responsible for these nosocomial infections, *Candida tropicalis* and *Candida glabrata* are reported to be the most prevalent. *Candida tropicalis* and *Candida glabrata* were frequently found in bone marrow transplant recipients who were neutropenic, as well as in nosocomial UTI cases, meningitis-causing pathogens, and infections in cancer patients. The ongoing development of multi-drug resistant (MDR) fungus strains has forced

researchers to look for new compounds with antifungal action from plant sources. The primary source of medicines for diverse chemotherapeutic uses is medicinal plants. Phytochemicals, a class of naturally occurring biologically active substances found in plants, have a wide range of biologically active properties, including neuropharmacological, anticancer, detoxifying, and antioxidant properties. The growing idea that plant-derived medications are more dependable and safer than pricey synthetic treatments, many of which have negative side effects, is partly to blame for the increased interest in using traditional medicines and the demand for these goods. Pharmacological research on several medicinal plants with potential biological activity is urgently needed and could result in the creation of safer and more effective medications.

Both medicinal and commercial value can be found in the Salicaceae and Fabaceae family's respective plants, *Flacourtia jangomas* and *Adenanthera pavonina*. Different plant parts are employed in various treatments for human ailments because they offer antifungal, antioxidant, and anti-cancerous properties. A firmly established platform for secondary metabolite profiling in plants is GC-MS. A research method for predicting whether one molecule will connect to another, often a protein, is computational molecular docking. The method can carry out predictions for protein-ligand, protein-protein, and protein-DNA docking, albeit the method used in each case varies greatly. Modelling the interaction between a protein and a ligand is used in protein-ligand docking. The ligand may bind the protein *in vitro* or *in vivo* if the pair has complimentary geometry and advantageous biochemical interactions. Molecular docking tools, which are important in the prediction of functional sites on protein molecular surfaces, structure-based drug design, and investigating the interaction between the protein and ligand molecules are some examples of computational methods currently being used to create novel lead molecules.

Candida

Candida species belong to the class Ascomycetes and comprises of a heterogeneous group of both yeast (unicellular) and hyphae (multicellular) forms (Bialkova and Subik, 2006). *Candida* is ubiquitous, dimorphic, diploid, naturally heterozygous opportunistic, yeast which remains in commensal association with many animals and also affects humans. *Candida* displays three modes of growth: yeast, hyphal and pseudo - hyphal modes of growth which are inter-convertible depending upon the environmental conditions. These

modes of growth differ in their morphology and pathogenicity, hyphal and pseudo - hyphal being the pathogenic forms (Navin *et al.,* 2012).

Candida tropicalis and *Candida glabrata* have become the two dominant species in the existing Non Albican species that cause maximum nosocomial infections. The data from previous studies also clearly showed the high incidence of Non Albican *Candida* species particularly *C. tropicalis, C. glabrata* and *C. parapsilosis* which are responsible for the high frequency of nosocomial candidemia (Verma *et al*., 2003; Malini *et al*., 2005). *Candida tropicalis* is found to be the main causative agent of candidemia and candiduria, and differs extensively based on geographical region and the specifics of the patient (Negri *et al*., 2012). Recent records show that *Candida tropicalis* cause severe infections in cancer patients especially those with neutropenia (Nucci and Colombo, 2007; Chander *et al*., 2013). There is a noticeable emergence of *Candida tropicalis* causing blood stream infections that lead to 52 to 70% mortality (Kontoyiannis *et al*., 2001). A recent finding on the involvement of the fungus *Candida tropicalis* was discovered in Crohn's disease wherein, a combination of two types of bacteria i.e., *Escherichia coli* and *Serratia marcescens* and the fungus was found to be strongly associated (Hoarau *et al*., 2016).

C. glabrata was known as a non-pathogenic fungus in the 1960s (Fidel *et al.,* 1999), however it was reported as the fourth most common cause of candida blood stream infections by the 1990s and became one of the most common causes of blood stream infections in the 2000s (Li *et al.,* 2007). *C. glabrata* is non-dimorphic yeast and is the only *Candida* species that does not form pseudohyphae at temperatures above 37 °C, thus existing as small blastoconidia under all environmental conditions as a pathogen. *C. glabrata* colonizes host tissues as well as abiotic surfaces as biofilms (Silva *et al.,* 2012). The occurrence of mucosal and systemic infections caused by *C. glabrata*, which is time and again the second or third most prevalent source of candidiasis after *C. albicans*, has increased significantly following the extensive and augmented use of immunosuppressive therapy along with broad-spectrum antimycotic therapy (Sinnot, 1987; Sobel, 1997). Apart from candidemia, candiduria is the most recurring *Candida* infection seen in hospitalized patients (Guler *et al*., 2006). The eradication of the NAC species is found to be tougher than *C. albicans* as they are well adapted to the urinary tract (Achkar and Fries, 2010).

Studies disclosed *C. tropicalis* and *C. glabrata* as the causative agents of nosocomial urinary tract infections (Jang *et al*., 2005; Sobel *et al.*, 2000). Cancer patients are particularly vulnerable to systemic infection caused by *C. glabrata* and *C. tropicalis* (Farmakiotis *et al.*, 2014; Canton *et al*., 2010). Contrary to *C. albicans* being the main causative organism of meningitis, reportedly an increase in meningitis caused by *C. tropicalis* has been noticed recently (Luciano and Rodrigo, 2010).*C. glabrata* and *C. tropicalis* infections being resistant to several azole antifungal agents, exclusively fluconazole is hard to treat (Hitchcock *et al.*, 1993; Perea and Patterson, 2002).

Selected Plants

Among the plants used in traditionalmedicine,*Flacourtia jangomas* and *Adenanthera pavonina* are two plants known for their ethnomedicinal uses. The various plant parts have curative properties and are less explored in research work.

1. Flacourtia jangomas

Flacourtia jangomas is commonly called as Coffee plum. It belongs to Salicaceae family. There are 4 genera that include 350-400 plant species. The genus *Flacourtia* comprises of trees or shrubs which are usually thorny. The tree is speculated to have its origin in tropical Asia. These are commonly found and largely cultivated in East Africa, Southeast and East Asia especially in the lowland areas. *Flacourtia jangomas* belongs to the mountain rainforest trees which are very common in India especially South India. They are endemic to Western Ghats especially the south and central Sahyadris. *Flacourtia jangomas* (Lour.) Raeusch locally known as Panial has both medicinal and economic values. Different parts of the plant are traditionally used and also employed in various therapies against human diseases. The fruits of the plant are used in healing biliousness, nausea and diarrhoea and liver related disorders. Roots are one of the best remedies to cure toothache (Ghani, 2003). It also promotes digestion. Raw fruits are eaten as such while the ripe fruits are used for making jams and culinary items. It is a cataplasm on skin rashes and sores. It also alleviates a swollen throat. The dried leaves of *Flacourtia jangomas* are used to treat asthma and also in relieving the difficulties of bronchitis (Ahmad *et al*., 1984). Diarrhoea and dysentery are treated using the decoction of the leaves. Antidiabetic properties of leaves and stem are made use of in different therapies (Singh & Singh, 2010). Both leaves and bark which are pungent and slightly acidic in nature are used in preventing dysentery, diarrhoea, weakness of limbs, bleeding gums etc. Young shoots have usage in increasing the

appetite and improving the functions of stomach. In addition, they have astringent properties too (Kirthikar and Basu, 1993).

2. *Adenanthera pavonina*

A. pavonina belongs to the family Fabaceae and is a genus with about 13 species. The plant is endemic to Southeast China and India with first reports being recorded in India (Roshetko and Gutteridge, 1996). The tree has been introduced extensively throughout the tropics as an ornamental plant and has become naturalized in Malaysia, Western and Eastern Africa as well as in most islands of both the Pacific and Caribbean regions (Balogun and Fetuga, 1985; GRIN Databases, 2009). In India its origin is south; it has been cultivated in many parts in southern region (The Wealth of India). *A. pavonina* is a fast growing, medium to large sized deciduous tree, 6-15 m tall and up to 45 cm diameter. Its wood being hard is used for constructing decorative wood products as well as for bridge and household construction, flooring, paving blocks and vehicle bodies. It may also be suitable for furniture and cabinet work and turnery (Benthal, 1946; Clark and Thaman, 1993; Zarnowski *et al.,* 2004).It can be used as a as source of fodder, green manure, and for improving soil nitrogen content through its rhizobial associations (Norani, 1983; Orwa *et al.,* 2009; PROSEA, 2012) and as substitute for true red sandalwood (*Pterocarpus santalinus*). In the Pacific Islands this species is known as a ‘food tree’as the roasted seeds are eaten by humans and young leaves are eaten as a vegetable (Orwa *et al.,* 2009). Nutritional studies show that the seed oil contains a high percentage of protein and fatty acids (Burkill 1966; Balogun and Fetuga, 1985). Various parts of *A. pavonina* have been used in traditional medicine for the treatment of diarrhoea, gout, inflammations, asthma, boil, rheumatism, tumour and ulcers, and as a tonic (Watt and Breyer-Brandwijk, 1962; Holdsworth, 1977; Kirtikar and Basu, 1981; Burkill, 1994; Jayasinghe *et al.,* 2006; Duke, 2009). The seeds of have been found to be effective in treating cardiovascular diseases in pregnancy. The ground seeds are used to treat boils and inflammatory reactions. Decoction of leaves is used to treat gout and rheumatism (Burkill, 1994; Adeyemi *et al.,* 2015). The bark of is traditionally used for treatment of various disease conditions in gonorrhea, haematuria and ulcers (Hussain *et al.,* 2011). The heartwood is used as an astringent, aphrodisiac, haemostatic, and is useful in dysentery, and haemorrhages (Khan *et al.,* 2007; Warrier, 2003).

Phytochemical Analysis of *F.jangomas* and *A. pavonina*

Most of the research works are done on the fruits of *F.jangomas*. In one of the studies, the methanolic fruit extract of this plant showed the presence of many of the secondary metabolites like flavonoids, phenols, tannins, terpenoids and saponins except alkaloids (Dutta & Bora, 2017). The leaves, bark and young shoots are found to be rich in tannins. High fibre content is seen in the fruits along with more protein content, Vit C and niacin. Stem and bark of *F. jangomas* shows the presence of two limonoids, i.e., limolin and jangomolide (Ahmad *et al*., 1984). Proteins, amino acids, carbohydrates, fats, phenolic compounds etc. were present in the fruits of *F. jangomas* (Ghani, 2003). Phytochemical analysis of the methanolic extracts of the four parts of *F. jangomas* showed the presence of various phytochemicals. The leaf extract of *F. jangomas* showed the presence of carbohydrates, alkaloids, tannins, terpenoids and glycosides. Root extract of the plant showed the presence of carbohydrates, flavonoids, saponins and steroids. Bark extract revealed the presence of carbohydrates whereas the flower extract showed the presence of carbohydrates and terpenoids (George *et al*., 2016). Chemical composition of *F. jangomas* fruits, quantitative analysis of sugars, amino acids and minerals were studied. Concentrations of amino acids, Na, Mn, Cu and Zn were also reported (Kermasha *et al*., 1987). The presence of steroids, flavonoids, phenolics and glycosides (except DCM) in the methanolic, n-butanol and DCM extract of *F. jangomas* fruits were found (Das *et al*., 2017).

Preliminary phytochemical analysis of the leaf of *A. pavonina* revealed the presence of alkaloids, tannins, flavonoids, saponins, glycosides, steroids and carbohydrates whereas the stem of the plant contained alkaloids, tannins, saponins, glycosides, steroids and carbohydrates (Bhadran *et al*., 2016). Phytochemical studies on *A. pavonina* have revealed the presence of various secondary metabolites including mainly flavonoids, steroids, saponins and triterpenoids (Gennaro andNasini 1972; Chandra *et al.,* 1982).Flavonoid compounds are the major constituents of the plant. The main important constituents are flavonoid compounds (Rastogi and Mehrotra, 1991). *A. pavonina* has been reported to contain a new five-membered lactone named pavonin with an exo-cyclic double bond,this has been isolated from the methanol soluble part of the plant (Ali*et al.,* 2005), sterols (β-sitosterol, β-sitosterol-3β -D- glucoside), triterpenes (nonacosane and hentriacontane) (Mesbah *et al.*, 2002) and saponins (sapogenins) (Yadav *et al.*, 1976).

Antifungal activity of *F. jangomas* and *A. pavonina*

Various plants have been screened for their biological activity against human pathogens like *C. tropicalis* and such studies have become new areas of research interest. The antimicrobial activity of ethanolic and aqueous extracts of seven plants against different species of *Candida* were reported in research studies (Hadid *et al.*, 2016). The ethanolic extracts of *Quercuc infectoria* showed the maximum activity against all species of *Candida* fungus especially against *C. tropicalis.*In the antifungal studies conducted on five medicinal plants, namely, *Cinnamomum porrectum, Lippia nudiflora, Cestrum nocturnum, Trachyspermum ammi* and *Sida carpinifolia*, the methanolic extracts exhibited noticeable antifungal activity against *C. tropicalis*(Bora, 2016).The methanolic extract of flowers of *F. jangomas* exhibited most remarkable antifungal activity against *C. tropicalis* with significant zone of inhibition as compared to the reference standard Fluconazole (George *et al*., 2016). The methanolic extracts of *F. jangomas* fruits that showed remarkable antibacterial activity amongst all solvents taken up for study (Das *et al*., (2017). Significant zone of inhibition was seen in case of Gram positive bacteria, *S. aureus* than the Gram negative bacteria, *E. coli*. The antibacterial activities of chloroform fraction of *Flacourtia jangomas* and *Flacourtia sepiaria* against two Gram positive and two Gram negative bacteria and the results obtained explained the antimicrobial effectiveness of this plant (Sarkar *et al*., 2011). *F. jangomas* exhibited better activity than *F. sepiaria* against all the tested bacteria with a zone of inhibition that was very close to zone of inhibition exhibited by Amoxicillin the reference standard taken for the study. The ethanolic extracts of *F. jangomas* taken up for study using disc-diffusion technique and microbroth dilution method against Gram positive and Gram-negative bacteria showed considerable inhibitory activity against *Shigella shiga* and *Bacillus megaterium* and moderate activity against *Bacillus cereus*. Poor activity against *Escherichia coli* was observed (Parvin *et al*., 2011). The investigations carried out on the antibacterial activity of n-hexane, ethyl acetate and chloroform extracts of leaves and fruits of *F. jangomas* on five strains of Gram-positive bacteria and eight strains of Gram-negative bacteria reported the effectiveness of chloroform and ethyl acetate extracts against most of the tested microorganisms (Bulbul, 2014).

Stem of *A. pavonina* exhibited potent antifungal activity (Bhadran *et al*., 2016). Scientific investigation of *A. pavonina* have exhibited that the crude extract has anti-inflammatory activities (Olajide *et al.*, 2007), blood pressure lowering effect (Adedapo *et al.,* 2009) antifungal, antioxidant and cytotoxic

(Rodrigo *et al.,* 2007). Significant antifungal activity was observed in the peptides extracted from *A. pavonina* seeds (Soares *et al.,* 2012). Significant anthelmintic activity was exhibited by the crude bark extract at different mg/mL when compared with standard drug piperazine citrate (Dash *et al.,* 2010). In the studies carried out on antimicrobial and anticancer efficacy of acetone and methanol extracts of seed and leaves of *A.pavonina*, the methanolic extract of seed and leaves exhibited efficacious antimicrobial and anticancer activity against various pathogens whereas acetone seed extract did not show any biological activity (Chauhan *et al.,* 2015).Phytochemical screening, analgesic, antimicrobial and antioxidant activities of bark extracts of *A. pavonina* was studied (Ara *et al.,* 2010).The bark of *A. pavonina* L. was extracted in petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EA) and methanol (ME) successively and evaluated for the pharmacological efficacies. The study revealed that the highest antimicrobial potential was in the crude ME extracts against gram-positive, gram-negative bacteria and fungi. The antibacterial and antifungal activity of chloroform, ethyl acetate and ethanol leaf extracts of *A. pavonina* were tested on microbes isolated from dairy cattle rearing unit in the research work where these extracts have significant antibacterial activity against *Salmonella enteritidis, K. pneumoniae, B. subtilis, S. aureus, E. coli* and *P. aeruginosa* but the two fungal strains *C. albicans* and *A. niger* were resistant against the extracts (Sophy *et al*., 2015).

GC-MS Analysis

GC MS analysis of *Flacourtia jangomas*

Twenty-one phytochemicals that may be responsible for the antifungal activity of the methanolic flower extract of *Flacourtia jangomas* against *Candida tropicalis* were detected after the extract underwent GC-MS analysis (Table 1). The methanolic flower extracts of *Flacourtia jangomas* was selected and subjected to GC-MS analysis based on the previous studies on their antifungal and antioxidant activities where *Flacourtia jangomas* flower extract displayed potent biological activities when compared to the other extracts.

Table 1: The 21 phytochemical constituents of *F.jangomas* obtained on GC chromatogram with NIST library match

Sl. No	R_{index}	Compound Name	Mol. Formula	Mol. Wt
GC Chromatogram with NIST library match of peaks with R.T 15.592min				
1	1060	Methyl benzoate / Niobe oil	$C_8H_8O_2$	136
2	1437	Benzohydrazine/Benzoyl hydrazide	$C_7H_8N_2O$	136
GC Chromatogram with NIST library match of peaks with R.T 18.225min				
3	533	Dimethylbutane	C_6H_{14}	86
4	1385	Isobutyl pentyl ester	$C_{11}H_{20}O_4$	216
5	1250	Oxalic acid, butyl propyl ester	$C_9H_{16}O_4$	188
GC Chromatogram with NIST library match of peaks with R.T 21.175min				
6	1235	2-Heptyl-1,3-dioxolane	$C_{10}H_{20}O_2$	172
7	578	1,3-Dioxolane	$C_3H_6O_2$	74
8	1204	Acetic acid, 3-[1,3]dioxolan-2-yl]propyl ester	$C_8H_{14}O_4$	174
9	1057	2,2'-bis[1,3-dioxolane]	$C_6H_{10}O_4$	146
GC Chromatogram with NIST library match of peaks with R.T 24.842min				
10	1648	Isopropoxy-1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576
11	1716	2-(2',4',4',6',6',8',8'-heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethyl cyclo pentasiloxane	$C_{16}H_{48}O_{10}Si_9$	652
12	578	1,3-dioxacyclopentane	$C_3H_6O_2$	74
13	1770	1,3-dioxolane	$C_{10}H_{16}BrCl_3O_2$	352
GC Chromatogram with NIST library match of peaks with R.T 32.708min				
14	1282	Methyl decanoate/ methyl caprate	$C_{11}H_{22}O_2$	186
15	1580	Methyl tridecanoate	$C_{14}H_{28}O_2$	228

GC Chromatogram with NIST library match of peaks with R.T 21.152 min				
17	1680	Methyl myristate	$C_{15}H_{30}O_2$	242
GC Chromatogram with NIST library match of peaks with R.T 35.842min				
18	863	Acetylhydrazide	$C_2H_6N_{20}$	74
19	637	Isonitropropane	$C_3H_7NO_2$	89
20	1219	1,2-diacetylhydrazine	$C_4H_8N_2O_2$	116
21	701	1-Nitropane	$C_3H_7NO_2$	89

GC-MS Analysis of *Adenanthera pavonina*

GC-MS analysis of the *A. pavonina* stem extract facilitated the identification of seventeen compounds which were predominantly responsible for the various biological activities found in the extract (Table 2).

Table 2: The 17 phytochemical constituents of *A. pavonina*obtained on GC chromatogram with NIST library match

S.No	R_{index}	Compound Name	Molecular formula	Molecular Weight (Grams)
GC Chromatogram with NIST library match of peaks with R.T 18.208 min				
1	544	Isobutyl nitrate	$C_4H_9NO_2$	103
2	1039	3,4 hexane dione	$C_9H_{16}O_2$	156
3	1250	oxalic acid butyl propyl ester	$C_9H_{16}O_4$	188
4	637	Isonitropropane	$C_3H_7NO_2$	8
5	1340	oxalic acid, alypentyl ester	$C_{10}H_{16}O_4$	200

6	715	2-methoxy-1,3-dioxolane	$C_4H_8O_3$	104
7	578	1,3-dioxolane	$C_3H_6O_2$	74
8	1312	2-benzyl-1,3-dioxolane	$C_{10}H_{12}O_2$	164
GC Chromatogram with NIST library match of peaks with R.T 24.842 min				
9	1716	cyclopentasiloxane, [(2,4,4,6,6,8,8-heptamethylcyclotetrasiloxan-2-yl)oxy]nonamethyl-	$C_{16}H_{48}O_{10}Si_9$	652
10	1235	2-heptyl-1,3-dioxolane	$C_{10}H_{20}O_2$	172
11	1612	3-ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris tatrasiloxane	$C_{17}H_{50}O_7Si_7$	562
12	1468	malonic acid, bis(2-trimethylsilylethyl ester	$C_{13}H_{28}O_4Si_2$	304
13	1566	acetic acid,[o-(trimethylsiloxy)pentyl]-,trimethylsilyl ester	$C_{14}H_{24}O_3Si_2$	296
GC Chromatogram with NIST library match of peaks with R.T 28.442min				
14	1406	methyl-alpha.-d-ribofuranoside	$C_6H_{12}O_5$	164
15	1714	3-methylmannoside	$C_7H_{14}O_6$	194
16	1436	2,3,4,5-tetrahydroxypentanal	$C_5H_{10}O_5$	150
17	1359	Methyl 4-O-methyl-d-arabinopyranoside	$C_7H_{14}O_5$	178

Molecular Docking studies

The design of pharmacological molecules is heavily influenced by computational biology and bioinformatics which increases the likelihood that the drug discovery process will be sped up.One of the efficient *insilico* techniques for identifying bioactive substances and their interactions with target proteins is molecular docking. It refers to predicting the bioactive conformation of a molecule in the binding site of a target structure and is a tool in structure-based drug design. Docking is a critical tool for anticipating the relationships between biologically significant molecules such nucleic acids, proteins, lipids, and carbohydrates in the context of molecular modelling, and it is crucial for signal transduction. It is widely used to determine the binding

orientation of the protein target by the drug candidates in order to anticipate the affinity and activity. Docking of the target receptor with the drug molecules exposes the ligand receptor interactions. This computational method makes use of several bioinformatics applications and technologies. The docking approach also provides data on complex stability and binding energy.Protein- Ligand interaction is crucial in the design of drugs with a structural basis (Sayre *et al*., 2001).

Protein-Ligand Docking Interactions: It is the method that is most frequently used to forecast where a protein will be when it interacts with ligand molecules, which can either operate as a promoter or an inhibitor. The selection of promising therapeutic candidates involves effectively scanning huge libraries of ligands (Smith *et al*., 2012). The interaction between protein and ligand involves high specificity along with induced fit within the interfaces increasing the rigidity. In the last three decades several docking algorithms have been developed (Gardiner *et al*., 2001; Gray *et al*., 2003). High specificity and induced fit within the interfaces, which increase rigidity, are involved in the interaction between the protein and the ligand. Several docking methods have been developed over the past thirty years. Most of these algorithms are too computationally intensive for large-scale investigations. As a result, the geometry-based molecular docking algorithm Patch Dock was created. It is an excellent approach for protein-protein and protein-small ligand docking (Duhovny *et al*., 2002). The programme can predict docking modifications that produce good complementarity in molecular shape. The chemical structure of molecules can be predicted using chemical structure drawing tools like ACD/ChemSketch or variants. The ChemSketch software has a number of templates that make it easier to input complex chemicals, organometallic complexes, and polymers. Additionally, it has a quick property generator that may display information on the weight of molecules, chemical formulas, percentages of their composition, and approximated macroscopic properties like density, molar refractivity, refractive index, molar volume, and others (Pagenkopf, 2005).

Selection and Preparation of Receptor: Building the receptor and identifying the active areas are included in the selection and preparation of the receptor.

Building the Receptor: For the purpose of displaying the three-dimensional structures of the fungal receptors, homology modelling was carried out using the free SWISS Model software. Modeler9v7 was used to create models of the proteins indicated above. To ensure that the receptors stay in a stable and

biologically active shape, the structural processing of the receptor utilizing the molecular docking tool was meticulous.

Identification of the Active Site: The active location for phytoligand binding within the receptors was found and examined computationally. The models were examined in greater detail on the Rampage Ramchandran plot server, and the best model was chosen and used for additional docking research**.**

Selection and Preparation of Ligand: From the phytocompounds recovered by GC-MS profiling, ten ligands were chosen, with higher peak values indicating higher concentrations in the methanol stem extract of *A. pavonina*. As ligands, the bioactive chemicals identified in the GC-MS study of the methanolic flower extract of *F. jangomas* with greater peak values corresponding to higher concentration were employed.The PubChem compound database at NCBI was used to retrieve the chemical structures of these phytocompounds, and Chemsketch was used to create the three-dimensional structures for the ligands for which the structures were not accessible. The structures that were downloaded as.mol files were stored as pdb files using the Argus lab programme.

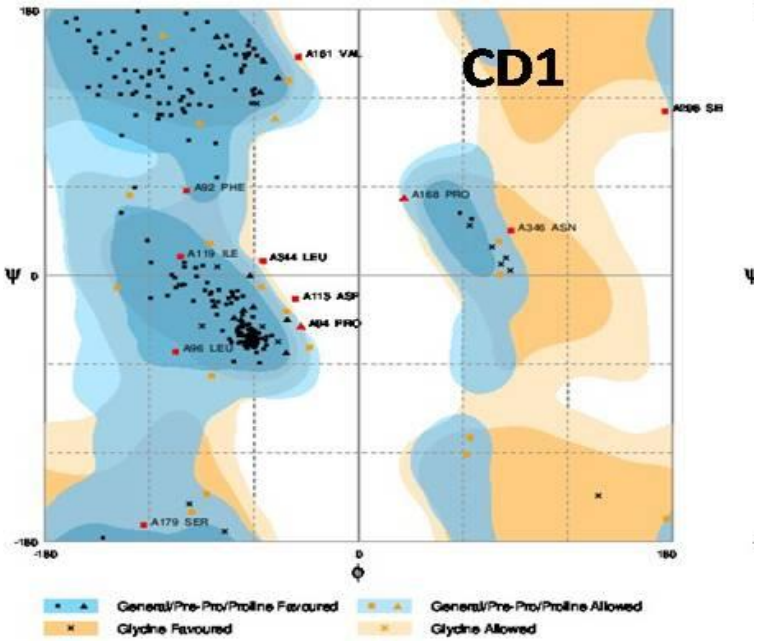
Docking studies: The best receptor models were chosen from Rampage, and the ligands were docked to those models using Patch Dock. Computational modelling was used to create the molecular interactions between the ligands and the receptors. The scoring function produced scores based on the interactions between ligands and receptors that fit best. By predicting the E value and comparing it to experimental E values, the anticipated ligand-receptor affinities were validated, and the scoring was completed. The stronger a molecule's affinity for a receptor, the larger its negative energy absorption value.

Docking solutions

1. Flacourtia jangomas

The receptors designed were used for molecular docking studies using the 21 ligands isolated from *F.jangomas* by GC-MS analysis for their affinity towards fungal proteins of *C. tropicalis* that are known targets for some antifungal agents with different mechanism of action. PATCHDOCK was used for the docking studies. The patchdock scores were taken into consideration. Fluconazole, the positive control used in the antifungal studies was also used in the *in silico* studies. *In silico* screening elucidates a ligand-receptor interaction and mark the compounds based on the binding energies or affinity and the Patch dock score. The docking values which are expressed in negative energy

values can be used as a conclusive factor for the drug design and development. Methyl myristate gave the best docking results among all the twenty-one bioactive compounds studied in the methanolic flower extract of *Flacourtia jangomas*. Among the seven receptors designed only three receptors namely chitin deacetylase model 1 and 3 (CD1, CD3) and FET3 were found to be best suited models for docking studies as per Ramachandran plot (Fig.1)The patchdock score of methyl myristate, the best fit ligand, was found to be 3688 with CD1,the receptor which was closest to the score 4096 obtained for the standard fluconazole (Fig.2, Fig.3). Molecular binding interaction observed in the *in silico* studies demonstrated that methyl myristate present in the flower extract has more specificity towards the fungal cell wall protein, chitin deacetylase 1 and could be a potent antifungal compound.



Number of residues in favoured region (~98.0% expected): 252 (95.3%)

Number of residues in allowed region (~2.0% expected): 12 (3.6%)

Number of residues in outlier region : 06 (1.1%)

Figure 1: Image showing the Ramachandran plot obtained from RAMPAGE for the CD1

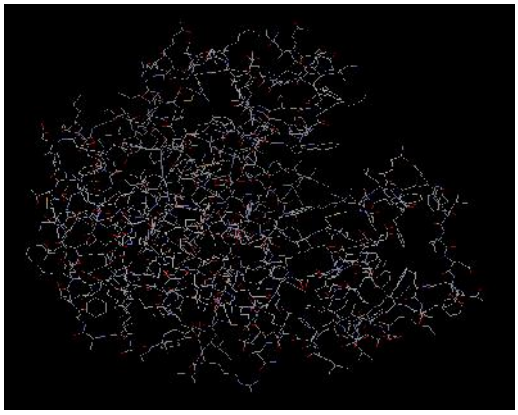


Figure 2: Receptor structure of CD1

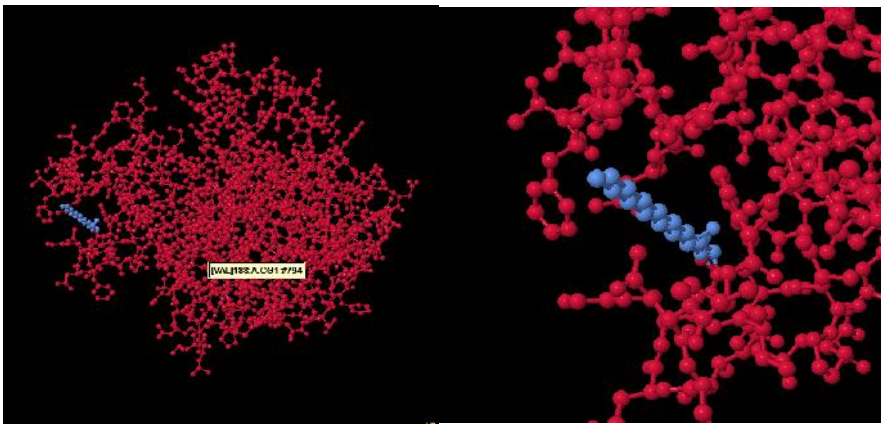
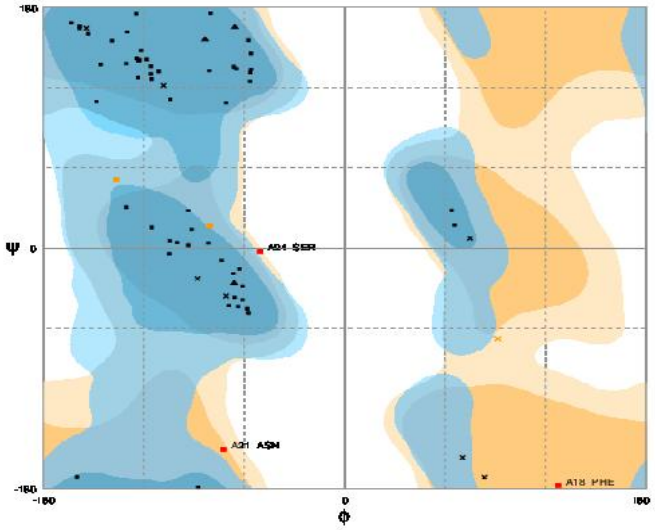


Figure 3: Docking solution of CD1 with Methyl myristate

2. Adenanthera pavonina

The contemporaneous investigation was employed for comparative comprehension of the mechanism of antifungal activity exerted by the standard antimycotic drug and the antimycotic compounds present in *Adenanthera pavonina* by screening for the possible target proteins on the cell wall of *Candida glabrata*. *In silico* analysis demonstrated that among the six receptors selected for the docking studies with the 10 phytoconstituents only three proteins viz. epithelial adhesin 6, Cell wall transcription factor ACE2 and cell wall integrity and stress response component 4 were displayed as the best suited models for docking as per analysis of Ramachandran plot

(Fig.4).Fluconazole demonstrated the least binding energy in the autodock results and hence has greater affinity towards the receptor. The protein- ligand interaction was best exerted by 2 heptyl 1, 3 dioxolane and cell wall integrity and stress response component 4 with more binding affinity and significant docking score compared to that between the fluconazole and the protein and hence was found to be the best solution in the present docking study (Fig.5 & Fig.6).



Number of residues in favoured region (~98.0% expected) : 75 (94.7%)

Number of residues in allowed region (~2.0% expected) : 2 (3.2%)

Number of residues in outlier region : 1 (1.1%)

Figure 4: Image showing the Rampage (Ramachandran Plot) results of Cell wall integrity and stress response component 4

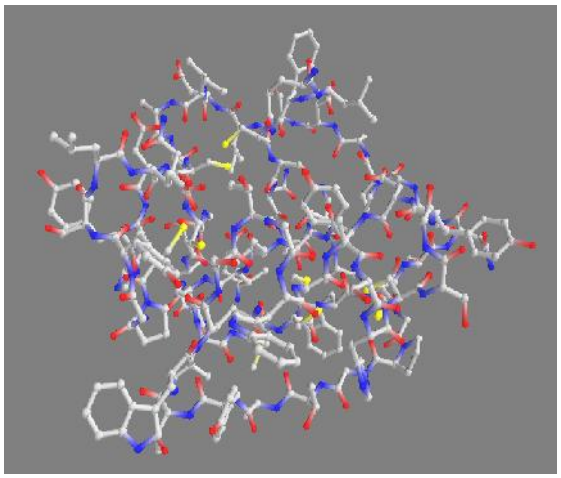


Figure 5: Receptor structure of Cell wall integrity and stress response component 4

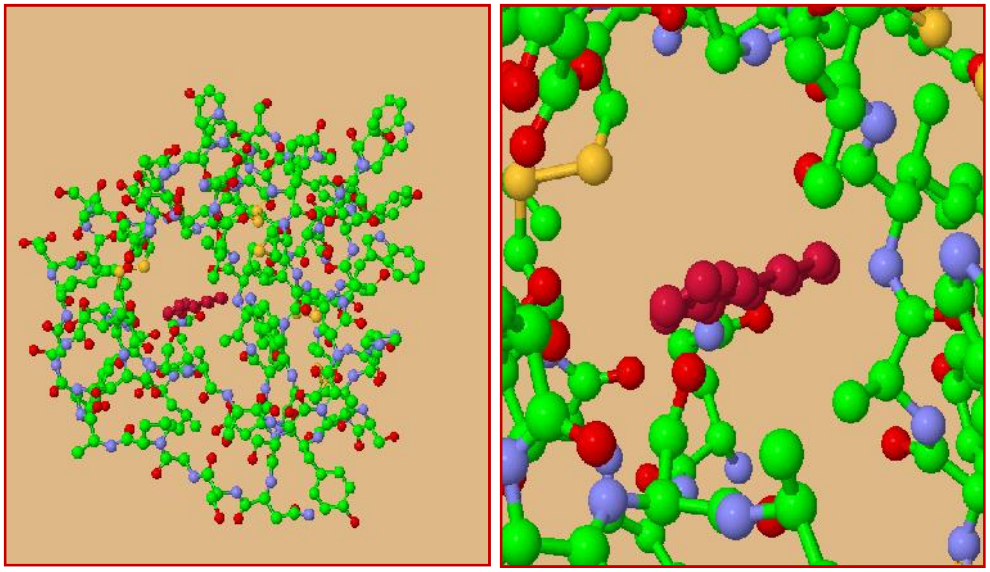


Figure 6: Docking solution between 2-heptyl-1,3-dioxolane and INT. Image retrieved from Hex 6.0

Conclusion

The nature of infectious diseases has endured reflective changes in the past few decades. Infections caused by non albican species of *Candida* like *Candida tropicalis* and *Candida glabrata* are on a rise as they are resistant to many azole antifungal agents. The selected plants *Flacourtia jangomas* and *Adenanthera pavonina* are known for various therapeutic uses.

The phytochemical profiling of the methanolic stem extract of *F. jangomas* and *A. pavonina* was carried out using GC-MS to identify the phytoconstituents. The bioefficacy of the identified phytoconstituents of *F. jangomas* and *A. pavonina* was screened on an *in silico* platform.The three-dimensional structure of the fungal receptors were derived by homology modeling using Modeller9v7 and the same for the ligands for which the structures were not available were drawn by ACD chemSketch. The docking of ligands and receptors were performed using PatchDock software. GC-MS analysis of the *A. pavonina* extract revealed the presence of 17 phytocompounds, of which 2 heptyl 1,3dioxolane had an excellent binding affinity signifying its potent antifungal activity. The methanolic stem extract of *A. pavonina* demonstrated good docking scores when docked with specific fungal cell wall receptorcell wall integrity and stress response component 4 and thus can prove to be appropriated for the lead molecule. Similarly, Methyl myristate gave the best docking results among all the 21 bioactive compounds studied in the methanolic flower extract of *Flacourtia jangomas* with the fungal cell wall protein, chitin deacetylase 1. Thus, 2 heptyl 1, 3 dioxolane and methyl myristate could be potent antifungal compounds against *Candida* species.

References

Adeyemi, O. A., Adedapo, A. D., Adedapo, A. A and Moody, J. O., 2015. Evaluation of the antimicrobial activity of crude extracts and chromatographic fractions of *Adenanthera pavonina* Linn (L) seeds. African Journal of Biotechnology, 14(12): 1067-1073.

Ahmad, J., Wizarat, K., Shamsuddin, K.,M., Zaman, A and Connolly, J.D., 1984. Jangomolide, a novel limonoid from *Flacourtia jangomas*. Phytochemistry, 23:1269-1270.

Ali, M. S., Ahmed, F., Azhar, I and Pervez, M. K., 2005. Pavonin: A new five-membered lactone from *Adenanthera pavonina* Linn. (Mimoaceae). Natural ProductResearch, 19(1): 37-40.

Ara, A., Saleheen, Md., Ahmed, N. U., Ahmed, M., Hashem, Md and Bachar, S.C., 2010. Phytochemical screening, analgesic, antimicrobial and antioxidant activities of bark extracts of *Adenanthera pavonina* L. (Fabaceae).Advances in Natural and Applied Sciences, 4(3): 352-360.

Bialkova, A and Subik J., 2006. Biology of the pathogenic yeast *Candida glabrata*. Folia Microbiologica (Praha), 51(1): 3-20.

Balogun, A. M and Fetuga, B. L., 1985. Fatty acid composition of seed oils of some members of the Leguminosae Family. Food Chemistry, 17: 175-182.

Benthall A. P., 1946. Trees of Calcutta and its Neighborhood. Thacker Spink and Co.,Calcutta, India.

Bhadran, S., Harini, B. P and George, S. A., 2016. Comparative Bio-Evaluation of Various Medicinal Plant Extracts and Their In vitro Antifungal Potency against *Candida glabrata*. European Journal of Biomedical and Pharmaceutical Sciences, 3(5): 265-269.

Bulbul, I. J., 2014. Comparative Antibacterial Study of n-Hexane, Chloroform and Ethyl Acetate Extracts of *Flacourtia jangomas* Leaves and Fruits. South East University Journal of Science and Engineering, 8 (1): 1-8.

Burkill IH, 1966. A Dictionary of the Economic Products of the Malay Peninsula, 2 (2nd edition): 2444.

Burkill, H. M. (1994). The useful plants of West Tropical Africa. Royal Botanical Gardens (2nd ed., Vol 2, 648)Kew, Richmond, United Kingdom. ISBN: 094764301x

Canton, E., Viudes, A and Pemán, J., 2001. Systemic nosocomial infection by yeasts. Ibero-American Journal of Mycology, 18 (2): 51-55.

Chander, J., Singla, N., Sidhu, S. K and Gombar, S., 2013. Epidemiology of *Candida* blood stream infections: experience of a tertiary care centre in North. Journal of Infection in Developing Countries, 7 (9): 670-675.

Chandra, S., Verma, M and Saxena, H., 1982. Triterpenoids of *Adenanthera pavonina* root. International Journal of Crude Drug Research, 20: 165-167.

Clark W. C. and Thaman R. R., 1993. Agroforestry in the Pacific Islands: Systems for Sustainability. United Nations University Press, Tokyo, Japan.ISBN 92-808-0824-9.

Chauhan, R., D'Souza, H. L., Shabnam, R.S and Abraham, J., 2015. Phytochemical and Cytotoxicity Analysis of Seeds and Leaves of *Adenanthera pavonina*. Research Journal of Pharmacy and Technology, 8(2): 198-203.

Das, S., Dewan, N., Das, K. J and Kalita, D., 2017. Preliminary phytochemical, antioxidant and antimicrobial studies of *Flacourtia jangomas* fruits. International Journal of Current Pharmaceutical Research, 9 (4): 86-91.

Dash, S., Das, C and Sahoo, D. C., 2010. Phytochemical and anthelmintic screening of crude bark extract of *Adenanthera pavonina* Linn. Pharmacie Globale, 2(10): 1-4.

Duhovny, D., Nussinov, R and Wolfson, H. J., 2002. Efficient unbound docking of rigid molecules. In: Guigo R., Gusfield D., editors. Proceedings of the Fourth International Workshop on Algorithms in Bioinformatics. Springer-Verlag GmbH Rome, Italy, 185–200.

Dutta, B and Borah, N., 2017. Studies on nutraceutical properties of *Flacourtia jangomas* fruits in Assam, India, Journal of Medicinal Plants Studies, 5 (1): 50-53.

Farmakiotis, D., Tarrand, J. J and Kontoyiannis, D. P., 2014. Drug-resistant *Candida glabrata* infection in cancer patients. Emerging Infectious Diseases, 20: 1833–1840.

Fidel, P. L., Vazquez, J. A and Sobel, J. D., 1999. *Candida glabrata:* Review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clinical Microbiology Reviews, 12:80-96.

Gardiner, E. J., Willett, P and Artymiuk, P. J., 2001. Protein docking using a genetic algorithm. Proteins, 44: 44–56.

Gennaro, A. M and Nasini, G., 1972. Flavonoids from *Adenanthera pavonina*. Phytochemistry, 11(4): 1515.

George, S. A., Harini, B. P and Bhadran, S., 2016. In vitro assessment of antifungal activity of selected botanicals on *Candida tropicalis*. International Journal of Recent Scientific Research, 7 (4): 9863-9866.

Ghani A., 2003. Medicinal plants of Bangladesh: Chemical Constituents and Uses. The Asiatic Society of Bangladesh, Dhaka, 2(2): 315.

GRIN Databases, 2009. USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network-(GRIN). National

Germplasm Resources Laboratory, Beltsville, Maryland. https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch

Gray J. J., Moughon S., Wang C., Schueler-Furman O., Kuhlman B., Rohl C. A and Baker, D., 2003. Protein–protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. Journal of Molecular Biology,331: 281–299.

Hadid, R. F., Seleman, T. T and Ahmed, S. S., 2016. Screening of some plant extracts for antifungal activity against *Candida* species. African Journal of Microbiology, 4 (3): 106-108.

Hoarau, G., Mukherjee, P. K., Rousseau, G. C., Hager, C., Chandra, J., Retuerto, M. A., Neut, C., Vermeire, S., Clemente, J., Colombel, J. F., Fujioka, H., Poulain, D., Sendid, B and Ghannoum, M.A., 2016. Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's Disease. mBio, American Society for Microbiology, 7 (5): e01250-16.

Hussian, A., Rizvi, A., Wahab, S., Zareen, I., Ansari, S and Hussain, S., 2011. Antibacterial screening of the bark of *Adenanthera pavonina* (L.). International Journal of Biomedical Research, 2 (2): 110-112.

Hitchcock, C. A., Pye, G.W., Troke, P. F., Johnson, E. M and Warnock. D. W. (1993). Fluconazole resistance in *Candida glabrata*. Antimicrobial Agents and Chemotherapy, 37: 1962–1965.

Holdsworth, D.K. and South Pacific Commission, 1977. Medicinal Plants of Papua New Guinea.

Jayasinghe, P. K. I. D. E., Bandara, B. M. R., Ekanayaka, E. W. M. A and Thevanesam. V., 2006. Screening for antimicrobial activity of *Acronychia pedunculata* (Ankenda) and *Adenantherapavonina* (Madatiya) against bacteria causing skin and wound infections in humans. Proceedings of the PeradeniyaUniversity Research Sessions, Sri Lanka, 11: 105.

Kermasha, S., Barthakur, N. N., Mohan, N. K and Mohan, N. P., 1987. Chemical composition and proposed use of two semi wild tropical fruits. Food Chemistry, 26 (4): 253-259.

Khan, A. I and Khanum, A., 2007. Herbal medicine for human diseases. Ukaaz Publications, Hyderabad, 3: 21-30. ISBN-10 : 8188279420.

Kirthikar, K. R and Basu, B. D., 1993. Indian Medicinal Plants, International Book Distributors, Dehradun, India.2nd Edition.

Kontoyiannis, D.P., Vaziri, I., Hanna, H. A., Boktour, M., Thornby, J., Hachem, R., Bodey, G.P., Raad II., 2001. Risk Factors for *Candida tropicalis* fungemia in patients with cancer. Clinical Infectious Diseases, 33(10):1676-1681.

Li, L., Redding, S and Dongari-Bagtzoglou, A., 2007. *Candida glabrata*: an emerging oral opportunistic pathogen. Journal of Dental Research,86: 204-15.

Luciano, Z.G., Rodrigo, P.S.,2010.*Candida tropicalis* as an emerging pathogen in Candida meningitis: case report and review. Brazilian Journal of Infectious Diseases, 14(6): 631-633.

Malini, R. C., Deepthi, N., Monorama, D., Pardeep, K. V., Lakshmi, S and Pushpa, A., 2005. Emergence of non-albicans Candida species and antifungal resistance in tertiary care hospital. Japanese Journal of Infectious Diseases, 58 (6): 344-348.

MesbahAhmad, Rehman U, Ataur M, Tabassum, Rumana, Naher Kamrun, 2002. Chemical constituents of leaves of *Adenanthera pavonina*. Journal of theBangladesh Chemical Society, 15(2):194-199.

Navin, K., Gupta, P., Gautam, P and Rai, N., 2012. An emerging hope to combat *Candida albicans*: Plant based therapeutics. Biotechnology International, 5(3): 85-114.

Negri, M., Martins, M., Henriques, M., Svidzinski, T., Azeredo, J and Oliveira, R., 2012. Insights into Candida tropicalis nosocomial infections and virulence factors. European Journal of Clinical Microbiology & Infectious Diseases, 31 (7): 1399 -1412.

Norani A, 1983. A preliminary survey on nodulation and VA mycorrhiza in legume roots. Malaysian Forester, 46(2):171-174.

Nucci, M and Colombo, A. L., 2007. Candidemia due to Candida tropicalis: clinical, epidemiologic and microbiologic characteristics of 188 episodes occurring in tertiary care hospitals. Diagnostic Microbiology and Infectious Diseases, 58 (1): 77-82.

Olajide, O. A., Echianu, C. A., Adedapo, A. D and Makinde, J. M., 2004. Anti-inflammatory studies on *Adenanthera pavonina* seed extract. 12(2): 196-202.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R and Simons, A., 2009. Agroforestree database: a tree reference and selection guide. Version 4.0. World Agroforestry Centre (ICRAF), Nairobi, Kenya.https://www.worldagroforestry.org/publication/agroforestree-database-tree-reference-and-selection-guide-version-40

Pagenkopf, B.,**2005.**ACD/HNMR Predictor and ACD/CNMR Predictor advanced chemistry development. Journal of the American Chemical Society,*127* (9): 3232.

Parvin, S., Kader, A, Sarkar, G.C., Hosain, S.B., 2011.*In vitro* studies of antibacterial and cytotoxic properties of *Flacourtia jangomas*. International Journal of Pharmaceutical Sciences and Research, 2(11): 2786-2790.

Perea, S and Patterson, T. F., 2002. Antifungal resistance in pathogenic fungi. Clinical Infectious Diseases, 35 (9): 1073–1080.

PROSEA, 2012. Plant Resources of South East Asia. http://www.prosea.nl/

Rastogi and Mehrotra, 1991. Compendium of Indian Medicinal plants (Vol. 2, 23) PID, New Delhi.

Rodrigo, S. K., Jayasinghe, U. L. B and Bandara, B. M. R., 2007. Antifungal, antioxidant and cytotoxic activity of *Acronychia pedunculata* and *Adenanthera pavonina.* Proceedings of the Peradeniya University Research Sessions, Sri Lanka, 12(1): 94-95.

Roshetko, J.M. Gutteridge, R.C., 1996. Nitrogen fixing trees for fodder production: a field manual. Winrock International, Arkansas, USA.125.

Sarkar, G. C. R. Z., Alam, B. M., Mohammed S. I., Mosaddik, M. A and Haque, M. E., 2011. Antibacterial activity of *F. jangomas* and *F. separia*. International Journal of Pharmaceutical and Life Sciences, 2 (7): 878-888.

Sayre, P.H., Moore, F.J.S., Fritz, T.A., Biermann, D., Gates, S.B., MacKellar, W.C., Patel, V.F.,Stroud, R.M., 2001. Multi-targeted antifolates aimed at avoiding drug resistance form covalent closed inhibitory complexes with human and *Escherichia coli* thymidylate synthase. Journal of Molecular Biology. 313(4): 813-829.

Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W and Azeredo, J., 2012. *Candida glabrata, Candida parapsilosis* and *Candida tropicalis*:

biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiology Reviews, 36(2): 288–305.

Singh, A. K and Singh, J., 2010. Evaluation of anti- diabetic potential of leaves and stem of *Flacourtia jangomas* in streptozotocin- induced diabetic rats. Indian Journal of Pharmacology, 42 (5): 301-305.

Sinnott, J. T., 1987. *Candida* (Torulopsis) *glabrata*. Infection Control, 8:334–336.

Smith, R. D., Engdahl, A.L., Dunbar, J. B. Jr and Carlson, H. A., 2012. Biophysical limits of protein-ligand binding. Journal of Chemical Information and Modelling, 52(8): 2098-2106.

Soares, J. R., Carvalho, A. Q., Santos, I. S. D., Machado, O. L., Nascimento, V.V., Vasconcelos, I. M., Ferreria, D. S. A. T., Perales, A. J. E and Gomes, V. M., 2012. Antimicrobial peptides from ADENANTHERA PAVONINA L. Seeds: characterization and antifungal activity. PROTEIN AND PEPTIDE LETTERS,19(5): 520–529.

Sobel, J. D and Chaim, W., 1997. Treatment of *Torulopsis glabrata* vaginitis: retrospective review of boric acid therapy. Clinical Infectious Diseases, 24: 649–652.

Sobel, J. D., Kauffman, C. A., McKinsey, D., Zervos, M., Vazquez, J. A., Karchmer, A.W, Lee, J., Thomas, C., Panzer, H and Dismukes, W. E., 2000. Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clinical Infectious Diseases, 30(1): 19-24.

Sophy, R. A. J., Fleming, A. T., Ronald, B. S. M., Shankar, K. G., Vidhya, R., Rajagopalan,V., Sheeba, A and Durgalakshmi, R., 2015. Antimicrobial activity of extracts of *Adenanthera Pavonina* and *Mussaenda Philippica* against isolated bacteria and fungi, 5(4): 22-27.

The Wealth of India. Raw Materials, 1985. CSIR, National Institute of Science Communication and Information Resources, New Delhi. I: 76.

Verma, A. K., Prasad, K. N., Singh, M., Dixit, A. K and Ayyagari, A., 2003. Candidemia in patients of a tertiary health care hospital from North India. Indian Journal of Medical Research, 117: 122–128.

Warrier, P. K., 2003. Indian medicinal plants: A Compendium of 500 species. Orient Longman Pvt. Ltd, Chennai. 4th Edition, 1: 58.ISBN-8125003037.

Watt, J. M and Breyer-Brandwijk, M. G., 1962. The Medicinal and Poisonous plants of Southern and Eastern Africa. E and S Livingstone Ltd. Edinburgh. 2nd Edition.

Yadav, N., Misra, G and Nigam, S. K., 1976. Triterpenoids of *Adenantherapavonina* bark. Planta Medica, 29: 176-179.

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Role of diet and Nutrition in Enhancing sporting performance

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Introduction

Sports and nutrition are interrelated. Without proper nutrition, an athlete cannot perform well, no matter how skilled they are. Any physical activity requires energy, and without proper nutrition, our body cannot release enough energy. Nutrition plays a very important role in our growth and development. It is important to maintain good health. Nutrition is the science of eating, where ingested food is digested, nutrients absorbed and distributed to tissues for use. The link between good health and good nutrition is well established. Today, the interest in nutrition and its effect on sports performance is a science in itself. Whether you are a competitive athlete, a weekend athlete or a dedicated daily exerciser, a nutritionally adequate diet is the foundation of better performance.Nutrition is increasingly recognized as a key component of optimal sporting performance, with both the science and practice of sports nutrition developing rapidly.[Burke et al.,2012]. The basic concept for sports nutrition for athletes requires proper eating strategies and need to have a command of general nutrition as well as exercise science. The second step is to gain the knowledge of how nutrition and exercise science are intertwined, emphasize that physical training and dietary habits are reliant on each other in order to produce optimal performance [Prochaska 1997]. The final step is the practical application of sport nutrition knowledge on the individual sports person who is participating in any sport or physical activity.Why study sports nutrition? An athlete challenges his body on a regular basis through physical training and competitions. In order to keep up with requirement of his activity or sport, he requires enough fuel for his body on day-to-day basis (Burke *et al*., 2011).

Nutritional needs

Training programs require a well-designed diet for active adults and competitive athletes. Research shows a balanced nutrition plan should include sufficient calories and healthy macronutrients to optimize athletic performance. The body will use carbohydrates or fats as the main energy source, depending on exercise intensity and duration. Inadequate caloric intake can impede athletic training and performance (Sharma *et al*., 2016).

The basic training diet should provide sufficient energy and nutrients for exercise and exercise needs, thatimproves adaptation and recovery between exercisesincludes a wide variety of foods such as whole grain breads and cereals, vegetables (especially leafy greens), fruits, lean meats and low-fat dairy products to promote long-term dietary patterns and behaviors,enables the athlete to achieve optimal weight and body fat percentage,Ensure adequate hydration to ensure maximum fluid intake before, during and after exercise, promote the health of athletes in the short and long term.

Athlete's diet

The athlete's diet should be similar to that recommended for the general public, and the energy consumption is divided as follows

45-65% carbohydrates

15-25% protein

20-35% fat

Athletes who exercise more than 60-90 minutes a day may need to increase their energy intake, especially from carbohydrates. There are also guidelines for carbohydrate and protein intake in grams per kilogram of body weight (g/kg).Current fat intake recommendations suggest that most athletes should follow general population recommendations and prefer fats from olive oil, avocado, nuts and seeds. Athletes should also take care to minimize the consumption of fatty foods such as cookies, cakes, pastries, French fries and fried foods.

Carbohydrates and sports

During digestion, all carbohydrates are broken down into sugars (mainly glucose), which is the body's main source of energy. After ingestion, glucose can be converted to glycogen and stored in the liver and muscle tissue. It can then be used as a primary source of energy during exerciseto fuel muscle tissue and other body systems. Athletes can increase their glycogen stores by

regularly eating carbohydrate-rich foods.If the intake of carbohydrates is limited in the diet, performance may deteriorate because the body is not supplied with enough glycogen. Inadequate protein intake can cause protein (muscle tissue) loss as the body begins to break down muscle tissue to meet its energy needs, and can increase the risk of infection and disease. Carbohydrates are essential for fuel and recovery Current recommendations for carbohydrate intake vary depending on the duration, frequency and intensity of exercise. Foods rich in unrefined carbohydrates, such as whole grain bread and cereals, should form the basis of an athlete's diet. Foods containing more refined carbohydrates (such as bread, jam and lollipops) can help increase total carbohydrate intake, especially in very active people. Athletes are advised to adjust the amount of carbohydrates they consume for fueling and recovery based on their performance. For example: - Light exercise (30 min/day): 3-5 g/kg/day - moderate intensity (60 min/day): 5-7 g/kg/day - Endurance training (1-3 hours/day): 6-10 g/kg/day.There may be situations in an athlete's training program that require limited carbohydrate intake. A recent strategy of some athletes is to train on a low volume and low carbohydrate basis (train low). There is growing evidence that carefully designed low-carb training can improve certain muscle adaptations to an exercise program. Carbohydrate ingestion has been shown to improve performance in events lasting approximately one hour (Jeukendrup, 2014).

Sports performance and the glycemic index

The glycemic index (GI) ranks foods and liquids according to how "carbohydrate" they are and how quickly they affect the body's blood sugar. GI is of increasing interest to athletes in the field of sports nutrition. In general, there is no evidence that manipulation of the GI has a significant effect on sports performance when the athlete's diet is adequate in carbohydrate and energy. However, the timing of consuming carbohydrate-rich foods with different glycemic indicators can be important in relation to exercise. There is evidence that pre-exercise low-GI foods may be beneficial in promoting sustained energy release, although the evidence for effectiveness is inconclusive. During exercise and early recovery, moderate to high GI foods and fluids may be most beneficial. However, it is important to adjust the type and timing of eating according to personal preferences and to maximize performance in the sport in which one participates. Eating before the event and eating before an event is an important part of an athlete's preparation for training. It is believed that a carbohydrate-rich meal 3-4 hours before training

has a positive effect on performance. Even a small snack 1-2 hours before training can have a positive effect on performance.

It is important to make sure you are well hydrated before the race. Consuming around 500ml of fluid in the 2-4 hours before a race can be a good general strategy. Some people react negatively to food right before exercise. A meal high in fat, protein or fiber is likely to increase the risk of indigestion. It is recommended to eat carbohydrate meals immediately before training, because they do not cause digestive problems. Suitable pre-workout meals and snacks include millet and low-fat milk, toast/buns/cookies, fruit salad and yogurt, pasta with tomato sauce, a low-fat breakfast or cereal bar, or low-fat rice with cream. Liquid supplements can also be especially beneficial for athletes who suffer from pre-competition jitters. For athletes competing in events lasting less than 60 minutes, rinsing the mouth with a carbohydrate-rich drink may be sufficient to improve performance. The benefits of this strategy seem to be related to effects on the brain and central nervous system. Eating during sportand during exercise lasting more than 60 minutes, consumption of carbohydrates is necessary to restore blood sugar and delay fatigue. Current recommendations suggest 30-60 g of carbohydrates, which can be consumed with lollies, sports gels, sports drinks, high-fat cereals and sports bars or bread sandwiches. It is important to start your carbohydrate intake early in your training and eat regularly during your training. It is also important to drink fluids regularly during a long workout to avoid dehydration. Sports drinks, diluted fruit juices and water are suitable for this. If you train for more than 4 hours, it is recommended to consume up to 90 grams of carbohydrates per hour. Eating after sports,after training, it is important to replenish glycogen stores quickly. Carbohydrate-rich foods and fluids should be consumed after exercise, especially within the first two hours after exercise. While adequate carbohydrate intake after exercise is important, the type of carbohydrate source can also be important, especially if a new exercise or competition is scheduled in less than 8 hours. In such cases, athletes should choose high GI carbohydrate sources (eg: bread, white rice, white potatoes) within the first half an hour after exercise. This should be continued until a normal eating pattern is restored. Suitable first meals include sports drinks, juices, cereal and low-fat milk, low-fat flavored milk, sandwiches, pasta, muffins/cookies, fruit and yogurt. Since most athletes become dehydrated during exercise, post-exercise hydration is also very important for optimal recovery. Athletes are advised to consume 1.25-1.5 liters of (non-alcoholic) fluid for every kilogram of body weight lost during exercise.

Protein and athletic performance

Protein is an important part of exercise nutrition and plays a key role in recovery and recovery after exercise. Most athletes meet (and often exceed) protein requirements by consuming enough energy in their diet. The amount of protein recommended for athletes is only slightly higher than the amount recommended for the general public.

Example:

For the general populace and physically active adults, the daily recommended protein intake is 0.8–1.0 g/kg (a 60 kg person should consume roughly 45–60 g of protein each day).Those who exercise for 45 to 60 minutes a day and are not endurance athletes should take in 1.0 to 1.2 g of protein per kilogram of body weight each day.People who work out for an extended period of time (more than an hour) or who participate in strength sports like weightlifting should ingest 1.2–2.0 g of protein per kilogram of body weight each day. Increased protein intake up to 2.0 g/kg/day can prevent muscle loss in athletes aiming to lose weight on a low-energy diet. Consume a high-quality meal before exercising if you want to increase your lean mass or muscle protein production.

Athletic performance with nutritional supplements

A well-planned diet covers the need for vitamins and minerals. Supplements are only useful if your diet is inadequate or if you have been diagnosed with a deficiency, such as a lack of iron or calcium. There is no evidence that additional vitamin supplements improve athletic performance. Dietary supplements are available in pill, tablet, capsule, powder or liquid form and include a variety of products including: Vitamins, Minerals, Herbs, Nutritional supplements and Sports nutrition products including natural foods. Before you start taking supplements, think about what else you can do to improve your athletic performancediet, exercise, and lifestyle changes are proven, costeffective ways to improve your performance. Relatively few nutritional supplements that promise performance have been scientifically proven. The use of vitamins and minerals is also potentially dangerous. Dietary supplements should not be taken without the advice of a qualified physician. An unbalanced diet can best be balanced by analyzing and changing the diet, not by taking supplements or pills. The ethical use of sports supplements is a personal decision for athletes and remains controversial. It is important to remember that when you take supplements, you are responsible for their use and for any potential health, legal or safety consequences. Taking supplements

also carries the risk of violating anti-doping rules, regardless of the level of your sport.

Aquatic and athletic activity

Dehydration can impair athletic performance and, in extreme cases, cause collapse and even death. Adequate hydration before, during and after exercise is very important. Don't wait until you're thirsty. Hydration is especially important in races lasting more than 60 minutes, high intensity or in hot conditions. Water is an appropriate drink, but sports drinks may also be necessary, especially at endurance events or in hot climates. Sports drinks contain some sodium to aid absorption. A sodium content of 30 mmol/l (millimoles per liter) seems appropriate for sports nutrition. While insufficient hydration is a problem for many athletes, overhydration can also be dangerous. In rare cases, athletes can consume too much fluid, which thins the blood too much and causes low sodium levels in the blood. This condition is called hypernatremia, which can lead to seizures, collapse, coma, or even death if not properly treated. A balanced diet A diet that contains the right amount of each nutrient, ie. carbohydrates, fats, proteins, etc., is called a balanced diet. A diet that contains all the necessary food components in the right proportions, ie. proteins, carbohydrates, fats, vitamins, minerals and water, is called a balanced diet. A balanced diet contains enough fiber and various nutrients (carbohydrates, fats, proteins, vitamins and minerals) to ensure good health. Food should also contain enough energy and enough water. Complete food contains sufficient amounts of all the important nutrients necessary for good growth and maintenance of the body. Nutrition is a dynamic process where the body becomes healthy by eating food. B. Nutrition: It is the acquisition and consumption of food or the breakdown of food and substances that are taken into the body as a source of energy. C. Nutrients: Energy rich foods in our diet.

Sports nutrition

It is the study and practice of nutrition and diet in relation to athletic performance. It deals with the type and amount of fluids and foods an athlete consumes, as well as nutrients such as vitamins, minerals and organic substances such as carbohydrates, proteins and fats. The body is made of 55-60% water, representing a nearly ubiquitous presence in bodily tissues and fluids. In athletics, water is important for temperature regulation, lubrication of joints and the transport of the nutrients to active tissues. It regulates the body’s

temperature, cushion and protects vital organs, aids the digestive system, acts within each cell to transport nutrients and dispel waste (Clark's Nancy,2008).

Macronutrients are mainly carbohydrates, proteins and fats and water, which are needed in large quantities and whose main task is to release energy in the body. Macronutrients are carbon, oxygen, hydrogen and nitrogen. Trace elementsare micronutrients, mainly vitamins and minerals that are needed in small amounts. However, both macro and micronutrients are important. Micronutrients include chlorine, iron, manganese, zinc, boron, sodium, copper, molybdenum and nickel.

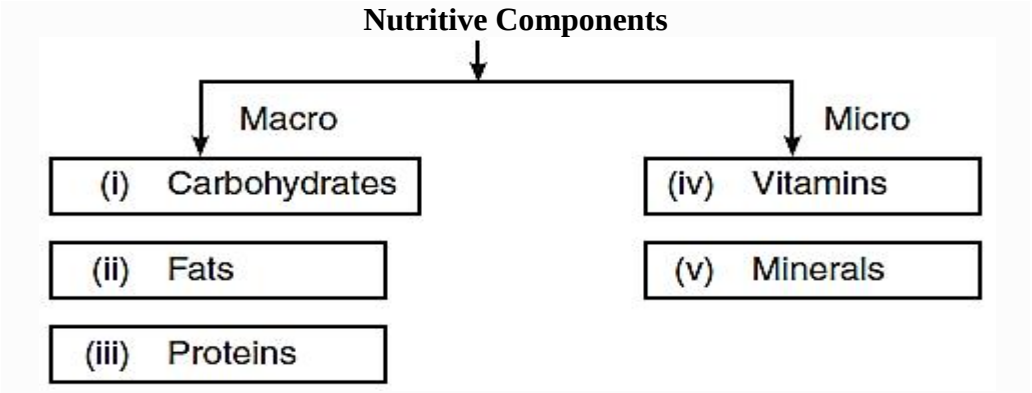

Figure 1: Nutritive Components of Diet
Courtesy:https://coolgyan.org/revision-notes/cbse-class-12-physical-education-notes-chapter-2-sports-and-nutrition

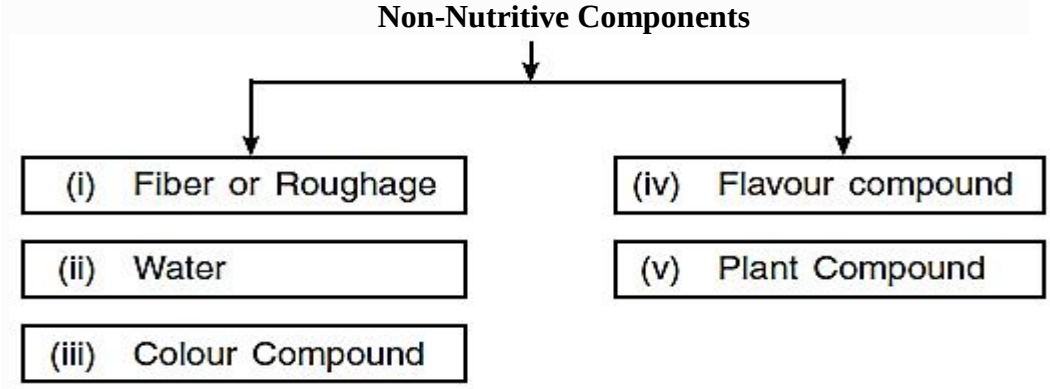

Figure 2:Non-Nutritive Components of Diet
Courtesy:https://coolgyan.org/revision-notes/cbse-class-12-physical-education-notes-chapter-2-sports-and-nutrition

Weight control

(i) A healthy weight is a weight that lowers your risk for health problems, generally body mass index (BMI) and waist size are good ways to achieve healthy weight.

Methods to calculate BMI = Weight in Kg/(Height in m)^2

Category	BMI
Under Weight	<18.5
Normal Weight Over Weight	18.5-24.9 25-29.9
Obesity Class I Obesity Class II	30-34.9 35-39.9
Obesity class III	>40

Table 1: BMI and Weight control

Courtesy:https://coolgyan.org/revision-notes/cbse-class-12-physical-education-notes-chapter-2-sports-and-nutrition

Nutritive components

Carbohydrates

Carbohydrates are needed to provide energy during exercise. Carbohydrates are stored mostly in the muscles and liver. Complex carbohydrates are found in foods such as pasta, bagels, whole-grain breads, and rice. They provide energy, fibre, vitamins, and minerals. These foods are low in fat. Simple sugars, such as soft drinks, jams and jellies, and candy, provide a lot of calories, but they do not provide vitamins, minerals, or other nutrients. Carbohydrates are stored in the body in a form of glycogen, which can be used during physical activity. Carbohydrate is necessary to meet the demands of energy needed during exercise, to maintain blood glucose level and replenish muscle glycogen store. During sub-maximal exercise, carbohydrates in the body are themajor source of fuel.

Protein

Protein is important for muscle growth and to repair body tissues. Protein can also be used by the body for energy, but only after carbohydrate stores have been used up. Only strength training and exercise will change muscles. Athletes, even bodybuilders, need only a little bit of extra protein to support muscle growth. Athletes can easily meet this increased need by eating more total calories (eating more food). Additional protein also helps muscles with maintenance, growth, and repair. For these reasons, athletes have higher protein needs than the general population. It is recommended that athletes consume 1.2 to 2.0 g/kg/day of protein in order to support these functions. Higher intakes may also be needed for short periods of intense training or when reducing energy intake (Thomas *et al*., 2016).

Fat

It provides the highest concentration of energy of all the nutrients. One gm of fat equals nine calories. One pound of stored fat provides approximately 3,600 calories of energy. Saturated fats are found primarily in animal sources like meat, egg yolks, yoghurt, cheese, butter, and milk. This type of fat is often solid at room temperature. Unsaturated fats include monounsaturated and polyunsaturated fats, which are typically found in plant food sources and are usually liquid at room temperature.Saturated fat, such as that found in butter and cream, as well as trans fat, which is found in snack foods and fried foods, have been shown to increase the risk for cardiovascular disease. While in turn, unsaturated fats, which are found in olive oil and canola oil, has been shown to decrease the risk of developing cardiovascular disease (Kinley, 2015). Fat is a secondary source of energy used during long-duration training sessions. Endurance athletes are more at risk for dehydration. Replacing fluids and electrolytes lost through sweat are necessary for peak performance (Pelly *et al*, 2014)

Vitamin

Vitamins are required in wide variety of bodily functions and operations which helps to sustain the body healthy and disease free. The function of minerals is for structural development of tissues as well as the regulation of bodily process (Srilakshmi, 2003). A well-planned and nutritionally adequate diet should meet an athlete‘s vitamin and mineral needs. Supplements will only be of any benefit if your diet is inadequate or you have a diagnosed deficiency, such as an iron or calcium deficiency. The use of vitamin

and mineral supplements is potentially dangerous, and they should not be taken without the advice of a qualified health professional.

Minerals

Minerals are categorized into major minerals (calcium, sodium, potassium, chloride, phosphorus, magnesium, and sulfur) and trace minerals (iron, zinc, copper, selenium, iodine, fluoride, molybdenum, and manganese) based on the total quantity required by the body on a daily basis. Similar to vitamins, minerals are found in a wide variety of foods, but mainly are concentrated in the meat and beans/alternative and milk/alternative groups (Holway, 2011).Minerals are very essential in our diet. Four percent of our body weight is made up of minerals. These are required for healthy teeth, bones, and muscles. It is also used by the body for various activities such as the transmission of nerve impulses, the formation of hormones, and the maintenance of the heartbeat. Macronutrients are nutrients that provide calories or energy to the body. The purpose of macronutrients is to promote healthy cellular growth, metabolism, and to maintain normal bodily functions. The macronutrients, as suggested by the name “macro,” are needed in the body in large amounts to provide the full and proper effect (Rowlands, 2015).

Macro minerals

a) Calcium: Calcium is among the top macro-minerals in terms of the growth and development of our bones and teeth. It helps with blood clotting. Its deficiency may cause rickets. The sources are cheese, milk, orange juice, eggs, green leafy vegetables, and cereals.

b) Potassium: Potassium is one of the most required minerals in a diet. It is helpful in keeping the nervous system and muscular system healthy and active all the time. It helps maintain the amount of water in the blood and tissues. Its main sources are bananas, tomatoes, green leafy vegetables, beans, etc.

c) Sodium: It helps in muscular activities. It also helps in the transmission of nerve impulses. The sources are table salt, pickles, butter, etc.

d) Magnesium: It repairs and maintains body cells. It is found in meat, brown rice, beans, whole grains, etc.

e) Phosphorus: Phosphorus helps in the formation of bones and teeth. It keeps the muscles and nerve activity normal. The sources are eggs, fish, liver, milk, unpolished rice, etc.

Micro minerals

a) Iodine: It produces the hormones for the thyroid gland. It is also significant for proper growth and development. Lack of iodine can cause goitre (swollen thyroid gland) and mental retardation. The sources are iodized salt, fish, and sea food.

b) Iron: it is essential in the production of haemoglobin. Its deficiency causes anaemia. The sources are meat, eggs, dry fruits, spinach, bananas, and green leaf vegetables.

c) Chromium: it is essential in the production of haemoglobin. Its deficiency may cause diabetes. The sources are soy beans, blackgram, carrot, tomato, groundnuts, bajra, and barley.

Non-Nutritious Components

a) Water

b) Roughage

c) Artificial sweeteners

d) Preservatives

e) Plant products

Fiber or roughage has no nutritive value. It is an undigested part of the food, or it can be said that it cannot be digested by the human intestinal tract. It consists of water and improves intestinal function by adding bulk to the food. It helps the individual satisfy their appetite. It prevents constipation.

Factors to Control Body Weight

There are many factors that contribute to a person's weight, including: diet, physical activity, genetics, environmental factors, health care support, medications, and illnesses (Goldman *et al*., 2020). The common factors involved in controlling Body weight includes, balanced diet, drinklots of water, eating a lot of fibrous food, regular medical checkup, avoidfats, medicine only by doctor’sadvice, physicalactivity, avoiddrinking, avoid junk food, meals in small shifts, follow hygienic habits, never try slimming pills, avoid over eating, balancing the intake and expenditure of calories(Mendenhall*et al* ., 2019).

Nutrition before competition

At least a week before the competition, athletes should take complex carbohydrate foods, which usually help increase glycogen stores. The fuel for

the muscles is usually provided in meals 3–4 days prior to the competition. The diet should depend on the intensity of the activity. The diet should be rich in carbohydrates and low in fat and protein. Two hours before the competition, a high-carbohydrate energy drink can be considered sufficient(Katzmarzyk *et al*., 2020).

Nutrition during competition

It is important to stay hydrated and maintain a healthy sugar level so that athletes do not experience fatigue. If the duration of the competition is more than 60 minutes, drink 12 to 1 cup of carbohydrate after 10–20 minutes, and if the duration is less than 60 minutes, drink carbohydrate after every 20–30 minutes.

Nutrition after competition

After competition, it is important to recover properly, so the first preference should be given to the replacement of fluid loss, which can be easily done by the intake of water or a replacement drink. Meals after competition should be taken within 2 hours. For best glycogen restoration, 100–200 grams of carbohydrate along with lean protein like meat or chicken should be taken. It will help in building, maintaining, and repairing muscles. At least 20 grams of protein are required after completion for complete recovery.

Advantages of Food Supplements

(a) Supplements can contribute to improving muscular strength, endurance, and overall physical performance.

(b) Some supplements are used in combination with drugs as a method of complementary or alternative treatment of health conditions.

(c) Food supplements give vitamins and minerals that protect the body from disease.

Disadvantages of food supplements

(a) Food supplements can cause adverse side effects if they are not consumed in the right quantity. They can damage the liver and reduce bone strength.

(b) Weight-loss supplements may contain numerous untested ingredients that have not been examined for safety or effectiveness in children. The possibility of product contamination is the main safety concern about

dietary supplements for both children and adults, but the danger may be greater for children.

Conclusion

Sports and nutrition are interrelated, and proper nutrition is crucial for athletes' growth and development. Nutrition is the science of eating that digests food, absorbs nutrients, and distributes them to tissues for utilization. Athletes should have a basic training diet that provides sufficient energy and nutrients for training and exercise, improves adaptation and recovery between training sessions, and includes a wide variety of foods such as whole grain breads, cereals, vegetables, fruits, lean meats, and low-fat dairy products.The diet of an athlete should be similar to that recommended for the general public, with energy intake divided into 45 to 65 % carbohydrates, 15 to 25 % protein, and 20 to 35% fat. Athletes who exert themselves for more than 60 to 90 minutes a day may need to increase their energy intake, especially from carbohydrates.Carbohydrates are essential for fuel and recovery, and recommendations vary depending on the duration, frequency, and intensity of exercise. Foods rich in unrefined carbohydrates should form the basis of the athlete's diet, while refined carbohydrates are useful for increasing total carbohydrate intake.Training with low carbohydrate availability may be necessary in an athlete's training program, but the benefits of this approach to athletic performance are currently unclear. The glycemic index (GI) ranks foods and liquids based on how "carbohydrate-rich" they are and how quickly they affect the body's blood glucose levels. Meal before the event is an important part of the athlete's preparation for training, with a carbohydrate-rich meal 3 to 4 hours before training having a positive effect on performance. A small snack one to 2 hours before training can also have a positive effect on performance.Eating during sports is necessary to replenish blood glucose levels and delay fatigue. Current recommendations assume 30 to 60 g of carbohydrates, which can be ingested in the form of lollipops, sports gels, sports drinks, low-fat cereals, sports bars, or sandwiches with white bread. It is important to begin carbohydrate intake at the beginning of exercise and consume regular amounts throughout the exercise period.Eating after sports is crucial for athletes' overall health and performance.

References

Burke LM, Hawley JA, Wong SH and Jeukendrup AE., 2011. Carbohydrates for training and competition, Journal of Sports Science 29(1): S17-S27.

Burke LM, Meyer NL, and Pearce J.,2013. National nutritional programs for the 2012 London Olympic Games: A systematic approach by three different countries. In: van Loon LJC, Meeusen R, editors. Limits of Human Endurance. 76:103–120.

Clark's Nancy, 2008. Sports nutrition guide book: The 1st Nutrition resources for active people, Health work fitness center chestnut hill, MA, USA, 103-105.

Goldman L, and Schafer AI, 2020. Goldman-Cecil Medicine (26th ed.). Philadelphia, PA: Elsevier. pp. 1418–1427. ISBN 978032353266.

Katzmarzyk PT, Martin CK, Newton RL, Apolzan JW, Arnold CL, Davis TC, 2020. Weight Loss in Underserved Patients - A Cluster-Randomized Trial. The New England Journal of Medicine. 383 (10): 909–918. doi:10.1056/NEJMoa2007448

Holway FE and Spriet LL.,2011. Sport-specific nutrition: practical strategies for team sports. Journal of Sports Science, 29:115-S125.

Jeukendrup A, 2014. A step towards personalized sports nutrition: carbohydrate intake during exercise. Sports Medicine:44(Suppl 1):S25–S33.

Kinley, 2015. Health center, Macronutrients: The importance of Carbohydrate, protein and fat.www.mckinley.illinois.edu/handouts/macronutrients.

Mendenhall E and Singer M, 2019. The global syndtic of obesity, undernutrition, and climate change. Lancet. 393 (10173): 741. doi:10.1016/S0140-6736(19)30310-1

Pelly F, Meyer NL, Pearce J, Burkhart SJ, and Burke LM., 2014. Evaluation of food provision and nutrition support at the London 2012 Olympic Games: the opinion of sports nutrition experts. International Journal of Sport Nutrition Exercise Metabolism, 24: 674-683.

Prochaska JO, and Velicer WF, 1997. The transtheoretical model of health behavior change. American Journal of Health Promotion 12: 38-48.

Rowland D,Houstham S,Musa-Veloso K, Brown F, Paulionis L, and Bailey D.,2005. Fructose- Glucose Composite Carbohydrates and endurance

performance: Critical review and future perspectives. Sports Medicine, 45:1561-1576

Srilakshmi B, 2003. Food Science. New Age International, New Delhi,375-380,ISBN 978-8122414813,

Sharma S, Sharma A and Bhushanam G.V.2016. Assessment of the knowledge of the adolescent female football players regarding the carbohydrate and its importance, Journal of Sports Science 4: 102-104.

Thomas, D. T., Erdman, K. A., & Burke, L. M., 2016. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance, Journal of the Academy of Nutrition and Dietetics, 116(3): 501-528.

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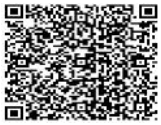	Subject: Food and Nutrition
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Enhancing the Sustainability and Performance of Bio-Concrete: A Comprehensive Review of Microbial-Based Construction Materials

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Introduction

Through the evolution of humans, concepts of building a shelter or tools for survival also changed over time. Early humans who lived inside caves and burrows, along with the development of the intellect began to construct buildings of habitations and various utensils by using different materials found in the surrounding environment. These materials used in construction were different according to the civilization and their location. Ancient civilizations used dried or burned clay bricks, carved stones, and limestone commonly in building larger shelters, which were combined with wooden structures to enhance structural integrity. Tools and utensils for their usage are also commonly derived either from wood, stone, or clay (Carran, et al., 2012; Lucas, 1935).

Initially recorded indications of the man-made concrete-like mixture were recorded in ancient Roman constructions, which is called lime mortar, it is a mixture of volcanic rock, limestone, sand, and water. This replaced the earlier invented clay and gypsum mortar which was used in Egyptian pyramids and buildings. In the 18^{th} and 19^{th} centuries, European architecture was built with mud-based building materials which are called ‘Pise’, and it was mixed with lime mortar to improve integrity. John Smeaton, an engineer from Leeds, England, commenced his investigations in 1755 to create a cement that surpassed the effectiveness of lime mortar previously employed. Through his

experiments, Smeaton formulated the fundamental concepts of hydraulicity which are the property important in the setting and waterproof ability of cement-like materials (Setchell, 1970; Wang, 2013). Similarly, in 1824, Joseph Aspdin, a skilled bricklayer hailing from England, made a significant breakthrough in the development of modern concrete. He was able to obtain a patent for producing what can be considered contemporary concrete, utilizing Portland cement. The 'Portland Cement' earned its name due to its resemblance in colour to the limestone found on the English Isle of Portland, once it solidified (Flatt, et al., 2012).

Initially, a suggested method to improve concrete's qualitieswas created by mixing different materials like rice husk ash, silica fume, fly ash, and a few other materials into the mixture of concrete. Also, these mixing components are required to be prepared in specific methods in order to be suitable to mix with concrete, for example, rice hull ash is subjected to controlled temperatures ranging from 500-700 , which will create a highly reactive form of ash that can serve as a pozzolanic material. Pozzolanic substances refer to materials that contain Silicon and Aluminium which undergo a chemical reaction with calcium hydroxide, resulting in the formation of compounds that exhibit cement-like properties. Considering to Aluminium concentration in rice hull ash, Silica concentration is much higher, which is around 80-85%. But it can vary depending on the burning temperature of the rice hull (Kishore, et al., 2011; Zain, et al., 2011). When the concentrations of rice hull ash mixed with cement are ranged between 5-20%, it increases the strength of cement and also, decreases the porosity of concrete and water absorption significantly (Ephraim, et al., 2012; Saraswathy, and Song, 2007). Similarly, fly ash can be mixed with cement replacing a portion of regular sand in the mixture, which will increase its strength and reduce porosity. Also, the addition of 15% Metakaolin into the cement mixture increases its compressive strength while reducing porosity, which is beneficial for the longevity of structures (Brooks, et al., 2001; Shannag, 2000).

But when these materials were replaced or used together with bacterial solutions, it showed a significant improvement in the qualities of concrete, which was a concept known as "Bioconcrete" developed by a microbiologist named Hendrik Jonker. He suggested the healing of concrete cracks similar to that of human bone cracks. Bacterial species which are capable of producing limestone is used with the mixture of concrete in the production of bioconcrete (Koushal, 2020). These organisms are commonly alkalophilic bacteria that can survive in harsh conditions with lacking nutritional content (Kumar, 2017)

Avoidance or reduction of micro-pores or complex pore structures inside the concrete is the main objective of the invention and production of bioconcrete. After completing the construction of a structure, certain external factors can affect the integrity of the concrete, which increases the deterioration process. Severe weather conditions, climate change, and elevated temperaturesare among the factors that influence the overall lifespan of concrete, resulting in its early degradation. After the solidification process of concrete, due to above mentioned natural phenomenon micro-cracks and pore structures form, and if ignored they will eventuallyincrease the permeability of the concrete(Ramachandran et al, 2001). This will allow external factors to interact with the concrete structure more effectively. The majority of these detrimental factors involve the chemical involvement on the concrete itself or on the reinforcing steel rebar contained within. Furthermore, the interlinked network of pores and the existence of micro-cracks facilitate the infiltration of harmful substances such as water, carbon dioxide (CO_2), and reactive ions like sulfate (SO_4^{2-}), chloride (Cl^-), and nitrate (NO_3^-) leading to the corrosion of the steel reinforcement (Achal, et al., 2015; Basheer, et al., 2001; De Muynck, et al., 2008). It is nearly impossible to prevent the formation of all these micro-scaled openings in a structure, but there are suggested methodologies in order to minimize or reduce the number of these cracks allowing for a decline in the deterioration rate. This can be achieved by using organic or inorganic filling material, which can be liquid concrete mixtures or pastes. Siliconized fillers, Latex fillers, Epoxy fillers, and other sealers. But these crack-repairing processes can be extra time-consuming as well as costly procedures (Yuan, et al., 2008).

It has been clear that the best solution for the above-mentioned difficulties is microbial-based concrete or bio-concrete production. Through introduction of this technology, it allowed to decrease the deterioration rate of concrete, enhance compressive strength, and decrease pore formation in concrete (Achal, et al., 2011). In this biotechnological method, bacterial species are used with the concrete, allowing them to precipitate Calcium carbonate ($CaCO_3$) (Gollapudi, et al., 1995; Kantzas,et al., 1992; Zhong,and Islam, 1995). This process is called microbiologically induced Calcium Carbonate Precipitation or MICP, which mostly focuses on establishing a self-repairing concrete (Jonkers, et al., 2010), which contains significantly higher compressive strength and stiffness value compared to conventional concrete without bacterial cells(Ramachandran, et al, 2001).

What aremicrobial-based construction materials?

Microorganisms-based construction material development can be considered a significant aspect in the evolution of construction materials. Unlike previous inventions of construction materials through mixing, in this method, microorganisms and their products are utilized in improving basic construction material properties. Throughout the world, microbial-based concrete usage has been reported under different research aspects.

Bioconcrete, also known as Self-mending concrete, has a rich history of application dating back to the 1990s when it was utilized to restore and preserve historically significant structures such as Thouars church tower, Angera Cathedral, Alcázar de Guadalajara, and Castillo de Chapultepec. Noteworthy research endeavors have extensively explored the use of diverse microorganisms like *B. subtilis*, BKH4 (alkaliphilic bacterium), *B. pseudofirmus*, *Bacillus cohnii*, *B. alkalinitrilicus*, *Diaphorobacter nitroreducens*, *Bacillus sphaericus*, and *Streptococcus aureus*. These microorganisms have proven effective in repairing cracks that measure less than 0.5 mm, through their targeted and specialized activities (Aguilera, et al. 2015;Adzami, et al. 2018; Ersan, et al. 2015; Jonkers, et al. 2010;Jroundi, et al. 2014; Khaliq, & Ehsan, 2016; Le Metayer-Levrel, et al. 1999; Perito, et al. 2014; Sarkar, et al. 2019; Shukla, et al. 2022; Tiano, et al. 1999; Wang, et al. 2012).

In the United States of America, Belgium, and India, microbial-based construction materials were suggested to be used as a better replacement for chemical polymers, allowing selective permeability in oil refining, where zones with significant water permeability could therefore be blocked off (Gollapudi, et al., 1995; Hart, 1960; Jack, et al., 1991; Lappin-Scott, et al., 1988). Similarly, a system of microbial mineral plugging based on Carbonate precipitation was proposed, which was initially used in reducing the permeability and pores of sand columns. This was later recognized to strengthen the structures as carbonates bind sand particles together (Jack, et al, 1993; Kucharski, et al, 2006; Whiffin, 2008; Zhong, and Islam, 1995). While developing this technique it was understood that it is suitable to utilize the urea hydrolysis pathway in obtaining the Carbonate ions, as bacterial species with the ability to process Urea are available in the soil, wastewater, and other environments, for example, *Sporosarcinaureae*, and *Sporosarcinapasteurii* (Fujita, et al, 2000; Mobley, and Hausinger, 1989), which was understood can be used in the consolidation of sand (Gollapudi, 1995;Kantzas, et al, 1992). Later this technique of strengthening sand by Calcite precipitation was used in repairing cracks in the granite. The mixture used contained bacterial species (*S.*

pasteurii), required nutrients, and, other filler materials such as sand, and silica fume (Zhong, and Islam, 1995). Oxygen presence and pH or alkaline condition of the concrete are also other important factors that can determine the proliferation of these bacteria. For example, *S. pasteurii* requires a pH level of 9 in order to be metabolically functional to carry out Calcite precipitation (Ramachandran, et al, 2001). Even though the regulation of oxygen is difficult in a concrete mixture, pH levels can be maintained by using certain compounds, such as silica fume, fly ash, polyurethane, sand, and lime. But the highest improvement in strength and durability can be achieved by using polyurethane. In order to understand the effectiveness of various methods which can be used in Calcite precipitation, research was carried out with microorganisms' incorporation, free enzymes (Urease), and immobilized enzymes (Urease). Where free enzymes had shown better rates of precipitation than the immobilized enzymes. Similarly, silica gel immobilized microorganisms such as *Bacillus sphaericus* were used in a study to investigate the carbonate precipitation abilities, where it was shown abilities to bridge cracks and form carbonate crystals, upon introduction of salts, urea, and Calcium Chloride (Bachmeier, et al, 2002;De Belie, and De Muynck, 2008).

Biological mechanism of biomineralization

The mechanism behind the bio-concrete is based on the biomineralization process of microorganisms. The process of biomineralization refers to the creation of minerals by microorganisms through biological means. Biomineralization is a common occurrence in various natural environments and involves microorganisms from different taxonomic groups with diverse metabolic pathways. Through microbial activity, minerals like sulfates, sulfides, oxides carbonates, phosphates, silicates, or hydroxides are formed, often with various cations such asMg^{2+}, MnO_2, Ca^{2+}, Fe^{3+}, and Fe^{2+}. Organic macromolecules found in the cells facilitate these productions by providing structural support and these molecules include proteins, polysaccharides, glycoproteins, and proteoglycans (Fu, et al., 2005;Ghosh, et al., 2009;Sarayu, et al., 2014).

In bacterial species, the biomineralization process can be categorized into three prominent mechanisms. They are Biologically Mediated Mineralization (BMM), Biologically Controlled Mineralization (BCM), and Biologically Induced Mineralization (BIM) (Weiner, and Dove, 2003).

a) BCM and BIM processes

In Biologically Controlled Mineralization (BCM), the microorganism's metabolic activity governs the processes of nucleation, composition, localization, and morphology of biominerals. This mechanism encompasses extracellular (BCMe), intracellular (BCMin), and intercellular (BCMint) processes. In the BCMeprocess, cellular cations are released through passive diffusion into the extracellular matrix, which will interact with anions forming biominerals. Similarly, in the BCMin process, cellular cations are transported to the epithelial surface by vacuoles and they will interact with secreted anions by another cell to form biominerals. BCMint involves the organic macromolecules such as exopolysaccharides (EPS) or vesicles in the biomineralization process. Where cation and anion interaction will take place inside the cell and formed minerals are carried outside and positioned on the cell surface by vacuoles (Weiner and Dove, 2003).

Biologically Induced Mineralization (BIM) involves the indirect precipitation of minerals through the interactions between microbial metabolic byproductsand ions in the surrounding environment such as Hydroxide (OH^-), Phosphate ions (PO_4^{3-}), and Carbonate ions (CO_3^-). However, microbial cells have limited involvement in the composition, localization, and nucleation of these minerals. The minerals produced by BIM exhibit a diverse range of particle sizes, low crystallinity, and distinct morphologies (Weiner, and Dove, 2003).

b) BMM Process

The BMM process does not involve intracellular or extracellular biological activities, but the organic or inorganic molecules or both interact with each other in an organic matrix. (Achal, et al., 2015; Dupraz,et al., 2009; Weiner, and Dove, 2003). In the 2009 records of Dupraz et al., he suggested using the term “Organomineralization” in understanding the mechanisms of this process and exclusion of Biologically Induced Mineralization (BIM) and Biologically Mediated Mineralization (BMM) from Biomineralization. The biominerals and organominerals that are most prevalent are primarily composed of calcium, as this element plays a crucial role in various essential cellular metabolic processes of organisms. From the total of biominerals and organominerals formed, Calcium containing minerals are above 50%, including Calcium Carbonate ($CaCO_3$), which is one of the most abundant minerals on Earth. Precipitation of Calcium Carbonate ($CaCO_3$) is known as Microbial Precipitation of Calcium Carbonate (MCP) which widely occurs in marine water sediments, soil, freshwater bodies, and other environments contributing to approximately 4% of the weight of the Earth’s crust. The Microbial

Precipitation of Calcium Carbonate (MCP) process can take place by either active or passive methods. The active form of MCP involves Microorganisms Induced $CaCO_3$ Precipitation (MICP), while the passive form is Biologically Mediated Mineralization, which occurs without direct biological activity. Instead, this relies on interactions between Calcium ions and Organic Extracellular Polymeric Substances (EPS) matrix. As a result of higher pH levels, deprotonation occurs inFunctional groups such as sulfate groups (R–O–SO_3H), sulfhydryl groups (–SH),hydroxyl groups (R–OH),carboxylic acids (R-COOH), and amino groups (R–NH_2), found inEPS produced by the cells. This deprotonation leads to an overall negative charge to the surface of the cell, facilitating their binding to metal ions like Calcium ions. The properties of the EPS Organic Matrix can determine the structural and mineralogical characteristics of Crystalized Calcium Carbonate (Dupraz,et al., 2009;Perito, andMastromei,2011;Sarayu, et al., 2014).

There are two major metabolic pathways involved in the biomineralization process found in microorganisms (Joshi, et al, 2017).

i. Autotrophic pathway

In this pathway, Caron dioxide is used as the main carbon source, where it is converted by microorganisms when Calcium ions are present in their surrounding environment. This can take place through oxygenic or anoxygenic photosynthesis and non-methylotrophic methanogenesis, where precipitation of Carbonates occurs (Castanier, et al, 1999/2000; Grengg, et al, 2015; Seifan, et al, 2016).

ii. Heterotrophic pathway

This pathway takes place inside microorganisms that are capable of carrying out Nitrogen and Sulphur cycles, resulting in Carbonate precipitation. These processes are anaerobic in nature and require ions like Calcium, Sulfate, and organic matter in abundance. Bacteria utilizing the Sulphur cycle are called Sulphate Reducing Bacteria or SRB, which carry out a dissimilatory reduction of Sulphate. In this process, Sulfate (SO_4^{2-}) acts as the final electron acceptor in the breakdown of organic matter while producing Bicarbonate ions (HCO_3^-) and Hydrogen sulfide (H_2S). It is understood that these bacteria carry out these reactions when the requirements are fulfilled. H_2S degasification is necessary in order to precipitate Calcium Carbonate ($CaCO_3$). In this biological process, degasification of H_2S occurs with the rise of pH level in the environment by the presence of ions like Calcium (Ca^{2+}) (Castanier, et al, 1999; Hammes, and Verstraete, 2002;Muyzer, andStams, 2008). Bacteria using the Nitrogen cycle

will precipitate Calcium Carbonate through three mechanisms, depending on the oxygen and nutrient requirement. Amino acid ammonification occurs in aerobic conditions with the presence of Calcium and organic matter, while dissimilatory nitrate reduction occurs in anaerobic conditions and requires Nitrate apart from Calcium and organic matter. The final mechanism, degradation of urea occurs in aerobic conditions in the presence of Urea, Calcium, and organic matter (Castanier, et al, 2000). As a result of these mechanisms, highly basic compounds like ammonia (NH_3) are formed which will contribute to elevation in the pH levels. These high pH levels result in more Carbonate ion (CO_3^{2-}) formation which will react with free Calcium ions (Ca^{2+}) to form Calcium Carbonate ($CaCO_3$). But it should be noted that the most commonly used pathway in the production of bioconcrete is the Urea hydrolysis pathway(Joshi, et al, 2017).

Microbial-based bio-concrete

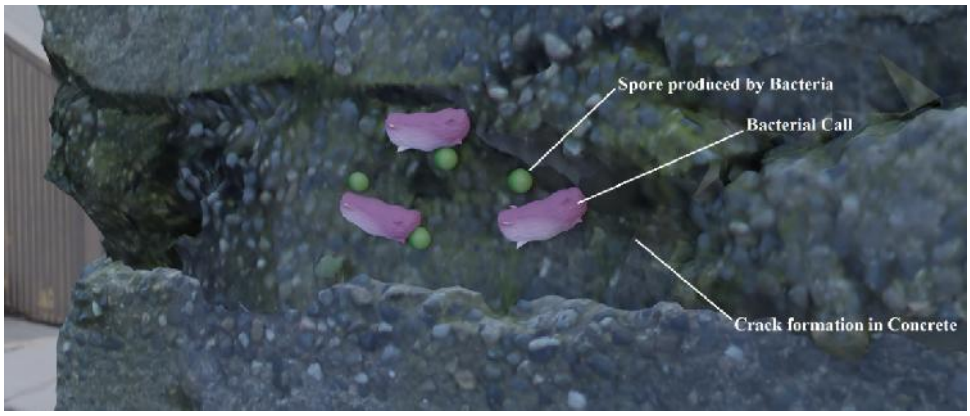

Figure - 1: Bacterial Cells and Spores on Concrete Cracks

The concept of producing self-repairing concrete was initially suggested in the 1990s and has since developed in different ways. As mentioned above, in this technique, selected bacterial strains which can carry out Calcium Carbonate precipitation. The main objective of this technique is to reduce the pore formation and control the pore amount of the concrete, after its solidification. Mostbacterial species used in this process are capable of Calcite, which is the carbonate mineral of the most stable form of Calcium Carbonate (Bang, et al., 2001;Dhami, et al.,2014). These bacteria are commonly alkali-resistant, spore-forming bacteria, which produce spores upon exposure to harsh conditions found inside the cement. But when the solidified concrete develops cracks and pores, previously inactivated spores are reactivated as they are

exposed to moisture and oxygen. As a result, these spores germinate by utilizing the substrate materials added to the cement mixture, which is present within the crack (Figure - 1).By the metabolic activity of these bacteria, these substrates can be transformedinto Calcium Carbonate viaa sequence of biochemical reactions.

This process leads to the formation of Calcium Carbonate precipitation, effectively accomplishing the objective of self-healing the crack (Bundur, et al, 2015; Luo, and Qian, 2016). This Calcium Carbonate ($CaCO_3$) precipitation improves the overall strength, durability, and compressive strength of the concrete compared to untreated concrete with bacterial species (Achalet al., 2013; Chahal, et al, 2012).

Initially, the concept of restoring cracks in a structure by using microorganisms was suggested by J. P. Adolphe and colleagues in 1990, while obtaining the patent for the technique which was mentioned as the Bioconcept of Calcite or Biodeposition. This is currently referred to as bioconcrete, where the incorporation of bacteria, which are capable of precipitating Calcium Carbonate or MICP. This unique property of bioconcrete enables it to effectively seal cracks that develop in the material, resulting in a self-healing characteristic (Wiktor, and Jonkers, 2011). Bioconcrete consists of three major components such as microorganisms capable of the MICP process, essential nutrients for microbial growth, and source of Calcium ions required for Calcium Carbonate (CaCO3) precipitation (Achal et al., 2015).

Essentials for quality bioconcrete

Throughout the past few years, the quality of bioconcrete has been tried to improve by adding different nutrients and other materials. Nutrients such as peptone, urea, yeast extract, calcium nitrate, and sucrose, are essential for the growthof microorganisms and other components such as sol-gel ceramsite sand, polyurethane, lactate and magnetic iron oxide nanoparticle (IONS) are also important in immobilizing and stabilizing bacterial cells. These strategies have been the subject of numerous studies over the past two decades. The main objective of these techniques is to help the bacteria to survive in high pH or nutrition-restricted and water ingress conditions inside the concrete matrix. The selection of suitable bacterial strain also, play an important role in the development of better concrete, several studies have shown the utilization of bacterial strainssuch as *Escherichia coli* and *Pseudomonas aeruginosa* is insignificant in establishing compressive strength, but usage of bacterial strains such as *Sporosarcinaureae, Shewanella, Bacillus mucilaginous L3, and*

Sporosarcinapasteurii are able to provide better compressive strengths to concrete (Achal, et al., 2009;Bang, et al., 2001;Chen, et al, 2016;Ghosh, et al, 2005; Ramachandran, et al, 2001).

It is necessary to provide specific nutrients, and other materials in appropriate amounts in order to obtain the highest compressive strength in bioconcrete. Certain materials can reduce the compressive strength rather than increase, for example, when nutrients likeyeast extract, calcium nitrate, and urea are used with encapsulated bacterial spores, compressive strength was reduced. Utilization of urea as a form of nutrient was more efficient in free spores than the encapsulated spores(Wang, et al, 2014;Xu, and Wang, 2018).But encapsulation can enhance the survival of these microorganismsand the efficiency of encapsulated microorganisms or spores depends on several factors such as the dispersal rate of the encapsulated cells, the size of the encapsulation, and the encapsulating materials (Castro-Alonso, et al, 2019).

The addition of certain external materials such as iron oxide nanoparticles or IONpsand Magnesium Carbonate hydrate ($MgCO_3 \cdot 3H_2O$) into the concrete mixture can enhance the precipitation efficiency of Calcium Carbonate ($CaCO_3$) and the growth of the bacteria (Ruan,et al, 2019;Seifan, et al, 2018). High costs due to the usage of bacterial growth nutrients can be reduced by the addition of cheaper counterparts such as Activated sludge, Lentil seeds, sugar, Corn Steep Liquor, wastewater of Tofu production, and Mother Liquor (Achal, et al, 2010;Charpe, et al, 2017;Fang, et al, 2019; Zhang, et al, 2017).

For the improvement of bioconcrete production genetically modified bacterial strains containing beneficial enzymes have been documented. For example, genetically modified *Bacillus subtilis* incorporated with a gene encoding Bioremediase enzyme has shown enhanced micro-crack repair ability and compressive strength (Sarkar et al, 2015).

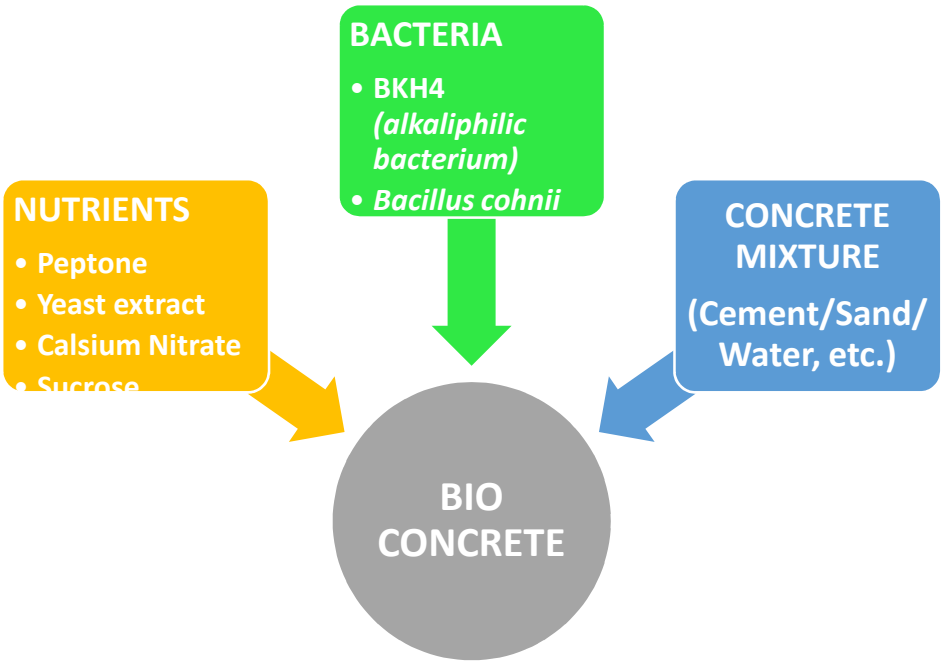

Figure - 2: Components of Bioconcrete

Advantages of bio-concrete

Conventional methods of producing building materials can cause health and environmental-related issues including depletion of natural resources. The search for resources in the case of construction material production has contributed to extensive deforestation endangering indigenous wildlife and plants. Granite is one of the main ingredients in producing conventional concrete mixtures, which can produce a high amount of fine dust that pollutes the air. This contaminated air can cause severe health complications in humans such as bronchitis, silicosis, asthma, and other respiratory conditions. Similarly, Marble dust is used in the process of the cement industry and masonry which can impact human health and the usability of farmlands (Jagadish, 2008).

The process of producing one ton of cement is estimated to emit one ton of Carbon dioxide (CO_2) which is a significant amount effect on the global atmosphere. Annually this amount is 7% of the total world CO_2 production (Mehta, & Monteiro, 2005). The production of bioconcrete is an eco-friendly and cost-effective technology as it reduces the overall CO_2 emission and expenses related to maintenance (Achal, et al., 2015).Bacterial presence in the concrete can provide enhancement in the concrete's resistance against alkali

agents, reactive sulfates, shrinking of concrete due to drying,and rapid cooling and heating effects. Also, the addition of bacterial species like *S. pasteurii*, and *Shewanella*, to the concrete mixture can improve the compressive strength (Ghosh, et al, 2005/ Ramachandran, et al, 2001). In recent years the focus of bioconcrete production has focused on bio-sequestration, where the greenhouse gases like CO_2 are absorbed in the process of making Calcite. Carbonic anhydrases and Ureases like enzymes catalyze this bio-sequestration process, which is produced by microorganisms (Alshalif, et al, 2022). Through another study, it was revealed that the usage particular concentration of fly ash content with selected microorganism solutions can significantly improve the compressive strength of concrete while preventing the formation of micro-cracks (Huseien et al, 2022).

References

Achal, V., Mukherjee, A., and Reddy, M. S., 2010. Biocalcification by *Sporosarcinapasteurii* using corn steep liquor as the nutrient source. Industrial Biotechnology, 6(3), 170-174.

Achal, V., Mukherjee, A., and Reddy, M. S., 2011. Microbial concrete: way to enhance the durability of building structures. Journal of Materials in Civil Engineering, 23(6), 730-734.

Achal, V., Pan, X., Lee, D. J., Kumari, D., and Zhang, D., 2013. Remediation of Cr (VI) from chromium slag by biocementation. Chemosphere, 93(7), 1352-1358.

Achal, V., Mukherjee, A., Kumari, D., and Zhang, Q., 2015. Biomineralization for sustainable construction–A review of processes and applications. Earth-Science Reviews, 148, 1-17.

Adolphe, J. P.,Loubière, J. F., Paradas, J., andSoleilhavoup, F., 1990. A method for the biological treatment of an artificial surface. European patent 90400G97. 0, 1989.

Aguilera, L. A. P., Zapata, J. A. N., and Morales, B. O. O., 2015. Bioprecipitation of calcium carbonate by native biota as a method of restoration. Nexo Scientific Journal, 28(01), 25-40.

Adzami, N. S., Ghazali, M. F., Ramli, A. H., Tajarudin, H. A., and Daud, Z., 2018. A new potential of calcium carbonate production induced by *Bacillus sphaericus* in batch fermentation. International Journal of Integrated Engineering, 10(9), 130-134.

Alshalif, A. F., Irwan, J. M., Othman, N., Al-Gheethi, A. A., and Shamsudin, S., 2022. A systematic review on bio-sequestration of carbon dioxide in bio-concrete systems: A future direction. European Journal of Environmental and Civil Engineering, 26(3), 1209-1228.

Bachmeier, K. L., Williams, A. E., Warmington, J. R., and Bang, S. S., 2002. Urease activity in microbiologically-induced calcite precipitation. Journal of Biotechnology, 93(2), 171-181.

Bang, S. S., Galinat, J. K., and Ramakrishnan, V., 2001. Calcite precipitation induced by polyurethane-immobilized *Bacillus pasteurii*. Enzyme and Microbial Technology, 28(4-5), 404-409.

Basheer, L., Kropp, J., and Cleland, D. J., 2001. Assessment of the durability of concrete from its permeation properties: a review. Construction and building materials, 15(2-3), 93-103.

Basheer, P.A.M., Chidiact, S.E., and Long, A.E., 1996. Predictive models for deterioration of concrete structures. Construction and Building Materials. 10 (1), 27-37.

Brooks, J. J., and Johari, M. M., 2001. Effect of metakaolin on creep and shrinkage of concrete. Cement and concrete composites, 23(6), 495-502.

Bundur, Z. B., Kirisits, M. J., and Ferron, R. D., 2015. Biomineralized cement-based materials: Impact of inoculating vegetative bacterial cells on hydration and strength. Cement and Concrete Research, 67, 237-245.

Carran, D., Hughes, J., Leslie, A., and Kennedy, C., 2012. A short history of the use of lime as a building material beyond Europe and North America. International Journal of Architectural Heritage, 6(2), 117-146.

Castanier, S., Le Métayer-Levrel, G., andPerthuisot, J. P., 1999. Ca-carbonates precipitation and limestone genesis—the microbiogeologist point of view. Sedimentary geology, 126(1-4), 9-23.

Castanier, S., Métayer-Levrel, G. L., andPerthuisot, J. P., 2000. Bacterial roles in the precipitation of carbonate minerals. Microbial sediments, 32-39.

Castro-Alonso, M. J., Montañez-Hernandez, L. E., Sanchez-Muñoz, M. A., Macias Franco, M. R., Narayanasamy, R., andBalagurusamy, N., 2019. Microbially induced calcium carbonate precipitation (MICP) and its potential in bioconcrete: microbiological and molecular concepts. Frontiers in Materials, 6, 126.

Chahal, N., Siddique, R., andRajor, A., 2012. Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete. Construction and Building Materials, 28(1), 351-356.

Charpe, A. U., Latkar, M. V., and Chakrabarti, T., 2017. Microbially assisted cementation–A biotechnological approach to improve mechanical properties of cement. Construction and building materials, 135, 472-476.

Chen, H., Qian, C., and Huang, H., 2016. Self-healing cementitious materials based on bacteria and nutrients immobilized respectively. Construction and Building Materials, 126, 297-303.

De Belie, N., and De Muynck, W., 2008. Crack repair in concrete using biodeposition. In Proceedings of the international conference on concrete repair, rehabilitation and retrofitting (ICCRRR), Cape Town, South Africa (pp. 291-292).

De Muynck, W., Cox, K., De Belie, N., and Verstraete, W., 2008. Bacterial carbonate precipitation as an alternative surface treatment for concrete. Construction and Building Materials, 22(5), 875-885.

De Muynck, W., De Belie, N., and Verstraete, W., 2010. Microbial carbonate precipitation in construction materials: a Review. Ecological engineering, 36(2), 118-136.

Dhami, N. K., Reddy, M. S., and Mukherjee, A., 2014. Synergistic role of bacterial urease and carbonic anhydrase in carbonate mineralization. Applied Biochemistry and Biotechnology, 172(5), 2552–2561.

Dupraz, C., Reid, R. P., Braissant, O., Decho, A. W., Norman, R. S., and Visscher, P. T., 2009. Processes of carbonate precipitation in modern microbial mats. Earth-Science Reviews, 96(3), 141-162.

Ephraim, M. E., Akeke, G. A., andUkpata, J. O., 2012. Compressive strength of concrete with rice husk ash as partial replacement of ordinary Portland cement. Scholarly Journal of Engineering Research, 1(2), 32-36.

Ersan, Y. C., Boon, N., and De Belie, N., 2015. Microbial self-healing concrete: denitrification as an enhanced and environment-friendly approach. In 5th International conference on Self-Healing Materials (ICSHM 2015).

Fang, C., He, J., Achal, V., and Plaza, G., 2019. Tofu wastewater as efficient nutritional source in biocementation for improved mechanical strength of cement mortars. Geomicrobiology Journal, 36(6), 515-521.

Flatt, R. J., Nicolas, R., andChristopher R. C,2012. Journal of the European Ceramic Society, 32 (11), p.2787-2798.

Fu, G., Valiyaveettil, S., Wopenka, B., and Morse, D. E., 2005. CaCO3 biomineralization: acidic 8-kDa proteins isolated from aragonitic abalone shell nacre can specifically modify calcite crystal morphology. Biomacromolecules, 6(3), 1289-1298.

Fujita, Y., Ferris, F. G., Lawson, R. D., Colwell, F. S., and Smith, R. W., 2000. Subscribed content calcium carbonate precipitation by ureolytic subsurface bacteria. Geomicrobiology Journal, 17(4), 305-318.

Ghosh, P., Mandal, S., Chattopadhyay, B. D., andPal, S., 2005. Use of microorganism to improve the strength of cement mortar. Cement and concrete research, 35(10), 1980-1983.

Ghosh, S., Biswas, M., Chattopadhyay, B. D., and Mandal, S., 2009. Microbial activity on the microstructure of bacteria modified mortar. Cement and Concrete Composites, 31(2), 93-98.

Gollapudi, U. K., Knutson, C. L., Bang, S. S., and Islam, M. R., 1995. A new method for controlling leaching through permeable channels. Chemosphere, 30(4), 695-705.

Grengg, C., Mittermayr, F., Baldermann, A., Böttcher, M. E., Leis, A., Koraimann, G., ... and Dietzel, M., 2015. Microbiologically induced concrete corrosion: A case study from a combined sewer network. Cement and Concrete Research, 77, 16-25.

Hammes, F., and Verstraete*, W., 2002. Key roles of pH and calcium metabolism in microbial carbonate precipitation. Reviews in environmental science and biotechnology, 1, 3-7.

Hart, R. T., 1960. The plugging effect of bacteria in sandstone systems. Can. Min. Metal. Bull., 495-501.

Huseien, G. F., Saleh, A. T., and Ghoshal, S. K., 2022. Effective Microorganism Solution and High Volume of Fly Ash Blended Sustainable Bio-Concrete. Biomimetics, 7(2), 65.

Jack, T. R., Stehmeier, L. G., Islam, M. R., and Ferris, F. G., 1991. Ch. F-6 Microbial Selective Plugging to Control Water Channeling. Developments in Petroleum Science, 31, 433-440.

Jack, T. R., Ferris, F. G., Stehmeier, L. G., Kantzas, A., and Marentette, D. F., 1993. Bug rock: bacteriogenic mineral precipitation systems for oil patch use. In Developments in Petroleum Science (Vol. 39, pp. 27-35). Elsevier.

Jagadish, K. S., 2008. Alternative building materials technology. New Age International. Pp. 1-39ISBN: 8122420370

Jonkers, H. M., Thijssen, A., Muyzer, G., Copuroglu, O., and Schlangen, E., 2010. Application of bacteria as self-healing agent for the development of sustainable concrete. Ecological Engineering, 36(2), 230–235.

Joshi, S., Goyal, S., Mukherjee, A., and Reddy, M. S., 2017. Microbial healing of cracks in concrete: a review. Journal of Industrial Microbiology and Biotechnology, 44(11), 1511-1525.

Jroundi, F., Gonzalez-Muñoz, M. T., Garcia-Bueno, A., and Rodriguez-Navarro, C., 2014. Consolidation of archaeological gypsum plaster by bacterial biomineralization of calcium carbonate. Acta biomaterialia, 10(9), 3844-3854.

Kantzas, A., Stehmeier, L., Marentette, D. F., Ferris, F. G., Jha, K. N., andMaurits, F. M., 1992. A novel method of sand consolidation through bacteriogenic mineral plugging. In PETSOC Annual Technical Meeting (pp. PETSOC-92). PETSOC.

Khaliq, W., and Ehsan, M. B., 2016. Crack healing in concrete using various bio influenced self-healing techniques. Construction and Building Materials, 102, 349-357.

Kishore, R., Bhikshma, V., and Prakash, P. J., 2011. Study on strength characteristics of high strength rice husk ash concrete. Procedia engineering, 14, 2666-2672.

Kucharski, E. S., Cord-Ruwisch, R., Whiffin, V. S., and Al-Thawadi, S., 2006. Microbial biocementation. Patent066326.

Koushal, K. K.,2020. A study on the innovative technology of smart road construction. Science, technology and innovation, pp. 12-19.ISBN: 978-81-949278-5-3

Kumar, M., Pandey, L., Singh, S., and Mishra, P., 2017. The Advance Technology of Smart Road Construction: Using Sensor, IJCRT Vol 5,(4) ISSN: 2320-2882.

Lappin-Scott, H. M., Cusack, F., andCosterton, J. W., 1988. Nutrient resuscitation and growth of starved cells in sandstone cores: a novel approach to enhanced oil recovery. Applied and Environmental Microbiology, 54(6), 1373-1382.

Lee, P., 2007. François Cointeraux and the School of "Agritecture" in Eighteenth-Century France. Journal of Architectural Education, 60(4), 39-46.

Le Metayer-Levrel, G., Castanier, S., Orial, G., Loubiere, J. F., andPerthuisot, J. P., 1999. Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. Sedimentary geology, 126(1-4), 25-34.

Lucas, A. W., 1935. Ancient Egyptian materials and industries,Nature. Vol-135, pp. 416

Luo, M., and Qian, C., 2016. Influences of bacteria-based self-healing agents on cementitious materials hydration kinetics and compressive strength. Construction and Building Materials, 121, 659-663.

Mehta, P. K., and Monteiro, P. J., 2005. Concrete: microstructure, properties, and materials. McGraw-Hill Education. 3rd Edition. Pages: 659, ISBN: 0071462899

Mobley, H. L., and Hausinger, R. P., 1989. Microbial ureases: significance, regulation, and molecular characterization. Microbiological reviews, 53(1), 85-108.

Muyzer, G., andStams, A. J., 2008. The ecology and biotechnology of sulphate-reducing bacteria. Nature reviews microbiology, 6(6), 441-454.

Perito, B., andMastromei, G., 2011. Molecular basis of bacterial calcium carbonate precipitation. Molecular Biomineralization: Aquatic Organisms Forming Extraordinary Materials, 113-139.

Perito, B., Marvasi, M., Barabesi, C., Mastromei, G., Bracci, S., Vendrell, M., and Tiano, P., 2014. A Bacillus subtilis cell fraction (BCF) inducing calcium carbonate precipitation: biotechnological perspectives for

monumental stone reinforcement. Journal of Cultural Heritage, 15(4), 345-351.

Ramachandran, S. K., Ramakrishnan, V., and Bang, S. S., 2001. Remediation of concrete using microorganisms. Materials Journal, 98(1), 3-9.

Ruan, S., Qiu, J., Weng, Y., Yang, Y., Yang, E. H., Chu, J., andUnluer, C., 2019. The use of microbial induced carbonate precipitation in healing cracks within reactive magnesia cement-based blends. Cement and Concrete Research, 115, 176-188.

Saraswathy, V., and Song, H. W., 2007. Corrosion performance of rice husk ash blended concrete. Construction and building materials, 21(8), 1779-1784.

Sarkar, A., Chatterjee, A., Mandal, S., and Chattopadhyay, B., 2019. An alkaliphilic bacterium BKH4 of Bakreshwar hot spring pertinent to bioconcrete technology. Journal of applied microbiology, 126(6), 1742-1750.

Sarkar, M., Adak, D., Tamang, A., Chattopadhyay, B., and Mandal, S., 2015. Genetically-enriched microbe-facilitated self-healing concrete–a sustainable material for a new generation of construction technology. RSC advances, 5(127), 105363-105371.

Sarayu, K., Iyer, N. R., and Murthy, A. R., 2014. Exploration on the biotechnological aspect of the ureolytic bacteria for the production of the cementitious materials—a review. Applied biochemistry and biotechnology, 172, 2308-2323.

Seifan, M., Samani, A. K., andBerenjian, A., 2016. Bioconcrete: next generation of self-healing concrete. Applied microbiology and biotechnology, 100, 2591-2602.

Setchell, J. R. M., 1970. Notes and Records of the Royal Society, 25 (1), p.79-86.

Shannag, M. J., 2000. High-strength concrete containing natural pozzolan and silica fume. Cement and concrete composites, 22(6), 399-406.

Seifan, M., Ebrahiminezhad, A., Ghasemi, Y., Samani, A. K., andBerenjian, A., 2018. The role of magnetic iron oxide nanoparticles in the bacterially induced calcium carbonate precipitation. Applied microbiology and biotechnology, 102, 3595-3606.

Shukla, A., Gupta, N., Singh, K. R., Kumar Verma, P., Bajaj, M., Khan, A. A., and Ayalew, F., 2022. Performance Evaluation of Bio Concrete by Cluster and Regression Analysis for Environment Protection. Computational Intelligence and Neuroscience, 2022.

Tiano, P., Biagiotti, L., andMastromei, G., 1999. Bacterial bio-mediated calcite precipitation for monumental stones conservation: methods of evaluation. Journal of microbiological methods, 36(1-2), 139-145.

Wang, F., 2013. The birth and use of concrete and reinforced concrete. In Advanced Materials Research (Vol. 712, pp. 955-960). Trans Tech Publications Ltd.

Wang, J., Van Tittelboom, K., De Belie, N., and Verstraete, W., 2012. Use of silica gel or polyurethane immobilized bacteria for self-healing concrete. Construction and building materials, 26(1), 532-540.

Wang, J. Y., Soens, H., Verstraete, W., and De Belie, N., 2014. Self-healing concrete by use of microencapsulated bacterial spores. Cement and concrete research, 56, 139-152.

Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. Reviews in mineralogy and geochemistry, 54(1), 1-29.

Whiffin, V. S., 2008. Microbial CaCO3 precipitation for the production of biocement, Pages: 168, ISBN: 363903872X

Wiktor, V., and Jonkers, H. M., 2011. Quantification of crack-healing in novel bacteria-based self-healing concrete. Cement and concrete composites, 33(7), 763-770.

Xu, J., and Wang, X., 2018. Self-healing of concrete cracks by use of bacteria-containing low alkali cementitious material. Construction and Building Materials, 167, 1-14.

Yuan, Y. C., Rong, M. Z., Zhang, M. Q., Chen, J., Yang, G. C., and Li, X. M., 2008. Self-healing polymeric materials using epoxy/mercaptan as the healant. Macromolecules, 41(14), 5197-5202.

Zain, M. F. M., Islam, M. N., Mahmud, F., and Jamil, M., 2011. Production of rice husk ash for use in concrete as a supplementary cementitious material. Construction and building materials, 25(2), 798-805.

Zhang, J., Zhou, A., Liu, Y., Zhao, B., Luan, Y., Wang, S., ... and Li, Z., 2017. Microbial network of the carbonate precipitation process induced by microbial consortia and the potential application to crack healing in concrete. Scientific reports, 7(1), 14600.

Zhong, L., and Islam, M. R., 1995. A new microbial plugging process and its impact on fracture remediation (SPE 30519). Proc. 70th Annual Technical Conference and Exhibition of the Society of Petroleum Engineers, Dallas, Texas, 1995:703-15.

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Immunotherapy: breakthrough in Cancer Treatment

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Introduction

Major advances have been made in the field of immunology in the past two decades. A better understanding of the molecular and cellular mechanisms controlling the immune system has opened the door to many innovative and promising new cancer therapies that manipulate the immune response.

Historically, the first successful immunotherapy to treat cancer involved the use of toxins from *Streptococcus erysipelatis* and *Bacillus prodigious* by William Coley in the 1890's. More recently, the development of vaccines to tumour-causing hepatitis B virus and papilloma virus are contributing significantly to preventing cancer in a large portion of the human population.

Also, a wide array of cell-based immunotherapies utilizing T cells, NK cells, and dendritic cells have been established. Furthermore, a rapidly expanding repertoire of monoclonal antibodies is being developed to treat tumours, and many of the available antibodies have demonstrated impressive clinical responses (Hossein et al, 2009).

Many immune cell types influence tumor growth in humans. The immune system is comprised of both innate cells that mediate immediate, short-lived responses [monocytes, macrophages, dendritic cells, and natural killer (NK) cells] and adaptive cells that develop long-lived responses and memory (T cells and B cells).

Immunotherapy uses our immune system to fight cancer. It works by helping the immune system recognise and attack cancer cells.Immunotherapy is a standard treatment for some types of cancer. And it is in trials for other types of cancer.There are different types of immunotherapies. These include monoclonal antibodies, checkpoint inhibitors, and vaccines. Some types of immunotherapies are also called targeted treatments or biological therapies.

Our immune system works to protect the body against infection, illness and disease. It can also protect us from the development of cancer. The immune system includes the lymph glands, spleen and white blood cells. Normally, it can spot and destroy faulty cells in the body, stopping cancer developing. But a cancer might develop when:

- the immune system recognises cancer cells but it is not strong enough to kill the cancer cells
- the cancer cells produce signals that stop the immune system from attacking it
- the cancer cells hide or escape from the immune system

Immunotherapy helps our immune system to fight cancer. There are different types of immunotherapy treatments. These work in different ways to help our immune system recognise and attack cancer cells.

Several types of immunotherapies are used to treat cancer. These include:

Immune checkpoint inhibitors, which are drugs that block immune checkpoints. These checkpoints are a normal part of the immune system and keep immune responses from being too strong. By blocking them, these drugs allow immune cells to respond more strongly to cancer.

When your immune system attacks invaders like bacteria and viruses, it uses a system of "brakes" called checkpoints to stop it from attacking your own healthy cells. Cancer cells sometimes turn these checkpoints on or off so they can hide. Immune checkpoint inhibitors are drugs that release the brakes on your immune system. Eight of these drugs are approved to treat cancer. They block the proteins PD-1, PD-L1, CTLA-4, and TF on the surface of immune cells, to let these cells go after the cancerous growth.

T-cell transfer therapy, which is a treatment that boosts the natural ability of your T cellsto fight cancer. In this treatment, immune cells are taken from thetumour. Those that are most active against thecancer are selected or changed in the lab to better attack your cancer cells, grown in large batches, and put back into the body through a needle in a vein. T cells are powerful white blood cells that fight infections. T cells are removed that have started to attack your tumour. They grow a large batch of these cells, called tumour-infiltrating lymphocytes (TILs), in a lab. They then put these activated fighters back into your body. T-cell transfer therapy may also be called adoptive cell therapy, adoptive immunotherapy, or immune cell therapy (Waldman*et al*., 2020).

Monoclonal antibodies, which are immune system proteins created in the lab that are designed to bind to specific targets on cancer cells. Some monoclonal antibodies mark cancer cells so that they will be better seen and destroyed by the immune system. Such monoclonal antibodies are a type of immunotherapy. Monoclonal antibodies may also be called therapeutic antibodies(Hafeez*et al*., 2018).

Monoclonal antibodies work in different ways:

Naked monoclonal antibodies are the most common type used in cancer treatment. They're called naked because they're unattached to anything. These antibodies boost your immune system's response against the cancer, or block antigens that help the cancer grow and spread.

Conjugated monoclonal antibodies have a chemotherapy drug or radioactive particle attached to them. The antibodies attach directly to cancerous cells. This reduces side effects and helps chemotherapy and radiation treatments work better.

Bispecific monoclonal antibodies attach to two proteins at once. Some attach to both a cancer cell and an immune cell, which helps the immune system attack the cancer. The leukaemia drug blinatumomab (Blincyto) attaches to a protein on leukaemia cells, and to a protein on T cells.

Cancer vaccines

These use your immune system to prevent or treat cancer. Cancer vaccines are made from dead cancer cells, proteins or pieces or proteins from cancer cells, or immune system cells(DeMaria, 2019).

Vaccines are approved to prevent cancer:

Cervarix, Gardasil, and **Gardasil-9** protect against the human papillomavirus (HPV), which is linked to cancers of the cervix, throat, vagina, vulva, anus, and penis.

Hepatitis B (HBV) vaccine (HEPLISAV-B) protects against HBV infections that can cause liver cancer.

Vaccines are FDA-approved to treat cancer:

- **Sipuleucel-T (Provenge)** treats advanced prostate cancer when hormone therapy doesn't work.
- **Talimogene laherparepvec (T-VEC)** treats melanoma skin cancer that has spread.

- **Bacillus Calmette-Guérin,** or **BCG,** treats early-stage bladder cancer.

Immune System Modulators

Other types of Immunotherapies boost the activity of your immune system in general. A more active immune system can better fight cancer. These drugs fall into a few classes:

Interleukins are a type of cytokine, a protein that some white blood cells make to control your immune system's response to cancer. A man-made version of the interleukin IL-2 increases the number of T cells and NK cells in your body. The IL-2 aldesleukin (Proleukin) is approved to treat advanced kidney cancer and metastatic melanoma.

Interferons are another type of cytokine that makes your immune cells more active against cancer. IFN-alpha treats cancers such as leukemia, sarcoma, lymphoma, and melanoma.

Immunomodulators (IMiDs) kick-start immune system reactions to treat some types of cancer. They include:

- Imiquimod (Aldara, Zyclara)
- Lenalidomide (Revlimid)
- Pomalidomide (Pomalyst)
- Thalidomide (Thalomid)

Immunotherapy has indeed emerged as a breakthrough in cancer research and treatment. It represents a revolutionary approach that harnesses the body's immune system to combat cancer.Traditionally, cancer treatments have focused on methods such as surgery, chemotherapy, and radiation therapy. While these approaches have proven effective to varying degrees, they can also have significant side effects and may not be successful for all types of cancer.

Immunotherapy, on the other hand, utilizes the immune system's inherent ability to recognize and destroy cancer cells. It works by stimulating or enhancing the body's immune response to specifically target cancer cells while sparing healthy cells. This approach offers several advantages, including potentially long-lasting effects and a more targeted attack on cancer cells (Hossein *et al*., 2009).

Immunotherapy has shown remarkable success in treating various types of cancer, including melanoma, lung cancer, kidney cancer, bladder cancer, and certain types of blood cancers, etc. It has led to significant improvements in overall survival rates and quality of life for many patients.

However, it is important to note that immunotherapy is not a one-size-fits-all solution, and its effectiveness can vary depending on the type and stage of cancer, as well as individual patient factors. Ongoing research and clinical trials continue to explore new strategies and combinations of treatments to improve outcomes and expand the applications of immunotherapy.

Overall, immunotherapy represents a major breakthrough in cancer research and has transformed the treatment landscape, offering new hope for patients and paving the way for more personalized and targeted approaches to fighting cancer.Some clinical trials are now using immunotherapy to treat nonmetastatic, early-stage cancers. Being able to achieve the same long-term outcome without the long-term side effects associated with chemotherapy would be a win for patients and oncologists alike (Brassil, 2019).

- Mesothelioma.
- Multiple myeloma.
- Non-Hodgkin lymphoma.
- Prostate cancer.
- Skin cancer.
- Soft tissue sarcoma.
- Stomach cancer.
- Endometrial cancer.
- For some patients with advanced uterine cancer, treatment with checkpoint inhibitors may be an option:

Immunotherapy can improve the quality and longevity of a cancer patient.The average cost of immunotherapy in India is around Rs. 1,50,000-4,50,000 per session.

Chemotherapy kills fast-growing cellsboth cancerous and non-cancerousin the body. Immunotherapy helps the immune system do a better job of identifying cancer cells so it can attack and kill them. **Immunotherapy may work when other treatments don’t.** Some cancers (like skin cancer) don’t

respond well to radiation or chemotherapy but start to go away after immunotherapy.

It can help other cancer treatments work better. Other therapies like chemotherapy, may work better than immunotherapy.**It causes fewer side effects than other treatments.** This is because it targets just your immune system and not all the cells in your body.When you have immunotherapy, your immune system learns to go after cancer cells if they ever come back. This is called immunomemory, and it could help to stay cancer-free for a longer time.

Conclusion

Although the principles of immunotherapy have been around for a long time, the field has gained momentum during the past decade due to multiple scientific advancements. Based on the overwhelmingly successful results of a number of clinical trials.Immunotherapy has emerged as a breakthrough in cancer treatment. Unlike other forms of treatment, immunotherapy is attractive because it can offer the potential for remarkable results with far fewer side effects.A growing number of people with cancer have benefited in recent years from immunotherapy, with some seeing dramatic and lasting responses to these new treatments. In rare cases, patients with advanced cancers have had their tumours disappear completely following treatment with immunotherapy.Although immunotherapy is primarily used in cancer, it is also used to treat autoimmune diseases and disorders. Researchers are exploring how immunotherapy may be used for genetic disorders, inflammation, diabetes, cardiovascular diseases and regenerative medicine.

References

American Society of Clinical Oncology (ASCO). Understanding immunotherapy.Accessed at https://www.cancer.net/navigating-cancer-care/how-cancer-treated/immunotherapy-and-vaccines/understanding-immunotherapy.

Brassil KJ, eds. Chemotherapy and Immunotherapy Guidelines and Recommendations for Practice. Pittsburgh, PA: Oncology Nursing Society; 2019:149-189.

DeMaria PJ, Bilusic M.,2019. Cancer vaccines. Hematology Oncology Clinics of North America; 33(2):199- 214.

DeSelm CJ, Tano ZE, Varghese AM, Adusumilli P S, 2017. CAR T-cell therapy for Pancreatic Cancer. Journal of Surgical Oncology, 16(1):63-74.

Hafeez U, Gan HK, Scott AM.,2018. Monoclonal antibodies as Immunomodulatory therapy againstCancer and Autoimmune Diseases. Current Opinion in Pharmacology, 41:114-121.

Hossein Borghaei, Mitchell R Smith, Kerry S Campbell, 2009. Immunotherapy of Cancer. European Journal of Pharmacology, 625(1-3):41-54.

National Cancer Institute (NCI). Immunotherapy to treat cancer. Accessed at https://www.cancer.gov/about-cancer/treatment/types/immunotherapy.

Waldman AD, Fritz JM, Lenardo MJ. 2020. A guide to cancer immunotherapy from T cell basic science to clinical practice, Nature Reviews Immunology; 20:651-668.

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Genetically Modified Organisms (GMOs): Balancing Benefits and Ethical Considerations

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Introduction

Would you like to have your roses glow in the dark? Would you rather have mosquitos spread around antibodies to fight diseases than the usual malaria? Or would you like to create crops that will not perish for a very long time and thus eradicate world hunger?

All of this could theoretically be achieved by creating GMOs. Genetically Modified Organisms (GMOs) have emerged as a significant topic of debate and controversy in recent years. As Theresa Phillips rightly mentioned in her paper, people have been creating new life forms with altered genomes for hundreds of years by selective breeding. That is how we have reached the current juicy tomato from its hard wild ancestors. Recently, we have established enough of a breakthrough in genetic engineering that we can incorporate a new gene in an entirely unrelated species while also targeting specific singular genes. (Theresa Phillips, 2008)

By altering the genetic makeup of organisms, scientists can enhance desired traits, such as increased crop yields, improved nutrition, and resistance to pests and diseases. While GMOs offer potential benefits for addressing global food security and sustainability, ethical concerns have also emerged regarding their potential risks to human health, biodiversity, and corporate control. This chapter talks about the ethical dimensions of GMOs, considering their benefits, potential risks, and the need for transparent decision-making processes.

The Talk

One can, hypothetically speaking, prance around like Dr Moreau from "The Island of Doctor Moreau" with their half human hybrids or like Dr Joseph

Heiter create a genetically altered version of The Human Centipede, which might not be appealing to the greater audience. But hey, that's what the rules are for. As once said by a very wise being of a fictional universe, "With great power, comes great responsibility." Or as aptly seen in this case, with great knowledge comes great ethical questions."

As the human urge to grow and learn more, to create grows the number of GM crops out there, written in Francis Dizon's paper, so does the concept of ethical eating. Ethical eating is not really a diet plan, but more like the morality and consequences of certain food choices (and not just the whole vegan and meat choices). This also concerns itself with the consequences of cross breeding between modified varieties and natural sources. These concerns are hence quenched by the tight leash of the numerous codes of ethics. But this is also doing its best to undermine the benefits of GMOs and disregard its utility in nations struggling with the global food shortages. (Francis Dizon et al, 2015)

The ethical dilemma surrounding genetically modified organisms (GMOs) stems from differing perspectives on their potential benefits and risks. Here are some key points and considerations that contribute to the ethical debate.

Potential Benefits of Genetically modified Crops

As said by Matin Qaim, 10% of the farmed land was used to grow GM crops. (Matin Qaim, 2010). Here are certain benefits of GM crops.

Increased crop yields

One of the primary arguments in favour of GMOs is their potential to increase crop yields and combat hunger on a global scale. By introducing traits like drought or pest resistance, GMOs can enhance agricultural productivity and food security. GMOs have the potential to enhance agricultural productivity, ensuring food security and addressing global hunger. For example, the modified BT cotton increased the production gain by over $ 2 billion per annum in India.

Improved nutrition

GMOs can be engineered to enhance the nutritional content of crops, such as adding essential vitamins or minerals to combat nutrient deficiencies. This has the potential to improve the health and well-being of populations, particularly in developing countries where nutrient deficiencies are prevalent. Example isthe creation of golden rice stemmed from the need to rectify vitamin A deficiency in developing countries.

Disease and pest resistance

Genetic modifications can confer resistance to pests, diseases, and environmental stresses, reducing the need for harmful pesticides and herbicides leading to more environmentally sustainable agricultural practices.An example being the GM crop of soybean and maize that have herbicidal properties.

Environmental sustainability

GMOs may enable the development of crops that require fewer natural resources, such as water and land, leading to reduced environmental impact. It also helps to clear out pollution levels from pesticide runoffs.

Methods of Creating Genetic Modification in Plants

There are several methods for introducing genetic modifications in plants:

1. **Agrobacterium-Mediated Transformation:** This involves using a bacterium called *Agrobacterium tumefaciens* to transfer genes into plant cells.
2. **Biolistic (Gene Gun) Transformation:** DNA-coated particles are shot into plant cells using a "gene gun."
3. **Electroporation:** Electric pulses are used to create pores in plant cell membranes, allowing DNA to enter.
4. **Protoplast Transformation:** Plant cell walls are removed, and DNA is introduced directly into the protoplasts.
5. **Viral Vector-Mediated Transformation:** Plant viruses can be engineered to carry desired genes into the plant cells.
6. **Crispr-Cas9 Technology:** This advanced method enables precise genome editing by targeting specific DNA sequences.
7. **RNA Interference (RNAi):** Introduction of double-stranded RNA molecules to suppress the expression of specific genes.
8. **Transposon-Mediated Transformation:** Mobile DNA elements called transposons can be used to insert genes into plant genomes.
9. **Chemical Methods:** Chemicals can be used to enhance the uptake of DNA by plant cells.

Each method has its advantages and limitations, and the choice depends on factors such as the plant species, the desired genetic modification, and the efficiency of the technique.

Potential benefits of Genetically Modified Animals:

1. **Improved Food Production:** GMAs can be engineered to possess desirable traits such as disease resistance, increased yield(milk, egg and meat), improved nutritional contentand exogenous proteins. This could potentially help in addressing food shortages and malnutrition.
2. **Biomedical Research:** Genetically modified animals serve as valuable models for understanding human diseases and developing treatments. They can be engineered to mimic specific diseases, which allows scientists to study the underlying mechanisms and test potential therapies.
3. **Conservation Efforts:** GMAs can play a role in conservation by aiding endangered species. For instance, genetic modifications could help improve reproductive rates or increase disease resistance in vulnerable populations.
4. **Medical Advances:** Genetic modifications in animals could lead to the production of biopharmaceuticals, like insulin and vaccines, in a more efficient and cost-effective manner.

5. Salmon: This genetically modified Atlantic salmon has been engineered to grow faster using genes from other fish species. The result is a fish that reaches market size more quickly, potentially reducing the environmental impact of fish farming.

6.Enviropig: Genetically modified pigs were developed to produce phosphorus-digesting enzymes in their saliva, which could reduce the environmental impact of pig waste on water systems.

7.Genetically Modified Chickens: Researchers have explored genetic modifications in chickens and cows to enhance meat and egg production, as well as improve animal health and welfare.

8.Transgenic Cattle: Some genetically modified cattle have been developed to produce milk with altered composition, such as increased levels of certain proteins or reduced allergens.

9.Disease Resistance: Genetic modification can be used to develop animals that are more resistant to specific diseases, reducing the need for antibiotics and enhancing animal health.

10.Nutritional Enhancement: There have been efforts to genetically modify animals to produce meat, milk, or eggs with improved nutritional profiles, such as higher omega-3 fatty acid content.

Methods of creating Genetically Modified Animals:

Creating genetically modified animals involves various methods, they have own advantages and disadvantages.

1. **Transgenesis:** Foreign DNA is introduced into the animal's genome, often using techniques like microinjection or retroviral vectors.
2. **Crispr-Cas9 Technology:** This revolutionary technique allows precise gene editing by altering specific DNA sequences.
3. **Somatic Cell Nuclear Transfer (SCNT):** The nucleus of a somatic cell is transferred into an egg cell from which the nucleus has been removed. This method was used to clone Dolly the sheep.
4. **Gene Knockout:** Specific genes are intentionally disrupted or "knocked out" to study their function or create specific traits.
5. **Gene Addition:** New genes can be introduced to provide animals with desirable traits or produce therapeutic proteins.
6. **RNA Interference (RNAi):** Like in plants, RNA molecules can be used to suppress the expression of specific genes in animals.
7. **Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs):** These are similar to Crispr-Cas9 and enable precise gene editing.
8. **Viral Vectors:** Viruses can be engineered to deliver desired genes into animal cells.
9. **Pronuclear Microinjection:** DNA is directly injected into a fertilized egg's nucleus, incorporating the new gene into the animal's genome.
10. **Homologous Recombination:** Specific genetic modifications are achieved by replacing a target gene with a modified version.
11. **Mitochondrial DNA Manipulation:** Genetic modifications can be made to the mitochondrial DNA of animals.

Each method has its own applications and ethical considerations. The choice of method depends on factors such as the animal species, the desired genetic modifications, and the intended purpose of the modification.

Potential Risks

With everything new, comes the fear of the mysteries and the limited understanding. While the benefits of GMOs are compelling, it is essential to acknowledge and address the potential risks and ethical concerns associated with their use.

As A. S. Bawa and K. R. Anila Kumar have debated in their paper, through all the benefits, not everyone is an eager participant and questions this new technology of genetic modification and the implications of playing the act of "mother nature". As it rightly should be, things to be questioned, their impacts and influence to be answered. The potential risks mainly concern the health of consumers, environmental risks and ecological dangers. (Bawa et al, 2019)

While the benefits of GMOs are compelling, it is essential to acknowledge and address the potential risks and ethical concerns associated with their use. It is crucial to prioritize rigorous scientific research to assess potential risks, such as allergenicity or the transfer of antibiotic resistance genes.

Unknown long-term effects

The long-term impacts of GMOs on human health and the environment are not yet fully understood, leading to concerns about unintended consequences.

Biodiversity and ecosystem disruption

GMOs may crossbreed with wild or non-GMO plants, potentially leading to the loss of biodiversity or altered ecosystems. Maintaining biodiversity is critical for ecological balance and resilience, and measures must be in place to mitigate the risks of gene flow from GMOs.

Corporate control

The issue of corporate control and farmer dependence is also a significant concern. Critics argue that GMOs are often developed by profit-seeking corporations, leading to seed patents, farmer reliance on specific seeds, and limited access for farmers in developing countries. Balancing intellectual property rights and ensuring the rights of farmers to save and exchange seeds is essential for a fair and sustainable agricultural system.

Lack of transparency

There have been concerns about inadequate labelling of GMO products, limiting consumer choice and the ability to make informed decisions about what they consume.

Ethical Considerations of Genetically modified Crops

Genetically Modified Organisms (GMOs) have emerged as a significant topic of debate and controversy in recent years. By altering the genetic makeup of organisms, scientists can enhance desired traits, such as increased crop yields, improved nutrition, and resistance to pests and diseases. While GMOs offer potential benefits for addressing global food security and sustainability, ethical concerns have also emerged regarding their potential risks to human health, biodiversity, and corporate control. This essay explores the ethical dimensions of GMOs, considering their benefits, potential risks, and the need for transparent decision-making processes.

Albert Weale, after studying the reports of The Nuffield Council on Bioethics (NCOS), said that their surveys showed that the major ethical issue behind GM crops stems from their "Unnatural origins". But the benefits behind GM crops in helping with global food sustainability, makes it necessary for exploration of this new technology with ethical responsibility. Especially when it comes to the growing population of developing countries with not the same exponential growth in food production, it is necessary to assist through the production of GM crops even if it's within the legal borders. (Albert Weale, 2010).

Human health

The potential risks to human health, such as allergenicity or the transfer of antibiotic resistance genes, should be thoroughly assessed before widespread adoption of GMOs.

Environmental impact

Evaluating the impact of GMOs on biodiversity, ecosystems, and the potential for unintended consequences is crucial to ensure sustainable agricultural practices.

Farmers' right

Ensuring that farmers have access to non-GMO seeds, protection from patent infringement claims, and the freedom to choose the farming methods they prefer.

Consumer choice and transparency

Allowing consumers to make informed choices by providing clear and accurate labelling of GMO products. Ethical decision-making around GMOs should prioritize transparency and empower consumers to make informed choices. Labelling GMO products accurately is crucial for consumer choice. Clear labelling provides individuals with the freedom to choose whether to consume GMOs or opt for non-GMO alternatives. Transparency also involves ensuring that adequate information is accessible to the public regarding the benefits, potential risks, and the decision-making processes governing GMOs.

Regulation and Decision-Making

Balancing these considerations requires careful regulation, scientific research, and transparent decision-making processes. Ethical frameworks such as utilitarianism, deontology, and precautionary principles are often applied to evaluate the risks and benefits of GMOs and guide policy decisions.

Balancing the benefits and ethical considerations of GMOs necessitates robust regulatory frameworks and decision-making processes. Governments should establish stringent regulations for the testing, approval, and labelling of GMOs. Scientific research should be independent and peer-reviewed, ensuring comprehensive risk assessments. Public engagement and participation should be encouraged in the decision-making processes, incorporating diverse perspectives and ensuring transparency.

As explained by Ishaan Sood in his column, the regulatory framework for genetically modified (GM) crops in India is primarily governed by the "Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells" (commonly known as the "1989 Rules") under the Environment Protection Act, 1986. The Genetic Engineering Appraisal Committee (GEAC), operating under the Ministry of Environment, Forest and Climate Change (MoEFCC), is responsible for the approval of GM crops for environmental release and commercial cultivation in India. Besides the GEAC, other agencies involved in the regulation of GM crops include the Review Committee on Genetic Manipulation (RCGM) and State Biotechnology Coordination Committees (SBCCs). (Ishaan Sood,2021)

Before commercial cultivation can start on a GM crop, in depth field trials are conducted on it to map its safety, efficacy and environmental impacts if used commercially. All of this is done with strict guidelines from the regulatory authorities. During the regulation process, public opinion is taken

into account as well. To maintain a certain level of transparency, in India there are regulatory laws placed on the labelling and traceability of each food product for the presence of GM crops.

Ethical Considerations of Genetically Modified Animals

Animal Welfare: The genetic modification process can cause harm to the animals involved. It may lead to unintended health issues or suffering, which raises concerns about the well-being of the animals and their moral status.

Environmental Impact: There are uncertainties about the ecological consequences of releasing genetically modified animals into the environment. The potential for unintended ecological disruptions is a significant ethical concern.

Unintended Consequences: Genetic modifications may have unforeseen and long-term effects, potentially altering ecosystems or introducing unintended genetic traits that could spread beyond the targeted population.

Ownership and Patents: Genetic modifications can be subject to intellectual property rights, leading to issues of ownership and control over living organisms. This raises questions about the ethical implications of treating animals as commodities.

Informed Consent: Ethical concerns arise when using genetically modified animals in research, especially if the animals are unable to provide consent for their participation.

Slippery Slope: Some critics argue that accepting genetically modified animals might lead to the acceptance of genetic modifications in humans, which opens up a whole new set of ethical dilemmas.

Balancing the benefits and ethical considerations of genetically modified animals is a complex task. Striking a balance requires careful evaluation, transparent and responsible research, adherence to ethical guidelines, and open public discourse. Regulation and oversight are essential to ensure that the potential benefits are realized while minimizing the risks and ethical dilemmas associated with genetic modification in animals.

Regulatory Boards for Making Laws of GMO Creation:

1. **United States:** In the U.S., GMOs are regulated by three main agencies: the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and the U.S. Department of Agriculture (USDA). The Coordinated Framework for the Regulation of Biotechnology outlines

their roles in assessing the safety of GMOs for human consumption and the environment.

2. **European Union:** The EU has strict regulations on GMOs. Any genetically modified organism, including plants and animals, must undergo a comprehensive risk assessment before they can be authorized for cultivation or marketing. Labeling of GMO products is mandatory in the EU.
3. **Canada:** Canada's approach to GMO regulation involves multiple government agencies, including Health Canada, the Canadian Food Inspection Agency (CFIA), and Environment and Climate Change Canada. They assess GMO safety, environmental impact, and market release.
4. **China:** China has regulations governing the research, production, and commercialization of GMOs. Any activities related to GMOs must receive government approval, and labeling requirements are in place for GMO products.
5. **Brazil:** Brazil has a regulatory framework overseen by the National Technical Commission on Biosafety (CTNBio). GMOs undergo safety assessments before commercial release is permitted.
6. **India:** In India, GMOs are regulated under the "Rules for the Manufacture, Use/Import/Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989." GMOs must undergo safety assessments before being approved for release.
7. **Australia:** GMOs in Australia are regulated by the Office of the Gene Technology Regulator (OGTR). They assess the risks associated with GMOs and have guidelines for containment and release.
8. **International Standards:** The Cartagena Protocol on Biosafety is an international treaty governing the movement of GMOs across national borders. It aims to ensure safe handling, transfer, and use of GMOs, with a focus on protecting biodiversity.

Recent Development

1. Yeast has been used for hundreds of years by the human civilization to create fermented food products but just recently researchers from Tufts University School of Engineering have created a modified yeast that can feed on agricultural waste biomass. This is a stepping stone for creating biofuels with very less carbon footprint. (Mike Silver, 2023)
2. The lifespan of an organism is a direct measure of the age of their cells. Researchers from University of California found out the pathways used by a cell during ageing and managed to genetically modify these pathways so the cells switch between them and have an extended life. (Zhen Zhou et al , 2023)
3. Spider silk is a fascinating product on it's own by being stronger than steel but also incredibly weightless. Now it has become even more fascinating as Professor Fuzhong Zhang has created modified microbes that can synthetically produce the spider silk. (Fuzhong Zhang et al 2023)
4. Scientists have been recently trying to modify the natural microbial flora of humans to improve their therapeutic assistance. Researchers at Massachusetts General Hospital successfully created *E. coli* strains that secrete proteins that are important therapeutically. (Jason P et al, 2023)

Conclusion

Although we are a long way from the feared animal-human hybrids from "Sweet Tooth" and might not even get there with all the laws and regulations behind GMOs, it is a necessity to continue the exploration of their ethical consequences. It is not just a black and white picture of a chess board with the corporate and the scared wing pulling at the leash, but a gray area that affects not just humanity but the entirety of life on this planet.

There's no doubt that everyone debating on the matter will think that their points are valid and nothing else matters. Even though the points might be valid, it is the closed box nature of their argument that is the principle issue of the debate. So many established theories and beliefs have been disapproved over the years and will continue to do so even on this particular topic and hence requires both sides to have a flexible faith on their beliefs that also carries the ability to encompass the other side of the argument.

The ethical dilemma surrounding GMOs requires careful consideration of their potential benefits and risks. GMOs hold promise for addressing global challenges like food security and sustainability. However, ethical concerns related to human health, biodiversity, corporate control, and consumer choice must be taken seriously. By prioritizing scientific research, transparent decision-making processes, and inclusive public engagement, society can navigate the ethical complexities of GMOs and strive for a balanced approach that maximizes benefits while minimizing risks.

References

Albert Weale,2010. Ethical arguments relevant to the use of GM crops, Ethical Arguments Relevant to the Use of GM Crops. New Biotechnology, 27(5):582-587.DOI:10.1016/j.nbt.2010.08.013

Bawa AS and Anilakumar KR., 2013. Genetically modified foods: safety, risks and public concerns-a review. Journal of Food Science and Technology, 50(6):1035-1046. doi: 10.1007/s13197-012-0899-1. Epub 2012 PMID: 24426015; PMCID: PMC3791249.

Dizon F, Costa S, Rock C, Harris A, Husk C, andMei J.,2016. Genetically Modified (GM) Foods and Ethical Eating. Journal of Food Science;81(2):R287-91. doi: 10.1111/1750-3841.13191. Epub 2015 Dec 28. PMID: 26709962.

Fuzhong Zhang Li J, Jiang B, Chang X, Yu H, Han Y, 2023. Bi-terminal fusion of intrinsically-disordered mussel foot protein fragments boosts mechanical strength for protein fibers. Nature Communications, April 14,. https://doi.org/10.1038/s41467-023-37563-0

Ishaan Sood, 2021. Sowing the 'seeds' of monopoly: GMO Regulations vis-a-vis the farm laws, Agricultural Laws: Breaking new grounds for Sustainable futures .RGNUL Student Research Review (RSRR): rsrr.in/2021/01/24/gm-crops-regulations-in-india-vis-a-vis-the-farm-laws/

Jason P. Lynch, Coral González-Prieto, Analise Z. Reeves, Sena Bae, Urmila Powale, Neha P. Godbole, Jacqueline M. Tremblay, Florian I. Schmidt, Hidde L. Ploegh, Vikram Kansra, Jonathan N. Glickman, John M. Leong, Charles B. Shoemaker, Wendy S. Garrett, Cammie F. Lesser. (2023)Engineered *Escherichia coli* for the in-situ secretion of therapeutic nanobodies in the gut. CELL HOST &MICROBE, DOI: 10.1016/j.chom.2023.03.007

Mike Silver (2023). Bioengineered yeast feed on agricultural waste: Result sets the stage for biomanufacturing of biofuels and other products with a very low carbon footprint. Tufts University. ScienceDaily. www.sciencedaily.com/releases/2023/07/230719112303.html

Phillips, T.,2008. Genetically Modified Organisms (GMOs): Transgenic Crops and Recombinant DNA Technology. Nature Education, 1, 213.

Qaim M, and Kouser S., 2013 Genetically modified crops and food security. PLoS One. 5;8(6):e64879. doi: 10.1371/journal.pone.0064879. PMID: 23755155; PMCID: PMC3674000.

Zhen Zhou, Yuting Liu, Yushen Feng, Stephen Klepin, Lev S. Tsimring, Lorraine Pillus, Jeff Hasty, Nan Hao. (2023);Engineering longevity—design of a synthetic gene oscillator to slow cellular aging. SCIENCE, 380 (6643): 376 DOI: 10.1126/science.add7631

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Detection of heavy metals in plants and its impact on environment

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Introduction

Industrial development and usage of chemical pesticides lead to environmental pollution. In this pollution, heavy metals cause serious problems to humans, animals and plants. When a heavy metal is highlighted for its potential toxicity, especially in environmental circumstances, it is said to be poisonous. Through inhalation, diet, and manual handling, heavy metals can enter plant, animal, and human tissues where they can bind to and impair the function of essential cellular components. Heavy metal toxicity is defined as an excess of the required concentration or it is unwanted that was found naturally on the earth and becomes concentrated as a result of human caused activities. Significant environmental contaminants included heavy metals.They are poisonous, which is a concern that is becoming more and more important for ecological, evolutionary, dietary, and environmental reasons. These include copper (Cu), manganese (Mn), lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), zinc (Zn), chromium (Cr), iron (Fe), arsenic (As), silver (Ag), and platinum, which have an atomic density greater than 4 g/cm3, or 5 times or more, than water. Environmental conditions are referred to as anything that surrounds a particular creature or group of organisms, specifically the collection of outside physical factors that have an impact on and influence how organisms grow, develop, and survive. They are generally found scattered among rock formations. Heavy metals were anthropogenically contributed to the biosphere by increasing industry and urbanization, with the greatest availability in soil and aquatic ecosystems and a very minor proportion in the atmosphere as particulate or vapors. Since many heavy metals are thought to be necessary for plant development, their toxicity in plants varies with plant species, individual metal, concentration, chemical form, and soil composition and pH. Certain of these heavy metals, including Cu and Zn, either operate as

cofactors or enzyme reaction activators.Heavy metals including Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are needed by organisms in very small levels, but too much of these elements can be detrimental. Pb, Cd, Hg, and as (a metalloid but commonly referred to as a heavy metal) are examples of heavy metals that do not benefit organisms and are therefore considered to be the "main threats" because they are extremely harmful to both plants and animals, pollute the air, water, and soil, and may be poisonous or toxic to living things.Plant roots are the main interaction point for heavy metal ions in the ecological food chain because they absorb the ions at the primary producer level and subsequently consume them at the consumer level. However, in aquatic systems, when plant bodies are exposed to these ions and particles are left on the foliar surfaces, heavy metals are directly absorbed into the leaves(Koller and Saleh et al, 2018).

Factors that Lead to Heavy Metal Contamination

Agriculture

In agriculture, chemical agents are frequently utilized to counteract any noxious element that can reduce crop yields. However, these chemicals indirectly pose serious dangers to people and plants. In order to guarantee that the plants receive an appropriate supply of nitrogen, potassium, and phosphorus, the fertiliser is routinely given to the soils. However, it also contains dangerous trace metals as impurities, such as cadmium and lead. In addition, the key components of a pesticide combination include lead and arsenic which is lead arsenate was used to control some parasitic insects. In addition, the mixture of copper, chromium, and arsenic was widely used to control the pest in fruits and these agricultural chemical sources disperse the contaminants, and various heavy metals are discharged from them. According to a study, heavy metal accumulation in the soil in Iran caused the bulk density of the soil to increase over time as a result of prolonged exposure to chemical fertilizers and pesticides. The soil's cadmium concentration also exceeded the recommended upper limit for safe agricultural soil, indicating that the crops grown there are unsafe for human consumption. Therefore, it is necessary to limit the use of pesticides and fertilizers in agriculture or replace them with safer substitutes for food production(Sikdar and Kundu,2018).

Heavy Metal and Consumer Health

In the human body, heavy metals can linger and build up to a significant level that will harm the body's systems. Because heavy metals do not decompose in the body, eating plants that have been contaminated with

them can upset the body's chemistry. They can stay within the body for a longer period of time because of their strong affinity. The most effective route for those heavy metals to enter the body of a person and cause disruption is through oral exposure. The heavy metals that are transferred into plants by contaminated soil and air also make their way into food chains, where they eventually affect consumers.Heavy metals can lead to serious illness and are cancer-causing.Lead exposure causes hemoglobin and anemia biosynthesis to be disrupted, a rise in blood pressure, miscarriage, disturbance of the neurological system, and brain damage in humans.An instance of the Itai-Itai sickness was also documented in Japan, where people had it after consuming locally grown rice that had been irrigated with water tainted with cadmium. Meanwhile, ingesting arsenic can result in serious cardiovascular and central nervous system disruptions, gastrointestinal symptoms, and other health issues(Mourato et al, 2015).

Air pollution

The industrialization, transportation, and automobile emissions all contribute to air pollution. When processed at high temperatures, metals like arsenic, cadmium, and lead may volatilize and may escape into the atmosphere. They become oxides and condense into little particles.The burning of fuel oils and tyres, as well as metals including smoke, can damage plants when people drive cars.Through the exposed portion of the plants, dangerous heavy metal-containing dust enters the plants. The majority of the plants grown close to roadways are poisoned with lead.The presence of heavy metal pollution from the contaminated air makes the plants are unsafe for human consumption even if they have not been treated with chemical pesticides or fertilizers(Mourato et al., 2015).

Mechanism of Heavy Metal Toxicity in Plants

When molecular oxygen takes electrons from other molecules, oxygen-free radicals are created. In addition, a number of intracellular processes convert oxygen to superoxide (O_2) or hydrogen peroxide (H_2O_2). The majority of the oxidative damage in biological systems may be caused by hydroxyl radicals (OH), despite the fact that these molecules aren't particularly reactive.Despite being thermodynamically undesirable, the one-electron reduction of molecular oxygen to the superoxide radical can nonetheless occur by contact with another para-magnetic center. As a result of their frequent unpaired electron density, transition metals like iron and copper (M) make excellent oxygen reduction catalysts.

Reactions:

$$Mn+O_2 \rightarrow Mn+1+O_{-2}$$

O_2 may form H_2O_2 in aqueous solutions with a pH of neutral, which can then undergo the Haber-Weiss reaction to produce -OH with the involvement of copper or iron (M) once again as follow:

$$Mn+1+O_{-2} \rightarrow Mn+O_2$$

$$Mn+H_2O_2 \rightarrow Mn+1+OH-+OH$$

This reaction summarized as :

$$O_2+H_2O_2 \rightarrow O_2+OH^-+OH$$

The Fenton reaction is the name given to the Haber-Weiss reaction in which iron serves as the transition metal. The Haber-Weiss process can generate hydroxyl radicals (-OH) that can oxidize biological molecules, causing severe cellular harm and eventually cell death. In close proximity to DNA, hydroxyl radicals can form, adding or taking away H atoms from DNA bases or the DNA backbone, respectively. Each day, one cell may have its DNA base modified as a result of their action.These hydroxyl radical-mediated DNA modifications require Fe 2+ ions, which can be free in solution, complexed to a phosphate residue, coordinated with ring nitrogen, or all three. Metal ions also significantly contribute to the oxidative changes of free amino acids and proteins through the Haber-Weiss process.The most frequent sites of oxidation in proteins are histidine, arginine, lysine, proline, methionine, and cysteine residues, and their primary oxidation products have been identified. Usually, oxidation only modifies one amino acid residue in a specific protein. The targets of certain amino acid residues at metal-binding sites are the mechanisms of heavy metal toxicity in plants, which relate to site-specific activities (Kalaivanan and Ganeshamurthy, 2016).

Mechanism of Plants to Cope with Metal Toxicity

Understanding the physiological and molecular processes governing plant tolerance to a specific heavy metal, as well as their genetic underpinnings, is crucial to the development of plants as phytoremediation agents. In order to survive excessive levels of heavy metals, plants have both constitutive and adaptive systems.To investigate the underlying processes of heavy metal accumulation, tolerance, and adaptive mechanisms to deal with heavy metal stress, physiological, biochemical, and molecular techniques are still used.

Some processes are commonplace because they are also necessary for overall metal homeostasis. They also reduce the harm done by high levels of heavy metals in plants through detoxifying, hence granting tolerance to heavy metal stress. Other systems specifically aim for certain metal ions.Some plants have many mechanisms to limit the buildup of particular metals; these mechanisms may entail excluding certain metals from the intracellular environment or sequestering hazardous ions inside compartments to keep them away from delicate cellular components.Many plants exposed to dangerous levels of metal ions try to avoid or diminish absorption into root cells as a first line of defense by limiting metal ions to the apoplast, binding them to the cell wall or to cellular exudates, or impeding long-distance transport. If this is unsuccessful, a variety of storage and detoxification techniques are used to deal with metals already present in the cell, including immobilization, the synthesis of particular heavy metal transporters, chelation, trafficking, and the sequestration of heavy metals by specific ligands (phytochelatins and metallothioneins).When all other options have failed, plants switch on oxidative stress defense mechanisms that counteract the effects of ROS and MG (such as upregulating the antioxidant and glyoxalase system) and produce stress-related proteins and signaling molecules, such as heat shock proteins, hormones, the biosynthesis of Pro, polyamines, and signaling molecules such as salicylic acid and nitric oxide(Sereginand Kozhevnikova, 2011).

Effects of Toxic Heavy Metals on Plants

Like all living things, plants are frequently sensitive to both a lack of and an abundance of some heavy metal ions as essential Mechanisms of Heavy Metal Toxicity in Plant micronutrients, while the same at higher concentrations and even more ions such as cadmium (Cd), mercury (Hg), and arsenic (As) are strongly toxic to the metabolic activities. Because of their potential negative ecological impacts, heavy metal contamination of agricultural soil has become a serious environmental problem. Due to their frequent prevalence and acute and chronic harmful effects on plants growing in these soils, these toxic substances are regarded as soil contaminants(Mohnish Pichhodeand Nikhil, 2016).

Heavy Metal Detection

1. Atomic Absorption Analysis (AAS)

AAS is a useful technique for figuring out very low metal concentrations. The determination of the presence of metals in the samples is quick and simple. Flame AAS (FAAS) and electrothermal AAS (ETAAS) are

two of the techniques it uses. FAAS will provide the absorption signal continuously, but ETAASsend the signal in a discontinuous trend and need two to four minutes each sample. Usually, a diluted acid solution or xylene solution must be used to dilute the material. Due to the inability of AAS to analyze solid samples directly, sample preparation takes longer. The samples must be in liquid form instead. This sample will be heated to cause atoms to vaporize, changing the sample's state from liquid to gas.The liquid sample must be vaporized at a high temperature (2300°C) in conventional AAS (FAAS). Then, the amount of each element will be determined by how much electromagnetic radiation is absorbed by each atom at a certain wavelength. AAS delivers low levels of interference and good detection sensitivity. The development of oxides in the flame, however, might result in chemical interference if the sample is exposed to heat. The sample dilution can be changed to eliminate this chemical influence. As a result, because AAS can only analyze one element at a time, it is not advised to analyze samples that include several different elements. The analysis will be lengthier as a result. Additionally, it is not utilized to detect light substances like halogens, inert substances, and H, C, N, O, P, and S(Rao et al., 2011, Nur and Azura,2020).

Source

In atomic absorption, hollow cathode tubes with one or more elements are often used as the source. Less commonly, a xenon arc's brilliant continuum has been employed as a source.When these atoms collide with an inert gas like argon, the metal atoms are excited, which leads to the emission of recognizable radiation.

Burner

The key variables that determine the outcome of examination by an atomic absorption device are the quality of the burner, the kind of fuel, and the ratio of fuel to oxidize. The burner is comparable to a spectrometer's sample cell.

Monochromator

The monochromator needs to have the ability to pass the resonance line while blocking others.

Phototube and amplifier

Aspects of the atomic absorption spectrometer include the following. In general, an organic solvent improves the absorption signal, which may change

the strength of the absorption.These have a strong connection to metal and have a tendency to lessen the signal's strength. EDTA might get rid of such effect.

Chemicals and reagents

Analytical-grade nitric acid (HNO_3), perchloric acid ($HClO_4$), hydrochloric acid (HCl), and sulfuric acid (H_2SO_4) can be used. The stock solution had a 1000 ppm concentration.

2. Neutron Activation Analysis (NAA)

Neutron Activation Analysis (NAA) is an instrument that use irradiation system to detect trace elements. The sample is introduced to neutrons in a nuclear sample. The nucleus in the sample absorbed the neutrons and converted into a radioactive nucleus. These radioactive nuclei emit specific gamma rays which are used as an indication of elements that presents in the sample. The samples are transparent to the probe (neutron) and analytical signal (gamma-ray). Thus, it eliminates matrix interference. However, other interferences might arise. An identical gamma-ray signal may be delivered by a different sample component, leading to an incorrect detection. The issue arises when some atoms are unable to absorb the neutron, despite the fact that NAA is a highly sensitive approach.

The undiscovered component will thus be regarded as being absent from the sample. Additionally, NAA does not call for any sample pre-treatment that might potentially contaminate the sample. It can also read a multi-element analysis in a single pass.

To determine the element component: The components of interest may be identified in the spectrum of -rays from the activated sample utilizing the characteristic -rays that are released by the sample in relatively high quantity. By adjusting the irradiation and decay times (i.e., how long the sample stays near a neutron source for and when the sample is examined), elemental measurements may be made more accurate. This is because the generation and decay rate of -radiation rely on the half-life of the nuclei (Weaver et al., 2004).

To determine the element concentration: Gamma(-) ray emission from an element in a sample occurs at a rate that is directly proportional to the element's concentration.

Types of NAA

NAA can be divided into two categories:

- **Destructive or Radiochemical NAA (RNAA):** an NAA technique that uses chemical separations to differentiate important processes from disruptive ones after the radiation has been delivered.
- **Non-destructive or Instrumental NAA (INAA**):The most used NAA technique, which uses no chemical processes either before or after the irradiation. Measurement after various decay durations and the use of specialized radiation detectors allow for the selection of activities of interest.

3.X-ray Fluorescent Spectrometer (XRF)

The X-ray Fluorescent Spectrometer (XRF) may also be used to find the presence of heavy metal elements. To excite the atoms, the sample will be exposed to X-ray beam irradiation. These atoms become unstable and release energy known as ad fluorescent radiation when they are stimulated. The features of a transition between two particular electron orbitals of an element are reflected in the fluorescence emission. Consequently, it is possible to determine that element's identification. There are several XRF techniques, such as total reflection X-ray fluorescence (TXRF), energy dispersive X-ray fluorescence (EDXRF), and direct in-situ portable X-ray fluorescence (PXRF).A thorough study of materials in both bulk and particle form is possible. A quick, non-destructive, and multi-element analysis technique is XRF. It has the capacity to analyze several main and trace components. But it is inappropriate to analyze atoms with atomic numbers below 11. Additionally, XRF occasionally needs additional equipment to analyze the same materials with different properties. The existence of isotopes, for instance, cannot be distinguished by XRF. To characterize the isotopes, further methods like TIMS or SIMS are required. In addition, wet chemical analysis or Mossbauer spectroscopy may detect the same elements with varying valence state of ions, although XRF cannot (Byers et al., 2019).

CPMS (Inductively Coupled Plasma Mass Spectrometry), which combines ICP and MS, is used to find analytes in samples that are present at low concentrations. The ICPMS has a cyclonic spray chamber, a quartz flame with a quartz injector tube, and a concentric nebulizer. Usually, samples need to be broken down from solid to liquid. The ICP's nebulizer is used to introduce the liquid samples. The liquid sample is then nebulized to create an

aerosol that is carried into the plasma torch by hightemperature argon gas.Ions generated during the process using mass spectrometry (MS), which is based on the mass to charge ratio. ICPMS is a quick and sensitive method that can identify analytes in samples at concentrations as low as 1 part per trillion. The high-temperature ion source is the inductive argon coupled plasma (ICP). Most metals may be ionized by the argon ICP with an efficiency of 80% to more than 90%.ICPMS is frequently used in heavy metal detection because, when compared to other methods, it is a quick procedure. ICPMS's multi-element feature makes it possible to determine numerous elements more quickly. By utilizing less than 2 ml of solution and an uptake rate of 1 ml min-1, for instance, 70 elements may be analyzed in less than 2 minutes. ICPMS can ionize the sample, however it is possible that some elements will only be partially ionized, which would lower the detection limit by a factor of 2 compared to fully ionized elements.When only one isotope of a multi-isotope element is found, the detection limit will also drop. Additionally, only 2% of the sample may reach the detector when it is examined using a traditional ICPMS that merely has a nebulizer and spray chamber. When the ions are separated using a quadrupole-based apparatus, mass discrimination may take place. This is due to the fact that ion transmission is not constant over the mass range. Additionally, the plasma sample site can influence the ICPMS's capacity for detection. When a sample travels through several processes in the plasma, the chemical makeup of the element in the solution might have an impact. As a result, the maximum ion density will be impacted. ICPMS is also favored because of its sensitivity. Compared to ICPAES, the detection limits are 10–100 times better. It is capable of detecting and analyzing both metal and non-metal elements, such as silicon, phosphorus, and all other halogens save fluorine. Since the majority of the periodic table's elements are readily ionized, ICP is a useful source of elemental ions for MS.Hydrogen, helium, fluorine, neon, and argon are the only 5 elements that ICPMS cannot directly analyze. Due to their greater ionization potentials than argon, which transforms liquid particles into ions, hydrogen, helium, fluorine, and neon cannot be identified. However, in an argon plasma, argon itself cannot be detected.The sample is delivered to the nebulizer in liquid form, which offers an acceptable degree of homogeneity control and calibration simplicity. A peristaltic pump is then used to transfer the sample to the nebulizer, which might lessen physical interference such variations in the sample's viscosity. However, the sample's solution, which contains dissolved solids, might potentially lead to erroneous detection.The sample will pass through an aperture in the plasma, which is a tiny component that is quickly obstructed by dissolving solids. ICPMS can

handle dissolved solid concentrations of 0.2%, however their existence can result in concurrent elements, which can either suppress or increase the analyte signal(Galiová et al., 2008).

Conclusion

Changes in the physiological and biochemical activity of plants growing in heavy metal-polluted soils result in a reduction in growth. This is particularly true when the heavy metal in question does not contribute in any way to the growth and development of plants. For the successful treatment of soil contaminated with heavy metals, bioremediation can be utilized. It is the most suitable approach of soil remediation when the remediated site is used for crop production since it is a nondisruptive one.Heavy metal-polluted soil can be efficiently treated via bioremediation. Since it is a nondisruptive type of soil remediation, it is best suitable when the remedied site is employed for crop production. In comparison to using microorganisms, using plants for bioremediation (phytoremediation) of heavy metals is more frequent. Different processes are used by plants to clean up heavy metalcontaminated soil.The most popular method of phytoremediation used to remediate heavy metal-polluted soils is phytoextraction. It guarantees that the contaminant is completely removed. The effectiveness of bioremediation is increased when microbes and plants are used together. Numerous phytoremediation programs have successfully included both mycorrhizal fungus and other PGPR. The types of microbes and plants involved, as well as the amount of the heavy metal in the soil, all have a role in how well these organisms work together. Sewage treatment is another option to reduce the soil pollution due to heavy metals. Removal of heavy metals is closely related to the food safety.

References

Ambika Asati, Mohnish Pichhode and Kumar Nikhil, 2016. Effect of Heavy metals on plants: An Overview, International Journal of Application or Innovation in Engineering and Management, 5(3): 56 – 66.

Byers, H. L., McHenry, L. J., and Grundl, T. J., 2019. XRF techniques to quantify heavy metals in vegetables at low detection limits. Food Chemistry*: X*, *1*, 100001.

Galiová, M., Kaiser, J., Novotnỳ, K., Novotnỳ, J., Vaculovič, T., Liška, M., Malina, R., Stejskal, K., Adam, V., & Kizek, R. (2008). Investigation of heavy-metal accumulation in selected plant samples using laser induced

breakdown spectroscopy and laser ablation inductively coupled plasma mass spectrometry. Applied Physics *A*, *93*, 917–922.

Kalaivanan, D., &Ganeshamurthy, A. N.,2016. Mechanisms of Heavy Metal Toxicity in Plants. In N. K. S. Rao, K. S. Shivashankara, & R. H. Laxman (Eds.), Abiotic Stress Physiology of Horticultural Crops (pp. 85–102). Springer India. https://doi.org/10.1007/978-81-322-2725-0_5

Koller, M., & Saleh, H. M., 2018. Introductory chapter: Introducing heavy metals. Heavy Metals, *1*, 3–11.

Labya Prabhas, Megha Agrawal, andKamal Nayan Mishra, 2018. Heavy metal detection using AAS(Atomic Absorption Spectrophotometer) in Leafy Vegetables: Part 1, International Journal of Advance Research in Science and Engineering,7 (2): 742 – 749.

Mohnish Pichhode, & Nikhil, K. (2016). Effect of Heavy Metals on Plants: An Overview. https://doi.org/10.13140/RG.2.2.27583.87204

Mourato, M. P., Moreira, I. N., Leitão, I., Pinto, F. R., Sales, J. R., & Louro Martins, L. (2015). Effect of heavy metals in plants of the genus Brassica. International Journal of Molecular Sciences, *16*(8), 17975–17998.

N. Hamsa and N B Prakash, 2020. Heavy metal contamination in soils and crops irrigated with lakes of Bengaluru, Current Science, 119(11): 2 -6.

Nur Illia Mohd Ruzaidy and Azura Amid, 2020. Heavy metal contaminationin vegetables and its detection: A Review, Science Heritage Journal, 4(1): 1 – 5.

Rao, M. M., KumarMeena, A., & Galib., 2011. Detection of toxic heavy metals and pesticide residue in herbal plants which are commonly used in the herbal formulations. Environmental Monitoring and Assessment, *181*, 267–271.

Seregin, I. V., & Kozhevnikova, A. D., 2011. Histochemical methods for detection of heavy metals and strontium in the tissues of higher plants. Russian Journal of Plant Physiology, 58, 721–727.

Sikdar, S., and Kundu, M. ,2018. A review on detection and abatement of heavy metals. Chembioeng Reviews, *5*(1), 18–29.

S K Gunavathy and H Benita Sherine, 2019. Determination of heavy metalsand phytochemical analysis of some selected medicinal plants, International Journal of Scientific Research in Biological Sciences, 6(3): 87 – 96.

Weaver, J. N., Hanson, A., McGaughey, J., & Steinkruger, F. J., 2004. Determination of heavy metals in municipal sewage plant sludges by neutron activation analysis. Water, Air, and Soil Pollution, *3*, 327–335.

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Expanding Treatment Frontiers: The Convergence of CAR-T Cell Therapy and Immune Checkpoint Inhibitors in TNBC

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Introduction

TNBC, is a subtype of breast cancer, that is distinguished by the lack of expression of the human epidermal growth factor receptor 2 (HER2), the estrogen receptor (ER), and the progesterone receptor (PR) (Gluz*et al*., 2009).In comparison to other breast cancer types, it tends to be more aggressive and is linked to a poor prognosis. Younger women are frequently affected by TNBC, which is more common in the African-American and Hispanic populations. Chemotherapy, radiation therapy, and surgery are the only therapeutic options available for TNBC due to the lack of targeted medicines such as hormone or HER2-targeted medications. However, current studies are concentrated on creating novel treatments and focused strategies to enhance outcomes for people with TNBC.

Between 2010 and 2014, 1,151,724 new cases of breast cancer were reported, with the triple-negative phenotype representing about 8.4% of all occurrences. When compared to non-Hispanic white women, non-Hispanic black women had higher odds of diagnosis (odds ratio [OR], 2.27; 95% CI, 2.23-2.31) and Hispanic women had higher odds of diagnosis (OR, 1.22; 95% CI, 1.19-1.25). Compared to women aged 50 to 64, women under 40 had higher odds of being diagnosed (OR, 1.95; 95% CI, 1.90-2.01). The likelihood of being diagnosed with TNBC increased with stage III and later American Joint Committee on Cancer diagnoses (OR for stage III, 1.69 [95% CI, 1.68-1.72]; and OR for stage IV, 1.47 [95% CI, 1.43-1.51]). (Scott et al., 2019) Compared to other breast cancer subtypes, TNBC has a worse prognosis. TNBC typically has worse survival rates, especially in the early stages of the illness. The outlook can change, though, with the right care. Compared to other breast cancer subtypes, TNBC has a higher chance of recurrence within the

first few years of diagnosis. This accentuates how crucial close observation and aftercare are for TNBC patients.

Age and menopausal status are two factors that can affect the chance of getting triple-negative breast cancer (TNBC). Compared to premenopausal women, postmenopausal women typically have a slightly greater chance of having TNBC. Hormonal changes during menopause, such as lower estrogen and progesterone levels, could increase the risk. It's crucial to remember that TNBC can still happen in premenopausal women. Major risk factors can be shortlisted as genetic ancestry, family history, and BRCA mutations (Rey-Vargas et al., 2019). It's critical to know that genetics and personal risk factors can have a big impact on a person's likelihood of acquiring TNBC. A family history of breast or ovarian cancer, specific genetic abnormalities (such as BRCA1 and BRCA2), and lifestyle variables including obesity and alcohol consumption are additional factors that can increase the risk of TNBC. Women who smoked for 40 packyears and drank 7 servings of alcohol per week had a greater risk of developing TNBC. Hard liquor and wine use were both markedly positively linked with ER+ breast cancer. (Kabat et al., 2011) According to data from an extensive group of postmenopausal women, drinking alcohol and smoking do not appear to raise the risk of TNBC to a higher extent, although they may slightly increase the risk of ER+ breast cancer (Kabat et al., 2011).

Compared to other breast cancer subtypes, triple-negative breast cancer (TNBC) diagnosis and prognosis are very difficult. Triple-negative breast cancer (TNBC) is distinguished by its aggressiveness, greater propensity for early recurrence, and metastasis, all of which may worsen prognosis. Hormonal and HER2-targeted therapy are ineffective because it lacks expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). The identification of therapeutic targets and medical decision-making is hampered by the lack of specific biomarkers. New biomarkers and molecular subtypes are being identified through ongoing research to enhance prognosis and direct TNBC therapy plans. These difficulties emphasize the need for novel strategies and individualized treatments to handle this aggressive breast cancer subtype. A significant effort has been made to identify usable molecular targets to treat patients with these cancers as a result of the dearth of targeted treatments and the dismal prognosis of patients with TNBC. (Bianchini et al., 2016). Massive parallel sequencing and other 'omics' technologies have revealed an unexpected degree of TNBC heterogeneity and have allowed for the discovery of several rare genomic

alterations, germline BRCA1/2 mutations, androgen receptor presence, among other potentially therapeutic molecular characteristics (Bianchini et al., 2016). Beyond the requirement for a correct diagnosis of TNBC, this form of tumour has varied clinical behaviour and frequently has a bad prognosis for its patients. Therefore, a concerted effort has been made to comprehend the molecular causes of heterogeneity and find TNBC-targeting molecules. Robust testing techniques are necessary for accurate diagnosis in order to rule out hormone receptor-positive or HER2-positive breast tumours. TNBC and other aggressive subtypes have similar clinical and pathological characteristics, making them difficult to differentiate. A problem is the lack of specialised markers, which limits the availability of tailored diagnostic testing. To improve the diagnosis and categorization of TNBC, research initiatives are still being done to find new biomarkers and molecular fingerprints. A large portion of these initiatives has been devoted to classifying TNBC genetic characteristics into subgroups with distinct disease trajectories and uniform patterns of sensitivity to chemotherapy or other treatments. TNBC is treated consistently with chemotherapy and is regarded as a single clinical entity, but molecular profiling using massively parallel sequencing and other 'omics' technologies has found unexpectedly high levels of heterogeneity as well as certain similar characteristics (Liu et al., 2018). The ideal strategy to the therapy of patients with this disease would strike a compromise between the straightforward practicality of the present clinical therapeutic guidelines and the biological intricacy of TNBC. Determining useful TNBC subgroups with consistently actionable molecular characteristics has so received a lot of research attention. There have been important recent developments in new therapies. Among them are poly adenosine diphosphate-(ADP)-ribose polymerase inhibitors (PARPi) for people with germline BRCA1/2 mutations (which is particularly important for TNBC), which the FDA approved in 2019 for metastatic disease and in 2021 for early disease. (Tutt et al., 2021)

Role of protein biomarkers in triple negative biomarkers

Protein biomarkers play a vital role in the diagnosis and treatment of various diseases, including Triple Negative Breast Cancer (TNBC). TNBC is an aggressive form of breast cancer characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. Protein biomarkers are proteins that can be detected in the tumour tissue or blood samples of TNBC patients, providing valuable information about the disease's progression and response to treatment. Some commonly studied protein biomarkers in TNBC include BRCA1, p53,

EGFR, and CK5/6. These biomarkers help in distinguishing TNBC from other breast cancer subtypes and can guide treatment decisions (Sukumar, et al., 2021). For instance, high levels of EGFR expression may indicate a potential response to anti-EGFR targeted therapies. Protein biomarkers have revolutionized TNBC management by enabling personalized treatment approaches and improving patient outcomes. Further research and validation of protein biomarkers are essential to enhance TNBC diagnosis, prognosis, and targeted therapy options (Anborgh, et al., 2018).

BRCA1 (Breast Cancer Susceptibility Gene 1) is a significant protein biomarker in TNBC. BRCA1 is frequently linked to hereditary breast and ovarian cancer syndromes and is essential for DNA repair. A portion of TNBC patients had mutations in the BRCA1 gene, which impairs DNA repair processes. Targeted medicines, such as PARP inhibitors, which take advantage of the faulty DNA repair mechanisms to cause cancer cell death, may be beneficial for these individuals. Patients who might benefit from these targeted medicines can be found by looking for the BRCA1 protein expression (Zhang, Li, et al,2015).

A tumour suppressor protein called p53 is a further protein biomarker of relevance in TNBC. Its malfunction is frequently seen in various malignancies, including TNBC. P53 is important in cell cycle regulation and DNA repair. High levels of p53 expression in TNBC are linked to a worse prognosis and treatment resistance (Hashmi, et al.,2018). As alternative therapy approaches, including targeted medicines or immunotherapies, may be more effective in treating tumours with altered p53 pathways, identifying p53 expression levels can help treatment decisions (Xing, Zeyu, et al.,2021)

In addition, TNBC commonly overexpresses the protein biomarker epidermal growth factor receptor (EGFR). EGFR encourages cell survival and development and is connected to TNBC's aggressive behaviour. Higher EGFR expression is linked to treatment resistance and worse overall survival. For TNBC patients with increased EGFR expression levels, targeted medicines against EGFR, such as monoclonal antibodies or tyrosine kinase inhibitors, may provide therapeutic alternatives (Zakaria, et al.,2019).

Along with the protein biomarkers that are frequently investigated in Triple Negative Breast Cancer (TNBC), there are a few atypical protein biomarkers whose expression has been noted in this aggressive subtype of breast cancer. These uncommon biomarkers offer novel understanding of the illness and may have consequences for diagnosis, prognosis, and future

targeted therapy. The protein biomarker glipican-3 (GPC3) is one such uncommon protein. Normally produced throughout embryonic development, the cell surface protein GPC3 is infrequently seen in the tissues of healthy adults. However, research has revealed that a fraction of TNBC patients had higher GPC3 expression. The prognosis is worse and the tumour phenotype is more aggressive in TNBCs with high levels of GPC3 expression. Furthermore, experimental investigations investigating the use of anti-GPC3 antibodies and targeted medicines to impede tumour growth have identified GPC3 as a possible therapeutic target in TNBC (Barman, et al.,2020).

Claudin-4 (CLDN4) is another unique protein biomarker in TNBC. A family of proteins called claudins is involved in the creation of tight junctions and is essential for preserving the polarity and barrier function of epithelial cells(Abd Elazeem, et al.,2018). Although CLDN4 is routinely expressed in a variety of tissues, a subgroup of TNBC patients have been found to have an overexpression of the protein. In TNBC patients, increased CLDN4 expression has been linked to bigger tumour growth, lymph node metastases, and worse overall survival. For TNBC patients with high CLDN4 expression, targeting CLDN4 with antibody-based treatments or particular inhibitors may be a promising therapeutic approach (Szasz, et al., 2011). A unique protein biomarker called Clusterin (CLU) has also revealed altered expression patterns in TNBC. CLU is a secreted protein that is involved in a number of physiological functions, including the control of apoptosis, cell adhesion, and immune response regulation. Studies have shown both overexpression and downregulation of CLU in TNBC, underlining its complicated function in the illness. A poorer prognosis and altered CLU expression in TNBC have been linked. To fully comprehend the precise mechanisms underpinning CLU's contribution to TNBC and investigate its potential as a therapeutic target, more study is required(Y.Wang, et al.,2018).

Table:1 Protein biomarkers expression in TNBC

Sl. no.	Protein biomarker	Expression	Place of study	Reference
1	BRCA 1	Overexpressed in 10-15% TNBC	USA	Sinha, Abhilasha, et al.,2017
2	P53	Abnormally expressed	Pakistan	Hashmi, Atif Ali, et al018
3	EGFR	Overexpressed	Malaysia	Zakaria, Zubaidah, et al.,2019
4	Glipican 3 (GPC-3)	Highly overexpressed	USA	Makkouk, Amani, et al.2021
5	Claudin-4	Over expressed	Egypt	Abd-Elazeemand Mona A, 2015
6	Clusterin	Over expressed	USA	Wang, Yihong, et al.,2018

Empowering the body's defences: Advancements in immunotherapy

Immune system has a significant role in eradication of oncogenic cells. It occurs in three different phases- response; elimination, equilibrium and escape (Schreiber, et al.,2011). Some of the cancer cells escape the elimination and enter an equilibrium state. In the equilibrium phase, adaptive immune system restricts tumour outgrowth and in escape state, tumour cells attain the property to circumvent immune recognition via a lot of mechanisms. So, in immunotherapy, body’s own innate and adaptive immune response is enhanced to target the cancer cells. Immunotherapy utilises body’s own cells to support the immune system and body to destroy the cancer cells. Immune cells that functions against infections are T-cells. The T-cells are modified with the help of CRISPR- Cas9 technology to express specific antigen receptor and then introduced into the patient (Razeghian, et al.,2021). This will allow T-cells to recognize the cancer cells and destroy them. This treatment is called chimeric antigen receptor (CAR) T- cell therapy. Although this treatment is associated with certain side effects like low blood pressure, fevers and seizures, continuous research is being performed related to this treatment because it is very effective. In B cell leukemia or lymphoma, notable results were observed

when cells were treated with CAR- T cells but many barriers restrict the efficiency of this treatment in solid tumors. These barriers include immune escape, proliferation of cancer cells and decreased release of vascular-associated factors. Chimeric antigen receptor attacks specific surface antigens present on the tumor and destroys them showing anti-tumor activity (Mardiana, et al., 2020).

Revolutionizing TNBC treatment: Harnessing CAR-T cell therapy potential

Triple Negative Breast Cancer is the cancer that does not have usual receptors. It lacks expression of progesterone receptor, estrogen receptor and HER 2 due to which it does not respond to conventional endocrine and anti-HER-2 targetted therapies (Dees, et al., 2019). CAR T cell therapy promises a therapeutic effect in TNBC. CAR constructs can be introduced into allogenic T- cells via CRISPR Cas9 technology (Bagley, et al., 2020, Martinez, et al., 2019).The cell surface targets present in TNBC for CAR T cell therapy are AXL, CD32A (FcγR), Chondroitin sulfate proteoglycan 4 (CSPG4), EGFR, Folate receptor alpha (FRα), Disialoganglioside GD2(GD2), Intracellular adhesion molecule-1 (ICAM- 1), Integrin $\alpha_v\beta_3$, Mesothelin, c-Met, MUC1, NKG2D ligand, ROR1, SSEA-4, TEM8, and TROP2. Receptor tyrosine kinase AXL is involved in tumour progression via downstream signalling cascades NF-κβ, MAPK and PI3K (Zhu C et al., 2019). In vitro cytotoxicity and cytokine secretion is observed in AXL-CAR-T cell therapy and also reduction in tumour growth is observed in MDA-MB-231 xenograft mouse model (HER2 negative, CK18 and EGFR positive) (Jing Wei, et al., 2018). Fc-gamma receptors can enable utilisation of multiple antibodies to divert T-cells to antigen expressing tumour cells. FcγRs in combination with antibodies that target tumour antigens can be used to eliminate solid tumours (Caratelli et al., 2017). CSPG4 is a cell surface proteoglycan in melanoma cells. It is overexpressed in solid tumours including TNBC. CSPG4-CAR T cell has shown anti-tumour activity in Hs578T (cell line of epithelial morphology isolated from 74-year-old female patient with breast cancer), MDA-MB-468 (epithelial cell line isolated from 51-year-old female patient having breast adenocarcinoma) and MDA-MB-231 TNBC cell thereby causing proinflammatory cytokine release and cytotoxicity to tumour cells (Beard et al., 2014). Receptor tyrosine kinase EGFR mediates growth, survival and invasion of cancer cells via oncogenic signalling cascades (Nakai k, et al., 2016). Patients with TNBC overexpresses EGFR and thereby EGFR - CAR T cells restricted tumour growth in patient-derived xenograft and cell line–derived

xenograft mouse models (Liu, et al., 2017). In cancers of epithelial origin, including breast tumours, folate receptor alpha is overexpressed, so it is a major therapeutic target for cancer (Cheung, et al.,2016). FR*α*-CAR-T cells has shown effect *in vitro* killing of TNBC cells andtumour regression in an MDA-MB-231 xenograft mouse model (HER2 negative, CK18 and EGFR positive) (Song, et al., 2016). Glycosphingolipid, GD2 help tumour cells to bind to extracellular matrix proteins. CAR-T cells are modified with a single-chain variable fragment (scFv) derived from dinutuximab beta to aim GD2 on cancer stem cells in TNBC to destroy tumour cell populations and avoid metastasis formation. GD2-CAR-Tcells has shown in vitro antitumor activity and target cell lysis. Effective antitumor immune response that arrested tumour growth was observed in an orthotopic xenograft mouse model (human TNBC cell lines has been inserted) of TNBC seen in human females (Seitz CM, et al., 2019). ICAM-1 (glycoprotein) plays a major role in cell-to-cell interaction. It is overexpressed in MDA-MB-231 TNBC cells. Recent research *shown* in vitro antitumor activity of CAR-T cells aims at ICAM-1 in 85% of MDA-MB-231 TNBC (Yang et al., 2019).

Empowering CAR-T cell efficacy: Novel approaches and techniques

A major barrier to the efficacy of engineered T cells is caused by triggering of inhibitory receptors cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) leading to an impaired antitumor activity. PD-1 and CTLA-4 inhibitors are antibodies that block immune checkpoints, allowing the immune system to attack cancer cells more effectively. While utilizing CAR T cell therapy to inhibit PD-1 and CTLA-4, it's important to understand that CAR T cells and immune checkpoint inhibitors have different mechanisms of action. CAR T cells primarily target cancer cells directly, while immune checkpoint inhibitors modulate the immune response by blocking inhibitory signals. However, there is ongoing research exploring combinational therapies that involve both CAR T cell therapy and immune checkpoint inhibitors to enhance the effectiveness of cancer treatment. Researchers are investigating the development of CAR T cells that express additional receptors for PD-1 or CTLA-4, on their surface. This approach aims to create CAR T cells that not only directly attack cancer cells but also have enhanced anti-tumour activity by blocking immune checkpoints. Sequential therapy can be used which involves administering CAR T cell therapy followed by immune checkpoint inhibitors or vice versa. The rationale is that CAR T cell therapy can eliminate a significant portion of tumour cells, which may increase the effectiveness of subsequent immune checkpoint inhibitors by

reducing the overall tumour burden. Combining CAR T cell therapy with immune checkpoint inhibitors simultaneously has shown promise in preclinical studies. By using both approaches, the CAR T cells can attack cancer cells, while the immune checkpoint inhibitors prevent inhibitory signals from dampening the immune response, leading to a potentially stronger and more sustained anti-tumour effect. To increase CAR-T-cell efficiency, T cells can be electroporated with both, mRNA coding for CAR specific for small-interfering RNAs (siRNAs) and chondroitin sulphate proteoglycan 4 (CSPG4) to downregulate CTLA-4 (siCTLA-4) and PD-1 (siPD-1). Also, activation-induced upregulation of both PD-1 and CTLA-4 was suppressed. In melanoma cells with antigen CSPG4, CAR-T cells transfected with siPD-1 showed enhanced cytokine secretion. Also, CAR-T cells incorporated with either siPD-1 alone or together with siCTLA-4 shown enhanced cytotoxicity. Very less adverse effects were seen when CAR-T cells were co-incorporated with siCTLA-4 only (Simon, et al., 2018). The CAR-T cells can be transformed to secrete single-chain variable fragments (scFv), which has an activity of blocking PD-1. In syngeneic and xenogeneic mouse models of PD-$L1^+$ hematologic and solid tumours, it has shown its activity in both paracrine and autocrine manner to enhance the anti-neoplastic activity of CAR-T cells. This approach could potentially prevent toxicities related to systemic checkpoint inhibition(Rafiq, et al., 2018). Another complication following infusion of CAR T cells is cytokine release syndrome (CRS) (Lee, et al., 2014). The symptoms associated with CRS include fever, malaise, anorexia, nausea, cardiac dysfunction, myalgia, tachycardia/hypotension. Following therapy, levels of IL-6, IL-10, granulocyte macrophage colony-stimulating factor and interferon-gamma were elevated (Maude, et al., 2016, Davila, et al.,2014, Lee, et al., 2014 and Mauda, et al, 2014). The disease burden is related with the degree of CRS severity as patients with more tumour burden has more CRS activity (Maude, et al., 2016, Davila, et al.,2014 and (Lee, et al., 2014). Immediate reversal of CRS symptomatology can be acquired with the use of tocilizumab which will provide IL 6 blockade. The symptoms can also be improved with systemic corticosteroid.

The major hindrance is the formation of tumour resistance to single antigen targeting chimeric antigen receptors. The CAR-T cells treated malignant cells show partial or complete loss of target antigen expression. This is called antigen escape. Disease resistance mechanism involving downregulation of CD19 antigen in majority of patients with repeated disease after treatment is shown by latest data (Majzner, et al., 2018, Maude, et al., 2015). In multiple myeloma patients treated with BCM targeted CAR T- cells

downregulation of BCMA expression has been observed (Green, et al., 2018) (Brudno, et al., 2018, Cohen, et al., 2019). Utilization of dual-targeted CAR-T cells (CD19/CD22 or CD19/BCMA) have shown favourable results (Zhang, et al., 2019, Lin, et al., 2019, Dai, et al., 2020, Hossain, et al., 2018). In adult patients with ALL and diffuse large B cell lymphoma, CD19/CD22 CAR-T cell therapy shown more efficacy as suggested by preliminary clinical trial results (Dai, et al., 2020, Hossain, et al., 2018). HER2 and MUC1 in breast cancer and HER2 and IL13Ra2 in glioblastoma in preclinical models were treated with dual targeting CARs and resulted in more prominent anti-tumor responses(Hege, et al., 2017, Wilkie, et al., 2012). Systemic cytokine levels increased in an alarming level in patients treated with CAR T cells including all of the clinical trials of anti-CD19 CAR T cells (Park, et al., 2018, Maude, et al., 2018, Schuster, et al., 2017). The adverse effects include macrophage activation syndrome (MAS), immune effector cell-associated neurotoxicity syndrome (ICANS), cytokine-release syndrome (CRS) and hemophagocyticlymph histiocytosis (HLH) (Lee, et al., 2019). Due to these toxic effects, major clinical modifications happened in the utilization of CAR T- cell therapy (Neelapu, et al., 2018). Instead of using corticosteroids, tocilizumab or siltuximab targeting IL-6 pathway has been used to prevent the harmful effects on the antitumor activity of the CAR T- cells (Brentjens, et al., 2013). The level of antigen exposed on the tumour cells, the co-stimulatory elements in the chimeric antigen receptor, the affinity of the scFv (or other antigen-binding domain) for the antigen and the overall tumour burden is determining the degree and kinetics of CAR T cell activation (Milone, et al.,2018,Van der Stegen, et al., 2015). Transformation of the transmembrane amino acid sequences and CD8α-derived hinge of a CD19-targeted CAR showed reduced CAR T cell proliferation and decreased levels of cytokine release (Ying, et al., 2019). The utilization of human antibody fragments for CAR preparation, instead of mouse antibodies, and alteration of the extracellular hinge region and transmembrane domain can decrease the immunogenicity of the CAR (Sommermeyer, et al., 2017, Hombach, et al., 2010, Jonnalagadda, et al., 2015). Blocking the monocyte-activating and macrophage-activating cytokine GM-CSF with the help of antibody lenzilumab rises CAR T cell activity while lowering the risk of CRS (Sterner, et al., 2019, Sachdeva, et al., 2019). In a mouse model of lymphoma, myeloid cell-specific deletion of tyrosine hydroxylase or inhibition of this enzyme with the help of metyrosine decreases circulating catecholamine and, thereby cytokine levels after anti-CD19 CAR T cell treatment (Staedtke et al., 2018). The toxicity of CAR T cells can be controlled by engineering of ‘suicide genes’ into the CAR

construct to deactivate CAR T cells if and when either cytokine-mediated or on-target, off-tumour toxicities happen. Apoptosis-triggering fusion protein containing caspase 9 attached to an altered form of the FK506-binding protein FKBP1A (iCasp9), which will help in dimerization and activation of the fusion protein via binding to a biologically inert and systematically administered small molecule (AP1903). The patients who acquired an allogeneic haematopoietic stem cell transplant for the acute leukaemia therapy followed by iCasp9-modified donor T cells had immune reestablishment and the dimerizing agent incorporated in patients who got graft versus host disease (GVHD) in clinical trial, caused the removal of more than 90% of the altered T cells in 30 minutes and treatment of GVHD, thereby showing efficacy of this method for rapid depletion of CAR T cells (Di Stasi et al., 2011).

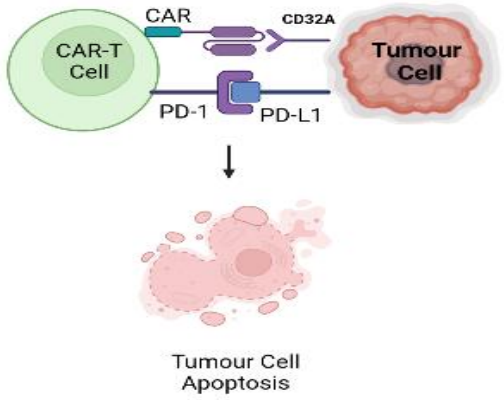

Figure:1 CAR-T attacking tumour cells and inducing apoptosis

Conquering obstacles: Overcoming challenges with resilience and innovation

Solid tumours often do not express a single tumour-specific marker or the expression of tumour associated antigens (TAAs) is not entirely unique to malignancy, which can lead to on target/off-tumor toxicity. Non-tumour side effects in CAR T lymphocyte therapies arise as a result of the expression of the target antigen on normal cells. Most CAR T-cell targets that have been tested

in humans are not exclusively tumour-specific, leading to the destruction of healthy cells that can damage vital organs (Santomasso,etal 2019).

Cytotoxicity

Human PBMCs are an important source of primary NK cells that have been used in many clinical trials. Use of NK cell isolation kits, a sufficient number of NK cells can be easily isolated directly from healthy donor PBMCs and then stimulated and expanded in NK cells in specific expansion media with cytokines for preclinical or clinical application at the level of good manufacturing practice (GMP). Activated PBMC-derived CAR-NK cells expressing a wide range of activating receptors can be administered without radiation which allows them to expand in vivo. PBMCs-derived NK (PB NK) cells, up to 90% of these are CD56dimCD16+ NK cells, typically showing mature phenotype, with increased cytotoxicity and decreased proliferation capacity (Xie G. et. al. 2020).

Furthermore, compared to PB NK cells, UCB NK cells show a less mature phenotype and lower cytotoxicity against tumour cells, with reduced expression of certain adhesion molecules, CD16, KIR, perforin and granzyme B and higher expression of inhibitory molecules such as NKG2A (Xie et al, 2020).

Proliferation

CAR T cells are also likely to be activated surrounding immune cells, such as macrophages, which in turn release inflammatory cytokines and contribute to the pathophysiology CRS. CRS typically presents with constitutional symptoms of fever, myalgia, stiffness, fatigue and loss of appetite, but can lead to multiorgan dysfunction in more severe cases. However, CRS is completely reversible if properly managed. Serum levels of IL-6 were correlates with CRS severity and IL-6 blockade with tocilizumab, an anti-IL-6 receptor antibody, can reverse CRS. Other cytokines and chemokines such as IL-8, IL-10, IL-15, IFNg and MCP-1 have also been shown to be associated with severe CRS. If therefore Blockade of IL-6 alone is not enough to manage CRS, corticosteroids are used because they can cause global suppression and reduced immunity in addition, the production of many different cytokines and chemokines directly affecting the proliferation and function of CAR T-cells (Neelapu, 2019).

Toxicity

Although CAR-T cell therapy has been revolutionary tool for cancer treatment, high level of toxicity in some death was prevented by CAR-T cell therapy become the treatment of first choice. One of the challenges in targeting solid tumour antigens is that solid tumour antigens are often also expressed normally tissues at different levels. Hence the choice of antigen essential in CAR design not only to ensure therapeutic efficacy but also to limit "on-target off -tumour" toxicity. Further development innovative strategies to reduce antigen leakage and selection antigens capable of inducing sufficient antitumor efficacy, and at the same time it will be necessary to minimize toxicity concerns with the aim of expanding the clinical use of CART cell therapies in haematological malignancies and solid tumours (Sterneret al, 2021).

Tumour infiltration

Major challenge associated with solid-state CAR-T cell therapy of cancer is inefficient transport to the tumour site, which has not been the case so far addressed in TNBC. Preparation of TNBC CAR-T cells for expression chemokine receptors such as CCR-2 and CCR-4 is one strategy that has potential to improve tumour trafficking and infiltration because TNBCs overexpress associated chemokine ligands. Actually, engineered anti-FITC CAR-T cells that co-express CCL-19 and IL-7 in other malignant tumours, an increase in tumour infiltration is considered. Further efforts to extend the duration of CAR-T cells in solid tumours take place and include incorporation constitutively activated IL-15 or IL-7 receptors into the CAR-T cell constructs (Dees S., et al., 2020)

Even after successful infiltration of CAR-T cells by the tumour into the surrounding microenvironment is endowed with harsh living conditions, including hypoxia, abnormal vascularization, compact extracellular matrix, nutritional depletion, acidic pH, oxidative stress and inhibition cytokines and soluble factors (eg, prostaglandin E2, adenosine, TGFβ, IL-4, and IL-10) that it prevents T cell expansion and persistence. Use of fourth generation CAR-T cells

(TRUCK cells), capable of secreting activating cytokines (e.g. IL-12, IL-15, IL-18), or fifth generation CARs, equipped with the ability to trigger endogenous cytokine signalling, could they help increase the proliferative capacity and survival of T cells (Abreu et al., 2019).

Cross reactivity

A panel of CAR affinity variants designed to evaluate cytotoxicity against cells with low antigen expression in vitro and in vivo. In the study it has been demonstrated that a reduced affinity of HER2 CAR T cells, failed to recognize physiological levels of HER2 (X. Liu, et. al. 2015). Additionally, it would be ideal to have animal models to evaluate the initial safety of CAR targets. For example, human B7-H4, one of the members of the B7 family, expression patterns and location is mostly similar to mouse B7-H4 protein expression. B7-H4 CAR can cross-react with both human and murine B7-H4 and B7- Treatment with H4 CAR T cells resulted in lethal toxicity in mice, suggesting that targeting B7-H4 homologue in mice may be a relevant preclinical model to address questions regarding B7-H4 function and targeting in vivo. In addition, the researchers created a clinically relevant model using transgenic mice expressing selected human antigens orthotopically, such as carcinoembryonic antigen (CEA) (M. Chmielewski. et al., 2012 and Xie, et al., 2020).

Complexities of CAR-T cell therapy

Although the enhanced efficacy of THZ1 is still unknown, the data imply that the effects of THZ1 on the group of IFN signalling genes are at least largely responsible by THZ1 of EGFR CAR T cells. It has been demonstrated that an "Achilles cluster" of super enhancer-associated, TNBC-specific genes is particularly responsive to CDK7 inhibition/THZ1. The decreased ineffectiveness of THZ1 may also be a result of the inhibitory effects of THZ1 on these genes. It showed that THZ1's enhanced efficacy is, at least in part, mediated by CDK7, it is important to note that THZ1 is a covalent inhibitor that also targets CDK12/13. The improved efficacy of THZ1 was investigate whether co-treatment with THZ1 attenuated EGFR CAR T-cell associated immune resistance in mice. SCID mice were subcutaneously implanted with MDAMB-231 cells and tumour growth/metastasis was monitored using bioluminescence imaging and size based on a caliper. Effect of EGFR CAR T cells and THZ1 co-treatment on TNBC was investigated using an immunocompetent model. Firstly, constructed a retroviral vector encoding an EGFR-targeted CAR designed for mice (EGFR mCAR) that had high expression in HEK293T cells after transfection (92.2%). Mouse T cells were infected with retroviruses encoding EGFR mCAR to generate EGFR mCAR T cells. EGFR mCAR T cells killed murine TNBC EMT6 cells in in a dose- and time-dependent manner. 1 THZ increased the efficacy of EGFR mCAR T cells

in killing EMT6 cells in vitro. EGFR mCAR T-cell induced expression of immunosuppressive genes was attenuated by THZ1 (Xia, et al., 2021).

Cetuximab and panitumumab as a model system for determining which FcgR variant-based CAR-T cell leads to optimal cytotoxicity. Results with the designed CD16A158F CAR-T cells in combination with cetuximab have shown significant in vitro antitumor activity against EGFR-overexpressing TNBC cells. On the other hand, CD32A131R CAR-T cells in combination with cetuximab or panitumumab led to the elimination of EGFR-positive MDAMB-468 TNBC cells, as well as pro-inflammatory secretion cytokines including IFNg and TNFa (Dees, S. et. al., 2020). CAR-T cells that exclude blocking immune checkpoint antibodies such as anti-CTLA-4 and anti-PD-1 would represent an attractive alternative to the combination therapy and is considered (Yoon, et al. 2018).

The use of combination therapy to simultaneously target different mechanisms of action has been shown to be a viable treatment approach cancer. CAR-T cell therapy for TNBC is unlikely to replace chemotherapy in the foreseeable future, but rather will be useful in combination therapy. In fact, the anti-PD-L1 immunotherapy atezolizumab is FDA-approved for use in combination with chemotherapy agent nab-paclitaxel for treatment metastatic TNBC (Schmid et al., 2019).

Clinical manipulations such as combinations with chemotherapy, radiotherapy and genetic engineering strategies to manipulate T cell trafficking to tumours may increase safety, efficacy and broad applicability of CAR T-cell technology. We believe that CAR T cells could show excellent safety and efficacy and improve the lives of TNBC patients' future (Xie et al., 2020). FRα CAR T application cell therapy in TNBC patients whose tumours express FRα, particularly at high levels. It is remarkable that the FRα gene is positively regulated by the glucocorticoid receptor agonist dexamethasone at the transcriptional level (promoter P4) and this profound regulation is further potentiated by inhibition of histone deacetylase (HDAC). This observation supports the view that efficacy of FRα CAR T cell therapy in patients with TNBC with low to moderate levels of tumours FRα can be improved in combination with dexamethasone and HDACs inhibitors that increase FRa gene expression. Therefore, co-administration of FRα CAR T cells and mesothelin CAR T cells may provide excellent antitumor properties effects on TNBC and also addresses potential tumours problems antigen heterogeneity and antigen loss (Song, et al., 2016).

Scalability

These CAR T cells are autologous lymphocytes from patients. However, this autologous patient-specific T cell paradigm is a significant limiting factor for large deployment of CAR technology as CAR T cells the product is individual and therefore varies by patient to the patient. It is therefore not a ready-to-use product conventional therapeutic agent. Individual the production process is costly and time-consuming. In in addition, generating a sufficient number of custom autologous T cells may not be feasible or successful in all cases, especially in infants or highly treated patients who are profoundly lymphogenic as a result of multiple prior chemotherapy and/or stem cell transplants. In addition, each CAR has a fixed antigenic specificity, e.g that each CAR T product can target only one epitope specific antigen, which limits efficacy due to heterogeneous tumour antigen expression and tumour antigen leakage. Universal "regular" CAR T cells that can be simultaneously or sequentially administered by multiple patients can effectively solve the above problems. This review summarizes recent advances in the designs and application of universal CAR T cells (Zhao,2018).

Future prospects

Despite adjuvant chemotherapy, the majority of TNBC patients recur within 1-2 years (Dent et al., 2007), which emphasizes the necessity for further therapy in early-stage TNBC to prevent rapid relapse. According to the hypothesis around cancer stem cells (CSCs), these cells are inherently resistant to chemotherapy and may later rebuild the tumour, causing recurrence (Deonarain et al., 2009). Therefore, medications that block major CSC active pathways including the PI3K and Wnt pathways may prevent them from causing tumours, which could be a viable therapeutic approach in the adjuvant setting of TNBC (Yu et al., 2011. The use of platinum-based CT in TNBC is supported by certain studies, notably in BRCA mutation carriers. How to boost pCR and how to improve outcomes for patients with residual illness are the two main axes on which clinical research is concentrated in the neoadjuvant context. There are now being developed new targeted therapies and immunotherapeutic medicines. Due to the significance of variability in TNBC, the difficulty is in directing investigations on more specialized patient populations. The most promising novel strategies include checkpoint inhibitor immunotherapy, PARP inhibition, and AR inhibition. (Collignon et al., 2016) Additionally, there is a continuing effort to find more precise targets in TNBC (Shu et al., 2016). The ultimate objective for a successful TNBC therapy is the

discovery of distinct genetic targets that are specific to a given subtype. (Garmpis et al., 2020)

The use of immune checkpoint-blocking antibodies, such as anti-PD-1 or anti-PD-L1 antibodies, is one of the more alluring immunotherapeutic strategies for TNBC. We may anticipate the continued development of innovative immune therapeutics, some of which will soon help TNBC patients. Furthermore, there is reason to assume that immunotherapy may soon be a viable therapeutic option for individuals with this untreatable disease and that it may well be the modality that brings about the improvements in the clinical management of TNBC that we have been working towards. Particularly, combining immunotherapy with current or novel therapies is an emerging trend and is most likely to influence future treatment methods (Jia et al., 2017).

References

Abd-Elazeem, M. A., & Abd-Elazeem, M. A. (2015). Claudin 4 expression in triple-negative breast cancer: correlation with androgen receptors and Ki-67 expression. Annals of diagnostic pathology, 19(1), 37-42. https://doi.org/10.1016/j.anndiagpath.2014.10.003

Abreu, T., Fonseca, N. A., Gonçalves, N., & Moreira, J. N. (2020). Current challenges and emerging opportunities of CAR-T cell therapies. Journal of Controlled Release, 319, 246–261. https://doi.org/10.1016/j.jconrel.2019.12.047

Akbari, P., Katsarou, A., Daghighian, R., van Mil, L. W., Huijbers, E. J., Griffioen, A. W., & van Beijnum, J. R. (2022). Directing CAR T cells towards the tumor vasculature for the treatment of solid tumors. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1877(3), 188701.https://doi.org/10.1016/j.bbcan.2022.188701

Anborgh, P. H., Lee, D. J., Stam, P. F., Tuck, A. B., & Chambers, A. F. (2018). Role of osteopontin as a predictive biomarker for anti-EGFR therapy in triple-negative breast cancer. Expert opinion on therapeutic targets, 22(8), 727-734. https://doi.org/10.1080/14728222.2018.1502272

Bagley, S., & O'Rourke, D. M. (2020). Clinical investigation of CAR T cells for solid tumors: Lessons learned and future directions. Pharmacology & Therapeutics, 205, 107419. https://doi.org/10.1016/j.pharmthera.2019.107419

Barman, U. D., Saha, S. K., Kader, M. A., Jamal, M. a. H. M., Sharma, S. P., Samad, A., & Rahman, M. S. (2020). Clinicopathological and

prognostic significance of GPC3 in human breast cancer and its 3D structure prediction. Network Modeling Analysis in Health Informatics and Bioinformatics, 9(1). https://doi.org/10.1007/s13721-020-00234-x

Beard, R. E., Zheng, Z., Lagisetty, K. H., Burns, W. R., Tran, E., Hewitt, S. M., Abate-Daga, D., Rosati, S. F., Fine, H. A., Ferrone, S., Rosenberg, S. A., & Morgan, R. A. (2014). Multiple chimeric antigen receptors successfully target chondroitin sulfate proteoglycan 4 in several different cancer histologies and cancer stem cells. Journal for Immunotherapy of Cancer, 2(1), 25. https://doi.org/10.1186/2051-1426-2-25

Bianchini, G., Balko, J. M., Mayer, I. A., Sanders, M. E., & Gianni, L. (2016). Triple negative breast cancer: challenges and opportunities of a heterogeneous disease. Nature Reviews Clinical Oncology, 13(11), 674–690. https://doi.org/10.1038/nrclinonc.2016.66

Caratelli, S., Sconocchia, T., Arriga, R., Coppola, A., Lanzilli, G., Lauro, D., Venditti, A., Del Principe, M. I., Buccisano, F., Maurillo, L., Ferrone, S., &Sconocchia, G. (2017). FCΓ Chimeric Receptor-Engineered T Cells: Methodology, Advantages, Limitations, and Clinical Relevance. Frontiers in Immunology, 8. https://doi.org/10.3389/fimmu.2017.00457

Cheung, A., Bax, H. J., Josephs, D. H., Ilieva, K. M., Pellizzari, G., Opzoomer, J. W., Bloomfield, J., Fittall, M., Grigoriadis, A., Figini, M., Canevari, S., Spicer, J., Tutt, A., &Karagiannis, S. N. (2016). Targeting folate receptor alpha for cancer treatment. Oncotarget, 7(32), 52553–52574. https://doi.org/10.18632/oncotarget.9651

Davies, A. M., & Sutton, B. J. (2015). Human IgG4: a structural perspective. Immunological Reviews, 268(1), 139–159. https://doi.org/10.1111/imr.12349

Davila, M. L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., ... &Brentjens, R. (2014). Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Science translational medicine, 6(224), 224ra25-224ra25. https://doi.org/10.1126/scitranslmed.3008226

Dees, S., Ganesan, R., Singh, S., & Grewal, I. S. (2020). Emerging CAR-T cell therapy for the treatment of triple-negative breast Cancer CAR-T cell therapy to target TNBC. Molecular cancer therapeutics, 19(12), 2409-2421.https://doi.org/10.1158/1535-7163.MCT-20-0385

Dees, S., Ganesan, R., Singh, S., & Grewal, I. S. (2020). Emerging CAR-T cell therapy for the treatment of triple-negative breast cancer. Molecular cancer therapeutics, 19(12), 2409-2421. https://doi.org/10.1158/1535-7163.MCT-20-0385

Dent, R., Trudeau, M. E., Pritchard, K. I., Hanna, W., Kahn, H. K., Sawka, C., Lickley, L., Rawlinson, E., Sun, P., & Narod, S. A. (2007). Triple Negative Breast Cancer: Clinical Features and Patterns of Recurrence. Clinical Cancer Research, 13(15), 4429–4434. https://doi.org/10.1158/1078-0432.ccr-06-3045

Deonarain, M., Kousparou, C., &Epenetos, A. A. (2009). Antibodies targeting cancer stem cells: A new paradigm in immunotherapy? mAbs, 1(1), 12–25. https://doi.org/10.4161/mabs.1.1.7347

Gluz, O., Liedtke, C., Gottschalk, N., Pusztai, L., Nitz, U., & Harbeck, N. (2009). Triple negative breast cancer—current status and future directions. Annals of Oncology, 20(12), 1913–1927. https://doi.org/10.1093/annonc/mdp492

Hashmi, A. A., Naz, S., Hashmi, S. N., Hussain, Z. F., Irfan, M., Khan, E. Y., Faridi, N., Khan, A., & Edhi, M. M. (2018). Prognostic significance of p16 & p53 immunohistochemical expression in triple negative breast cancer. BMC Clinical Pathology, 18(1). https://doi.org/10.1186/s12907-018-0077-0

Howard, F. D., Moingeon, P., Moebius, U., McConkey, D. J., Yandava, B., Gennert, T. E., &Reinherz, E. L. (1992). The CD3 zeta cytoplasmic domain mediates CD2-induced T cell activation. Journal of Experimental Medicine, 176(1), 139–145. https://doi.org/10.1084/jem.176.1.139

Jain, M. D., Bachmeier, C. A., Phuoc, V., & Chavez, J. C. (2018). Axicabtageneciloleucel (KTE-C19), an anti-CD19 CAR T therapy for the treatment of relapsed/refractory aggressive B-cell non-Hodgkin ’s lymphoma. Therapeutics and Clinical Risk Management, Volume 14, 1007–1017. https://doi.org/10.2147/tcrm.s145039

Jain, P., & Wang, M. (2019). Mantle cell lymphoma: 2019 update on the diagnosis, pathogenesis, prognostication, and management. American Journal of Hematology, 94(6), 710–725. https://doi.org/10.1002/ajh.25487

Jia, H., Truica, C. I., Wang, B., Wang, Y., Ren, X., Harvey, H. A., Song, J., & Yang, J. (2017). Immunotherapy for triple-negative breast cancer: Existing challenges and exciting prospects. Drug Resistance Updates, 32, 1–15. https://doi.org/10.1016/j.drup.2017.07.002

Wei, J., Sun, H., Zhang, A., Wu, X., Li, Y., Liu, J., Duan, Y., Xiao, F., Wang, H., Lv, M., Wang, L., & Wu, C. (2018). A novel AXL chimeric antigen receptor endows T cells with anti-tumor effects against triple negative breast cancers. Cellular Immunology, 331, 49–58. https://doi.org/10.1016/j.cellimm.2018.05.004

Kabat, G. C., Kim, M. Y., Phipps, A. I., Li, C. I., Messina, C. R., Wactawski-Wende, J., Kuller, L. H., Simon, M. S., Yasmeen, S., Wassertheil-Smoller, S., & Rohan, T. E. (2011). Smoking and alcohol consumption in relation to risk of triple-negative breast cancer in a cohort of postmenopausal women. Cancer Causes & Control, 22(5), 775–783. https://doi.org/10.1007/s10552-011-9750-7

Leddon, S. A., Fettis, M. M., Abramo, K., Kelly, R. L., Oleksyn, D., & Miller, J. (2020). The CD28 transmembrane domain contains an essential dimerization motif. Frontiers in Immunology, 11. https://doi.org/10.3389/fimmu.2020.01519

Liu, J., Lichtenberg, T. M., Hoadley, K. A., Poisson, L., Lazar, A. J., Cherniack, A. D., Kovatich, A. J., Benz, C. C., Levine, D. A., Lee, A. V., Omberg, L., Wolf, D. M., Shriver, C. D., Thorsson, V., & Hu, H. (2018). An integrated TCGA Pan-Cancer Clinical Data resource to drive High-Quality Survival Outcome Analytics. Cell, 173(2), 400-416.e11. https://doi.org/10.1016/j.cell.2018.02.052

Liu, Y., Zhou, Y., Huang, K., Liu, Y., Fang, X., An, L., Wang, F., Chen, Q., Zhang, Y., Shi, A., Yu, S., & Zhang, J. (2019). EGFR-specific CAR-T cells trigger cell lysis in EGFR-positive TNBC. Aging, 11(23), 11054–11072. https://doi.org/10.18632/aging.102510

Chmielewski, M., Hahn, O., Rappl, G., Nowak, M. A., Schmidt–Wolf, I. H., Hombach, A., &Abken, H. (2012). T cells that target carcinoembryonic antigen eradicate orthotopic pancreatic carcinomas without inducing autoimmune colitis in mice. Gastroenterology, 143(4), 1095-1107.e2. https://doi.org/10.1053/j.gastro.2012.06.037

Maddocks, K. J. (2018). Update on mantle cell lymphoma. Blood, 132(16), 1647–1656. https://doi.org/10.1182/blood-2018-03-791392

Mardiana, S., & Gill, S. (2020). CAR T cells for acute myeloid leukemia: State of the art and future directions. Frontiers in Oncology, 10. https://doi.org/10.3389/fonc.2020.00697

Martínez, M., & Moon, E. K. (2019). CAR T cells for Solid Tumors: New strategies for finding, infiltrating, and surviving in the tumor microenvironment. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.00128

Maude, S. L., Barrett, D. M., Teachey, D. T., & Grupp, S. A. (2014). Managing cytokine release syndrome associated with novel T Cell-Engaging therapies. The Cancer Journal, 20(2), 119–122. https://doi.org/10.1097/ppo.0000000000000035

Maude, S. L., Frey, N. V., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N., Chew, A., Gonzalez, V., Zheng, Z., Lacey, S. F., Mahnke, Y. D., Melenhorst, J. J., Rheingold, S. R., Shen, A., Teachey, D. T., Levine, B. L., June, C. H., Porter, D. L., & Grupp, S. A. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. The New England Journal of Medicine, 371(16), 1507–1517. https://doi.org/10.1056/nejmoa1407222

Nakai K, HungMC, YamaguchiH, (2016). A perspective on anti-EGFR therapies targeting triple-negative breast cancer. Am J Cancer Res; 6:1609–23 https://pubmed.ncbi.nlm.nih.gov/27648353

Neelapu, S. S. (2019). Managing the toxicities of CAR T-cell therapy. Hematological Oncology, 37(S1), 48–52. https://doi.org/10.1002/hon.2595

Nimmerjahn, F., &Ravetch, J. V. (2008). Fcγ receptors as regulators of immune responses. Nature Reviews Immunology, 8(1), 34–47. https://doi.org/10.1038/nri2206

Nobari, S. T., Nojadeh, J. N., & Talebi, M. (2022). B-cell maturation antigen targeting strategies in multiple myeloma treatment, advantages and disadvantages. Journal of Translational Medicine, 20(1). https://doi.org/10.1186/s12967-022-03285-y

Rafiq, S., Yeku, O., Jackson, H. J., Purdon, T. J., Van Leeuwen, D. G., Drakes, D. J., Song, M., Miele, M. M., Li, Z., Wang, P., Su, Y., Xiang, J., Ma,

X., Seshan, V., Hendrickson, R. C., Liu, C., &Brentjens, R. J. (2018). Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. Nature Biotechnology, 36(9), 847–856. https://doi.org/10.1038/nbt.4195

Razeghian, E., Nasution, M. K. M., Rahman, H. S., Gardanova, Z. R., Abdelbasset, W. K., Aravindhan, S., Bokov, D. O., Suksatan, W., Nakhaei, P., Shariatzadeh, S., Marofi, F., Yazdanifar, M., Shamlou, S., Motavalli, R., &Khiavi, F. M. (2021). A deep insight into CRISPR/Cas9 application in CAR-T cell-based tumor immunotherapies. Stem Cell Research & Therapy, 12(1). https://doi.org/10.1186/s13287-021-02510-7

Rey-Vargas, L., Sanabria-Salas, M. C., Fejerman, L., & Serrano-Gómez, S. J. (2019). Risk Factors for Triple-Negative Breast Cancer among Latina Women. Cancer Epidemiology, Biomarkers & Prevention, 28(11), 1771–1783. https://doi.org/10.1158/1055-9965.epi-19-0035

Roberts, Z. J., Better, M., Bot, A., Roberts, M., &Ribas, A. (2017). Axicabtageneciloleucel, a first-in-class CAR T cell therapy for aggressive NHL. Leukemia& Lymphoma, 59(8), 1785–1796. https://doi.org/10.1080/10428194.2017.1387905

Santomasso, B., Bachier, C., Westin, J. R., Rezvani, K., &Shpall, E. J. (2019). The other side of CAR T-Cell therapy: cytokine release syndrome, neurologic toxicity, and financial burden. American Society of Clinical Oncology Educational Book, 39, 433–444. https://doi.org/10.1200/edbk_238691

Schmid, P., Rugo, H. S., Adams, S., Schneeweiss, A., Barrios, C. H., Iwata, H., Diéras, V., Henschel, V., Molinero, L., Chui, S. Y., Maiya, V., Husain, A., Winer, E. P., Loi, S., Emens, L. A., & Investigators, I. (2020). Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncology, 21(1), 44–59. https://doi.org/10.1016/s1470-2045(19)30689-8

Schreiber, R. D., Old, L. J., & Smyth, M. J. (2011). Cancer Immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science, 331(6024), 1565–1570. https://doi.org/10.1126/science.1203486

Scott, L., Mobley, L., Kuo, T., &Il'yasova, D. (2019). Update on triple-negative breast cancer disparities for the United States: A population-based study from the United States Cancer Statistics database, 2010 through 2014. Cancer, 125(19), 3412–3417. https://doi.org/10.1002/cncr.32207

Seitz, C., Schroeder, S., Knopf, P., Krahl, A., Hau, J., Schleicher, S., Martella, M., Quintanilla-Martinez, L., Kneilling, M., Pichler, B. J., Lang, P., Atar, D., Schilbach, K., Handgretinger, R., & Schlegel, P. (2019). GD2-targeted chimeric antigen receptor T cells prevent metastasis formation by elimination of breast cancer stem-like cells. OncoImmunology, 9(1). https://doi.org/10.1080/2162402x.2019.1683345

Simon, B., Harrer, D. C., Schuler-Thurner, B., Schaft, N., Schuler, G., Dörrie, J., & Uslu, U. (2018). The siRNA-mediated downregulation of PD-1 alone or simultaneously with CTLA-4 shows enhanced in vitro CAR-T-cell functionality for further clinical development towards the potential use in immunotherapy of melanoma. Experimental Dermatology, 27(7), 769–778. https://doi.org/10.1111/exd.13678

Song, D. G., Ye, Q., Poussin, M., Chacon, J. A., Figini, M., & Powell, D. J. (2016). Effective adoptive immunotherapy of triple-negative breast cancer by folate receptor-alpha redirected CAR T cells is influenced by surface antigen expression level. Journal of Hematology& Oncology, 9(1). https://doi.org/10.1186/s13045-016-0285-y

Sterner, R. C., & Sterner, R. M. (2021). CAR-T cell therapy: current limitations and potential strategies. Blood Cancer Journal, 11(4). https://doi.org/10.1038/s41408-021-00459-7

Sukumar, J., Gast, K. C., Quiroga, D., Lustberg, M. B., & Williams, N. (2021). Triple-negative breast cancer: promising prognostic biomarkers currently in development. Expert Review of Anticancer Therapy, 21(2), 135–148. https://doi.org/10.1080/14737140.2021.1840984

Szasz, A. M., Nemeth, Z., Győrffy, B., Micsinai, M., Krenács, T., Baranyai, Z., Harsányi, L., Kiss, A., Schaff, Z., Tőkés, A. M., & Kulka, J. (2011). Identification of a claudin-4 and E-cadherin score to predict prognosis in breast cancer. Cancer Science, 102(12), 2248–2254. https://doi.org/10.1111/j.1349-7006.2011.02085.x

Tasian, S. K., & Gardner, R. (2015). CD19-redirected chimeric antigen receptor-modified T cells: a promising immunotherapy for children and adults with B-cell acute lymphoblastic leukemia (ALL). Therapeutic Advances in Hematology, 6(5), 228–241. https://doi.org/10.1177/2040620715588916

Tutt, A., Garber, J. E., Kaufman, B., Viale, G., Fumagalli, D., Rastogi, P., Gelber, R. D., De Azambuja, E., Fielding, A., Balmaña, J., Domchek, S. M., Gelmon, K. A., Hollingsworth, S. J., Korde, L. A., Linderholm, B., Bandos, H., Senkus, E., Suga, J. M., Shao, Z., . . . Geyer, C. E. (2021). Adjuvant Olaparib for Patients with BRCA1- or BRCA2-Mutated Breast Cancer. The New England Journal of Medicine, 384(25), 2394–2405. https://doi.org/10.1056/nejmoa2105215

Vinay, D. S., & Kwon, B. S. (2014). 4-1BB (CD137), an inducible costimulatory receptor, as a specific target for cancer therapy. Journal of Biochemistry and Molecular Biology, 47(3), 122–129. https://doi.org/10.5483/bmbrep.2014.47.3.283

Vitale, C., & Strati, P. (2020). CAR T-Cell therapy for B-Cell non-Hodgkin lymphoma and chronic lymphocytic leukemia: Clinical Trials and Real-World Experiences. Frontiers in Oncology, 10. https://doi.org/10.3389/fonc.2020.00849

Van Den Ende, N. S., Nguyen, A. H., Jager, A., Kok, M., Debets, R., & Van Deurzen, C. H. (2023). Triple-Negative breast cancer and Predictive markers of response to neoadjuvant chemotherapy: a systematic review. International Journal of Molecular Sciences, 24(3), 2969. https://doi.org/10.3390/ijms24032969

X. Liu, S. Jiang, C. Fang, S. Yang, D. Olalere, E.C. Pequignot, A.P. Cogdill, N. Li, M. Ramones, B. Granda, (2015). Affinity-tuned ErbB2 or EGFR chimeric antigen receptor T cells exhibit an increased therapeutic index against tumors in mice. Cancer research, 75 (2015) 3596-3607.https://doi.org/10.1158/0008-5472.CAN-15-0159

Xia, L., Zheng, Z., Liu, J. Y., Chen, Y. J., Ding, J., Hu, G. S., ... & Liu, W, (2021). Targeting Triple-Negative Breast Cancer with Combination Therapy of EGFR CAR T Cells and CDK7 Inhibition Targeting TNBC with EGFR CAR T Cells and CDK7 Inhibition. Cancer immunology

research, 9(6), 707-722.https://doi.org/10.1158/2326-6066.CIR-20-0405

Xie, G., Han, D., Liang, Y., Ham, J. D., Romee, R., & Chen, J. (2020). CAR-NK cells: A promising cellular immunotherapy for cancer. EBioMedicine, 59, 102975. https://doi.org/10.1016/j.ebiom.2020.102975

Xie, Y., Hu, Y., Zhou, N., Yao, C., Wu, L., Liu, L., & Chen, F. (2020). CAR T-cell therapy for triple-negative breast cancer: Where we are. Cancer Letters, 491, 121–131. https://doi.org/10.1016/j.canlet.2020.07.044

Xing, Z., Zhang, M., Liu, J., Liu, G., Feng, K., & Wei, X. (2021). Overexpression of IncRNA SAMMSON Promotes Triple-Negative Breast Cancer Cell Proliferation by Interacting with p53. Critical Reviews in Eukaryotic Gene Expression, 31(6), 1–8. https://doi.org/10.1615/critreveukaryotgeneexpr.2021039534

Yang, Y., Vedvyas, Y., McCloskey, J. E., Min, I. M., & Jin, M. M. (2019). Abstract 2322: ICAM-1 targeting CAR T cell therapy for triple negative breast cancer. Cancer Research, 79(13_Supplement), 2322. https://doi.org/10.1158/1538-7445.am2019-2322

Yoon, D. H., Osborn, M. J., Tolar, J., & Kim, C. J. (2018). Incorporation of Immune Checkpoint Blockade into Chimeric Antigen Receptor T Cells (CAR-Ts): Combination or Built-In CAR-T. International Journal of Molecular Sciences, 19(2), 340. https://doi.org/10.3390/ijms19020340

Yu, X., Lin, Y., Xie, Y., Tian, Q., Li, L., & Lin, E. H. (2011). CD133, Stem cells, and Cancer stem cells: myth or reality? Current Colorectal Cancer Reports, 7(4). https://doi.org/10.1007/s11888-011-0106-1

Zakaria, Z., Zulkifle, M. F., Hasan, W. Z. W., Azhari, A. K., Raub, S. H. A., Eswaran, J., Soundararajan, M., & Husain, S. N. a. S. (2019). Epidermal growth factor receptor (EGFR) gene alteration and protein overexpression in Malaysian triple-negative breast cancer (TNBC) cohort OncoTargets and Therapy, Volume 12, 7749–7756. https://doi.org/10.2147/ott.s214611

Zhang, L., Fang, C., Xu, X., Li, A., Cai, Q., & Long, X. (2015). Androgen receptor, EGFR, and BRCA1 as biomarkers in Triple-Negative Breast

Cancer: A Meta-Analysis. BioMed Research International, 2015, 1–12. https://doi.org/10.1155/2015/357485

Zhao, J., Lin, Q., Song, Y., & Liu, D. (2018). Universal CARs, universal T cells, and universal CAR T cells. Journal of Hematology& Oncology, 11(1). https://doi.org/10.1186/s13045-018-0677-2

Zhu C, Wei Y, Wei X. AXL, (2019). Receptor tyrosine kinase as a promising anti-cancer approach: functions, molecular mechanisms and clinical applications. Mol Cancer; 18:153. https://doi.org/10.1158/0008-5472.CAN-15-0159

Zola, H., Macardle, P. J., Bradford, T. M., Weedon, H., Yasui, H., & Kurosawa, Y. (1991). Preparation and characterization of a chimeric CD 19 monoclonal antibody. Immunology and Cell Biology, 69(6), 411–422. https://doi.org/10.1038/icb.1991.58

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Medicinal potential of Fabaceae family members

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Introduction

Since ancient times, herbs and spices have played a crucial role in India's medicinal practices. Ayurveda, an ancient system of medicine, has described around two thousand plants with medicinal properties, and at least five hundred of these are integrated into our daily lives. According to the World Health Organization, medicinal plants are defined as those possessing therapeutic properties or exhibiting beneficial pharmacological effects on the human or animal body. The use of plants for medicinal purposes dates back to the earliest history of mankind and remains an integral part of our healthcare traditions.

Remarkably, a significant proportion of modern drugs, approximately 67%, can be traced back to natural origins, showcasing the enduring importance of plant-based medicine (Newmann and Cragg, 2007).

Among the various plant families, the Fabaceae or Leguminosae family holds immense significance. Commonly known as the legume, pea, or bean family, it comprises a large group of flowering plants that are agriculturally vital. Many plants belonging to this family are classified as pulses, such as Horse gram, Bengal gram, and green gram, which are essential components of our diet and contribute to our overall well-being.

General taxonomic position of Fabaceae

Kingdom- Plantae
Subkingdom- Tracheobionta,
Class-Dicotyledon,
Subclass-Polypetalous,
Order-Leguminates,
Family-Fabaceae.

Lablab purpureus (Indian bean)

Characteristic features of Fabaceae Family

This family consists of spices, bushes, plants, or moving by twining or ringlets. The root contains root knobs that have nitrogen-fixing microscopic organisms (rhizobium) and regularly non protein amino acids are found. Leaves are generally pinnatus and are spirally organized, pulvinus of leaf and individual pamphlets are advanced. Blossoms are normally sexually unbiased; petals are standard or unpredictable cup molded hypanthium. Petals are by and large five in number. This is imbricate or basally connate, standard, and normally valvate. The androecium is with one to various stamens however typically ten, concealed by perianth to long-applied and in some cases gaudy fibers particular or connate. On the off chance that connate is monadelphous or diadelphous , dust grains are tricolporate, tricolpate or triporate Gynoecium of one carpel, particularly, is normally prolonged and with a short gynophore. The ovary is prevalent with parietal (peripheral) placentation. Ovules are anatropous to campylotropous. Natural products are normally vegetables. Seeds are hard external, incipient organisms directly bent with no or less endosperm.Their floral characters includes Inflorescence racemose, Calyx: sepals five, gamosepalous; imbricate aestivation, Corolla: petals five, polypetalous, papilionaceous, comprising of a back norm, two sidelong wings,

two foremost ones framing a fall (encasing stamens and pistil), vexillary aestivation, Androecium: ten, diadelphous, anther dithecous. Gynoecium: ovary predominant, mono carpellary, unilocular with numerous ovules, style single.

The family of leguminosae is divided into three subfamilies namely Papilionoidea, Caesalpinioideae,Mimosoideae.The identification of these subfamily are done by their flowers .The legume of this family is used economically for nitrogen fixation, the family has association with the bacterium of the genus Rhizobium located in root nodules found in many species of Fabaceae, converting atmospheric nitrogen into ammonia, a soluble form used by other plants, as suppliers of natural fertilizers, in addition to their considerable importance in agriculture representing their ability to occupy different habitats and diverse lifeform(Mitchell Andrews et al ., 2017).Species of this family range from herbs, vines, trees, and shrubs with usually alternate, stipulate, pinnately to palmately compound leaves which act as atraditional medicine for curing humans as well as livestock(Melese Mengisto Asfaw et al, 2021)

Subfamilies of Fabaceae, Uses and Examples

Family: Papilionaceae (Fabaceae)

The members exhibit most varied properties, some are amylaceous, other oleaginous, many yield resins, balsams and dyes, a few are astringent, acrid and bitter, narcotic and poisonous, emetic and purging, tonic and restorative. The seeds are often anti periodic and the root anthelmintic,some of the important genera of this family are Abrusspecies, Alhagi species, Arachis species, Butea species, Cajanus species, Cicer species, Derris species, Glycine species, Glycyrrhiza species, Medicago species, Pisum species,Phaseolus species.

Family: Caesalpiniaceae

The members exhibit mostly tonic, astringent and mucilaginous properties, some have a pectoral and laxative or cathartic action; others are anthelmintic, antiseptic, antipyretic, and styptic. Some of the important genera are Bauhinia species, Cassia species, Cynometraspecies.

Family: Mimosaceae

The member exhibit tonic and astringent, emetic, antiperiodic and anthelmintic properties. Many yields demulcent gums. Some of the important genera are Acacia species, Albizzia species, Entada species, Leucaena species,

Neptunia species, Prosopis species, Parkia species, Mimosa species, Pithecellobium species. (Meghendra Sharma et al., 2013).

Some of the Medicinal Plants Belonging to Fabaceae Family

(Adjanohoun et al., 1996, Deepak et al., 2017, Meghendra et al., 2013)

Botanical name (species)	Uses or comments about activity or indication	Part used
Accia raddiana Savi	Respiratory diseases, skin ailments, toothache	Flower, fruits
Afzeliabella Harms.	Mumps	seeds
Amphimas ptericarpoides Harms.	Napkin rash	Bark
Astragalus mareoticus Del.	Scorpion bite	Whole plant
Brownea latifolia	Female complaints	Flower, Leaves
Calycotmevillosa L	Cicatrizing	Leaves
Cassia obovata Colladon	Laxative	Leaves
Chamaecristamimosoides (Linn.)	Dysentery	Whole plant
Cicer arietinum L.	Antiseptic, kidney diseases	Leaves
Desmodiumadscendens (Sw.)	Abdominal pains, urinary tract infection, cough, dysentery.	Whole plant, leaves
Detariummicrocarpum Guill. & Perr.	Vulvovaginitis	Stem bark
Entada polystachya	Menstrual pain	Twigs
Erythina senengalensis DC.	Fracture	Whole plant
Erytrophleumguineense G. Don.	Elephantiasis (non - filarial)	Stem bark
Glycyrrhiza glabra L.	Inflammatory diseases, constipation, respiratory diseases, digestive diseases, ulcer, cancer, kidney diseases, heartburn	Roots, undergro und, stem

Guibourtiatessmennii (Harms.)	Gastroenteritis, malaria, pneumonia.	Bark, roots
Milletiasanagana Hams.	Malaria	Roots
Mimosa pudica	Antiviral, wound healing, diuretic	Roots
Ononis natrix L.	Toothache	Leaves
Piptadeniastrumafricanum (hook. F.)	Pelvic inflammatory disease, rheumatism, strangulated hernia	Bark, root bark
Pterocarpus soyauxii Taub	Gastralgia	Outer bark
Scorodophleuszenkeri	Used for loss of appetite, abdominal pain	Stem bark
Senna alata (linn.) Roxb.	Constipation, jaundice, gastroenteritis, intestinal helmentiasis	Leaves, stems
Tamarindus indica Linn	Jaundice	Bulb
Tetrapleuratetaptera (Schum et Thonn.)	Treatment of post-partum haemorrhage, abdominal pains, epilepsy, placenta retention	Fruit, root tuber
Tephrosia vogelii Hook.	splenomegaly	Stem bark
Trigonella foenum graecum L.	Cancer, fever, digestive diseases, respiratory problems, eye diseases	Seeds
Vicia faba L.	Digestive diseases, skin diseases	Fruits
Zornia latifolia	Pharyngitis	Whole plant

Extraction of antioxidant, antibacterial, and insecticidal compounds from the Fabaceae family has been explored. In a recent study conducted byDr.PrashithKekuda T.R, the methanolic compounds present in *A. pulchellum* were examined. For the extraction, 50g of shade-dried leaves and powdered leaf material were taken, and 100 ml of methanol was added to the mixture. The solution was sonicated for 30 minutes and left at room temperature overnight. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure to a pasty mass. The methanol extract underwent preliminary phytochemical screening, revealing the presence of tannins, saponins, steroids, flavonoids, and glycosides. The

results indicated that the methanolic extract of *A. pulchellum* possesses the potential to prevent damages caused by free radicals and infections caused by pathogenic bacteria. Additionally, it was observed that the presence of carbohydrates, saponins, phytosterols, and phenols imparted larvicidal activity, while Prenylated xanthones, tetracyclic phenols, and saponins were effective in controlling mosquitoes (K.S Vinayak et al., 2009).

In light of global malaria prevalence and drug resistance, discovering new candidates for malaria control is vital. This review focuses on *Mimosa pudica* and Fabaceae due to traditional usage and phytochemical properties, revealing their potential for antimalarial and insecticidal effects. The study found that *Mimosa pudica* extracts exhibited activity against *P. falciparum* and insects. Advanced research on other Fabaceae species examined various extract forms and compounds, including nanoparticles. Notably, aqueous, methanol, and water/methanol extracts of *Mimosa pudica* aerial parts displayed promising antimalarial activity against *P. falciparum* FCR-3 strain. Additionally, its leaf extracts showed potential against Anopheles mosquito species. (MH Zacka et al., 2022).

A study on traditional medicinal plants used by tribal people to combat diabetes. Diabetes mellitus is a prevalent metabolic disorder arising from insulin malfunction, ranking as a major cause of mortality alongside cardiovascular disease and cancer. While costly and potentially harmful antidiabetic drugs exist, interest has grown in an ethno-botanical approach, investigating the anti-diabetic properties of plants used by different ethnic groups globally. The study surveyed 33 plant species from various families and genera, categorized into herbs, trees, shrubs, and climbers. Prominent species for diabetes treatment included *Ageratum conyzoides, Andrographis paniculata, Annona squamosa, Argemone Mexicana, Azadirachta indica, Centella asiatica, Coccinia cordifolia, Ficus racemosa, Momordica charantia, Moringa oleifera, Syzygiumcumini, Tinospora cordifolia, and Xanthium indicum*. Comprehensive information on each species' scientific and local names, family, habits, mode of use, and parts utilized were recorded. The data was collected through informal interviews with knowledgeable individuals such as local Kabiraj/Herbalists, Ojha, and elderly people. Plant specimens were collected, processed, and identified using standard herbarium techniques and expert consultation. (A.H.M. Mahbubur et al ., 2015, Permender et al ., 2010).

The stem bark of *Erythrina lysistemon* from the Fabaceae family, traditionally used for "women remedies," has been assessed for its estrogenic activity. The ethyl-acetate extract of the stem bark of *E. lysistemon* exhibited estrogenic activities. In vivo testing on young ovariectomized Wistar female rats after a 7-day treatment showed increased vaginal epithelial height by 47.23% and a weak increase of uterine epithelial height by 6.76% following oral administration of 200 mg/kg BW/d of E. lysistemon extract. The estrogenic effects were not as pronounced as those elicited by the positive control of 100 microg/kg BW/d of ethinylestradiol given orally. Overall, the results suggested that the extract of *E. lysistemon* contains secondary metabolites endowed with estrogenic activity (F. S. F. Tanner et al., 2006). Similar estrogenic properties have been reported in several other Central African medicinal plants, including *Millettia conraui* (Leguminosae), *Millettia drastic* (Leguminosae), *Bridelia ferruginea* (Leguminosae), and *Erythrina poeppigiana* (Fabaceae) (Njamen et al., 2008).

Food legumes and seeds from certain plant species of the Fabaceae family serve as a major source of edible nutrients, including proteins, lipids, carbohydrates, mineral elements, fatty acids, amino acids, fiber, and vitamins (Deshpande et al., 2001), all of which are important for human and animal health. Legume lectins, carbohydrate-binding proteins widely distributed in various leguminous plants, have gained attention in cancer research. Some plant lectins have demonstrated the ability to destroy cancer cells, making them potential candidates for cancer treatment and biomarker screening. These lectins bind specifically to carbohydrates on cancerous cells, as observed through histochemistry in vitro and in vivo, making them a natural source for cancer diagnostics and therapeutics (Ajai Kumar Gutam et al., 2020).

The significance of medicinal herbs and their phytoconstituents in contributing to human health, particularly in the context of cancer prevention and treatment. The passage highlights the use of natural sources to discover bioactive molecules for anticancer therapeutics and emphasizes the need for further research in this area. Here's a breakdownof the main points:

1. Introduction to Medicinal Plants and Phytoconstituents: The study acknowledges the historical use of plants and their bioactive compounds in traditional medicine. These phytoconstituents have been utilized for their potential health benefits since ancient times.

2. Discovery of Cancer Drugs from Natural Sources: The discussion starts with the discovery of Podophyllotoxin in the late 1960s, which marked the beginning of the search for cancer-fighting compounds from natural sources.
3. Anticancer Potential of Phytoconstituents in Legumes: The study highlights the use of legume extracts for investigating their anticancer properties. Isoflavones found in legume sprouts are noted to work as phytoestrogens, inhibiting tumorigenesis. The mechanisms of action include DNA repair, apoptosis induction, and regulation of cell processes like proliferation, migration, and invasion.
4. Lectins and Their Anticancer Properties: Lectins, abundant proteins found in legume tree barks, are identified as having potential antitumor and anticancer properties. Studies suggest cytotoxicity or tumor inhibition mechanisms against various cancer cell lines.
5. Anticancer Activities of Legume Saponins: The potential anticancer effects of legume saponins against melanoma, colon cancer, and cervical cancer. These saponins are believed to suppress cancer metastasis and regulate apoptosis pathways.
6. Phenolic Compounds and Antioxidant Properties: Phenolic compounds, found in significant amounts in peas, are associated with antioxidant properties. These compounds are believed to play a role in preventing oxidative stress-related diseases like cancer and cardiovascular diseases.
7. Purification of Active Phyto Molecules: The passage discusses the process of isolating active phytoconstituents using strategies such as bioassay-guided fractionation. Analytical techniques like TLC, HPLC, FTIR, mass spectrometry, and NMR are utilized to separate active fractions. Different solvents and matrices can be used for fractionation.
8. Examination of Anticancer Effects and Drug Development: After purification, the isolated phytoconstituents are tested for their in vitro or in vivo anticancer effects. If promising results are achieved, further research is necessary, including investigations into pharmacokinetics, pharmacodynamics, safety profiles, drug interactions, and dosage determination.
9. Association of Phenolic Compounds with Disease Prevention: The passage underscores the connection between phenolic compounds'

antioxidant properties and their potential in preventing oxidative stress-related diseases, including cancer and cardiovascular diseases.

Overall, the passage highlights the potential of natural compounds derived from medicinal plants, particularly legumes, for developing novel anticancer therapeutics through in-depth research and careful investigation (Rani et al., 2020).

Several species of the Fabaceae family are also utilized in the treatment of various human and livestock diseases. Studies conducted in Ethiopia reported the use of 127 medicinal plants from the Fabaceae family, with different authors and regions highlighting their role in treating health problems such as snake bites, evil eye, wounds, and more. These plants also provide valuable resources like wood, dyes, resins, insecticides, fibers, and fodder, with species like *Millettia ferruginea* being frequently cited (Melese Mengistu Asfaw et al., 2021).

Traditional medicinal practices involving indigenous plants are employed to manage poultry health.In the preparation of remedies, simplicity and minimal equipment are favoured by farmers, leading to the prevalent practice of crushing plant parts, grinding or crushing plant components in wooden or stone mortar and pestles is a widespread method for drug extraction. The choice of preparation method varies depending on the intended medical application and administration route. Water is frequently employed as a solvent for herbal concoctions, consistent with its cost-effectiveness and versatility for extracting plant compounds. While water effectively extracts numerous plant properties, high-proof alcohol is required for resins.The Fabaceae family (*Colophospermum mopane, Senna italica, and Cassia abbreviata*) being perennial plants available year-round. This availability likely contributes to their common utilization. Notably, these Fabaceae members are employed for treating various poultry diseases (Vimbai et al., 2022).

The methanolic extract from the stems of *Mimosa pigra*, a member of the Fabaceae family, was investigated for its potential antihyperglycemic (blood sugar-reducing) and antinociceptive (pain-relieving) effects. This plant is commonly utilized in traditional folk medicine in Bangladesh due to its reputed abilities to lower blood sugar levels in diabetes patients and alleviate pain. To assess its potential, the stems' methanol extract underwent various preparation steps, including drying, pulverization, and successive maceration in different solvents. The resulting extract was then tested in vivo using adult male Wistar albino rats to determine its potential anti-diabetic properties.

Furthermore, phytochemical screening revealed the presence of diverse bioactive compounds within the plant, such as Saponins, Tannins, Flavonoids, Anthraquinones, Terpenes, and Steroids. These compounds are known for their potential to reduce high blood sugar levels and provide pain relief. This study enhances our comprehension of the antihyperglycemic and antinociceptive properties inherent in *Mimosa pigra* stems. The study underscores the plant's potential medicinal applications and warrant further exploration of its bioactive components for potential drug development, as highlighted in the research (Tasnuva et al., 2012 and Nkemdirim Elechi et al., 2023).

Some prevalent Fabaceae species found throughout India are as follows.

1. *Butea monosperma* O Kuntze ('Palas'): Used in Madhya Pradesh, Bihar, and Maharashtra to treat urinary complaints, diarrhoea, piles, tumours, dysentery, and herpes because of the presence of Alloxan and flavonoids such as Palasitrin, and prunetine , Tannis , Polyphenols etc .
2. *Crotalaria pallida* Dry. Syn. C.Striata DC ('Thankur'): Utilized in Assam to eliminate intestinal worms.They have revealed the presence of Alkanes Alkaloids, Pterocarpanoids ,Terpens and Fatty acids.
3. *Bauhinia variegata* Linn. ('Guiral'): Employed in the Garhwal Himalayas for dysentery, diarrhea, skin diseases, ulcers, piles, and leprosy because of the presence of the Glycosides, Terpenoids, Reducing Sugars, Steroids, Tannis etc.
4. *Entada pursaetha* DC. ssp. sinohimalyenesis Grierson and Long. Syn. E.scandensAuct. ('Pangra'): Utilized in Sikkim to treat mumps and in Madhya Pradesh to cure paralysis because of the presence of Phenolic compounds, Saponins, Diterpenes, and Triterpenes.
5. *Acacia chundra* (Rottl.) Willd. Syn. Acacia sundra DC. ('Kair'): Used by the Bhils, Nayakas, and other tribal communities in Gujarat for leucoderma. A paste of the wood is applied locally as it contains alkaloids such as Quercetin and kaempferol along with other compounds such as Catechin, Gallate, Tannis and Protocatechui acid etc.
6. *Cassia auriculata* Linn. ('Anwal, Avaram' Syn Senna auriculata): Employed in India as an astringent and anthelmintic, used for urinary complaints, skin afflictions, diabetes, and ophthalmia as they constitute of phytochemical compounds such as Anthraquinones, flavonoids, flavon-3-ol derivatives, alkaloid, glycosides, tannin, saponin, terpenoids, reducing sugar and steroids.

7. *Bauhinia purpurea* Linn. ('Khairwal'): Tribes in Raigarh (Sisrangha) use the stem bark decoction for body pain and fever as they consist of Glycocides, Oxepins, Phytosterols, Triterpenoids, Phenolic compounds etc.
8. *Crotolaria spectabilis* Retz ('Sonokai'): Bihar's Oraon and Khond tribes use the plant paste for curing rheumatism as they consist of Pyrrolizidine alkaloids such as Crotaline and Monocrotalline etc.
9. *Acacia nilotica*: Commonly found in Rajasthan and holds great medicinal value as they consist of alkaloids such as dimethytryptamine and N-methytryptamine and other active compound such as gallic acid, dicatechin, quercetin, robidandiol, β-amyrin, hentriacontane, betulin, sitosterol, kaempferol-3 chlorogenic acid, and glucoside isoquercetin (Meghendra Sharma et al., 2013).

Conclusion

In-depth examination of various species within the Fabaceae family, showcasing their wide-ranging applications in treating diverse diseases, not only in India but also in numerous other countries where they have been utilized as traditional or natural remedies. The therapeutic potential of these plants stems from the abundance of phytochemicals, phytomedicine, and phytonutrients present in their shrubs, barks, leaves, flowers, roots, and fruits. Remarkably, these bioactive compounds have demonstrated potent antioxidant, anticancer, antibacterial, anti-venomous, and anti-inflammatory properties, making them particularly valuable for addressing health issues, including menopausal problems.

Beyond their medicinal significance, these Fabaceae species hold economic importance in sectors such as lumber, oil, color, and beet production. Additionally, they offer a wealth of nutritional benefits, being rich in protein, complex carbohydrates, iron, unsaturated fats, and other essential nutrients, making them highly advantageous for both human and livestock consumption.

In conclusion, the Fabaceae family stands out as a remarkable source of natural treatments, exhibiting diverse therapeutic potential and fostering economic and nutritional advantages worldwide.

References

A.H.M. Mahbubur Rahman, 2015. Ethno-Botanical Survey of Anti-Diabetic Medicinal Plants Used by the Santal Tribe of Joypurhat District,

Bangladesh, International Journal of Research in Pharmacy and Biosciences; 2(5): 19-26.

Adjanohoun, E. J., 1996. Tropical biodiversity and the development of pharmaceutical industries, Cab International publication; ref. (9): 506-518.

Ajay Kumar Gautam, Divakar Sharma, Juhi Sharma and Khem Chand Saini, 2020. Legume lectins, Potential use as diagnostics and therapeutics against the cancer, International Journal of Biological Macromolecules; (142): 474-483.

Cragg, G.M. and Newman, D.J, 2009. Nature: a vital source of leads for anticancer drug development. Phytochemical Review; 8: 313–331.

Deepika Rana and Nitesh Kumar, 2017. The Documentation of medicinal plants (Fabaceae family) in Hamirpur district (H.P) as pharmaceutical/herbal drugs, CPUH-Research Journal; 2(2): 17-25.

F S F Tanee, D Njamen, C B Magne Ndé, J Wanji, O Zierau, Z T Fomum and G Vollmer, 2007. Estrogenic effects of the ethyl-acetate extract of the stem bark of *Erythrina lysistemon* Hutch (Fabaceae), National Center for Biotechnology Information; 14(2-3): 222-226.

Germain Jean Magloire, Ketcha Wanda, Franklin, Zemo Gamo and DieudonnéNjamen, 2015. Medicinal Plants of the Family of Fabaceae used to Treat Various Ailments in Fabaceae: Classification, Nutrient Composition and Health Benefits, Science Publishers; Ref (12): 1-20.

K.S. Vinayaka, S.P. Swarnalatha, H.R. Preethi, K.S. Surabhi, T.R. Prashith Kekuda and S.J. Sudharshan, 2009. Studies on In vitro Antioxidant, Antibacterial and Insecticidal Activity of Methanolic Extract of Abrus pulchellus Wall (Fabaceae), African Journal of Basic & Applied Sciences; 1(5-6): 110-116.

MH Zacka Aditama, Nisa Fauziah, AfiatBerbudi and Hesti Lina Wiraswati, 2022. The Potential of Plants of Family Fabaceae with Emphasis on Putri Malu Medicinal Plant *Mimosa pudica* Fabaceae as an Antimalarial & an Insecticide for Malaria Vectors: A Review, Journal of communicable diseases; 54(4): 85-103.

Meghendra Sharma and Ashwani Kumar, 2013. Leguminosae (Fabaceae) in Tribal Medicines, Journal of Pharmacognosy and Phytochemistry; 2(1): 127-134.

Melese Mengistu Asfaw and Firew Bekele Abebe, 2021. Department of Plant Science, College of Agriculture and Natural Resource, Wolkite University; School of Natural Resources Management, College of Agriculture and Environmental Sciences, Haramaya University, Dire Dawa, Ethiopi . International Journal of plant Biology; 12(1): 8473.

MitchellAndrews and Morag E Andrews, 2017. Specificity in Legume-Rhizobia Symbioses, International Journal of Molecular science; 18(4): 705.

Mubo A Sonibare, Tolulope A Oke and Mike O Soladoye, 2014. A pharmacobotanical study of two medicinal species of Fabaceae, Asian Pacific Journal of Tropical Biomedicine; 4(2): 131-136.

Muhamad Noor Alfarizal Kamarudin, Md Moklesur Rahman Sarker, Habsah Abdul Kadir and Long Chiau Ming, 2017. Ethnopharmacological uses, phytochemistry, biological activities, and therapeutic applications of *Clinacanthus nutans* (Burm. f.) Lindau: A comprehensive review,Journal of Ethnopharmacology; (206): 245-266.

Nkemdirim Elechi and Onyeka Evelyn Unamba, 2023. Anti-diabetic activity of *Mimosa Pigra* Linn (Fabaceae) methanol leaf extract on alloxan-induced diabetic rats, GSC Advanced Research and Reviews; 14(03): 16–20.

Onyenibe Sarah Nwozo, Enor Magdalene Effiong, Patrick Maduabuchi Aja and Chinaza Godswill Awuchi, 2023. Antioxidant, phytochemical and therapeutic properties of medicinal plants, International Journal of Food Properties; 26(1): 359-388.

P Masoko, SS Gololo, MP Mokgotho, J.N Eloff, R.I. Howard and L.J Mampuru, 2010. Evaluation of the antioxidant, antibacterial and antiproliferative activities of the acetone extract of the acetone extract of the roots of Senna italica (fabaceae), African Journal of Traditional, Complementary and Alternative Medicines; 7(2): 138-148.

Permender Rathee, Hema Chaudhary, Sushila Rathee, DharmendarRathee and Vikas Kumar , 2010. Antidiabetic Potential of Fabaceae family; An overview, Betham Science publisher; 6(3): 161-175.

Rani Sebastian, Dr. B Jaykar and Dr. V Gomathi, 2020. Current status of anticancer research in fabaceae family, The Pharma Innovation Journal; 9(8): 52-60.

Robert Zarnowski, Ewa D. Zarnowska and Arkadiusz Kozubek, 2000. Alkylresorcinols in the family Fabaceae, Acta SocietaisBotanicorumPoloniae; 70(1): 25-29.

Tasnuva Ahmed, Khondokar Md. Sharif Uddin Imam, Shiblur Rahman, Sadia Moin Mou, Md. Shahed Choudhury, MostafiJumrut Mahal, Sharmin Jahan, Md. Shakhawat Hossain and Mohammed Rahmatullah, 2012. Antihyperglycemic and antinociceptive activity of Fabaceae family plants – an evaluation of *Mimosa pigra* L. stems, Advances in Natural and Applied Sciences; 6(8): 1490-1495

Vibhas S. Deshpande, Piotr A. Wielopolski, Steven M. Shea, James Carr, Jie Zhengand Debiao Li, 2001. Journal of Magnetic Resonance Imaging; 13: 676–681.

Vimbai Gobvu, William Pote, Xavier Poshiwa and Mudadi Albert Benhura, 2022. Medicinal Plants Used for the Treatment of Poultry Diseases in Zimbabwe. Research square Journal; 1: 1-18.

Xuegin Ma, Chengjian Zheng, Changlinf Hu, Khalid Rahman and Luping Qin, 2011. The genus Desmodium (Fabaceae) – traditional uses in Chinese, medicine, phytochemistry and pharmacology, Journal of Ethnopharmacology; 138(2): 314-332.

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Application of Artificial Intelligence in Biochemistry

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Introduction

A subfield of computer wisdom called artificial intelligence (AI) focuses on using tackle and software to interpret mammogram results and intimately convert patient maps into individual data that can be used to prognosticate, among other effects, the propensity to develop conditions like bone and utmost cancers (Avijeet,2021). The term" intelligent agents" refers to any agent or technology having the capability to see and understand their surroundings and take applicable action to ameliorate their chances of success (Chan,2019). Unlike mortal intelligence or the intelligence of other living species, it's machine intelligence (Ciallella et al, 2019). This kind of intelligence is frequently appertained to as machine literacy. These biases can mimic mortal study processes in literacy and analysis, which makes them useful in problem-working (Chan, 2019). These tools are helpful in problem-working because they can pretend mortal cognitive processes in literacy and analysis (Chan, 2019). Neural networks, CAD algorithms, fuzzy sense, decision trees, and direct programming ways are exemplifications of artificial intelligence (AI) methodologies that are innovative in the conception of sensible structures. Artificial intelligence is used in a broad variety of fields throughout life. For illustration, AI is used in the healthcare sector to develop sophisticated widgets that can spot conditions and fete cancer cells. AI may help in the study of habitual conditions using lab and other medical data to insure early identification. AI integrates literal data with technical medical moxie in order to identify innovative medicines (Fan et al, 2015)). Two of the biggest problems faced by commerce businesses are credit card theft and bogus reviews. Credit card fraud is less likely with the help of AI. After reading client evaluations, numerous shoppers decide whether or not to buy a product or service. The use of artificial intelligence (AI) can help identify and manage fake reviews. Businesses that use machine literacy algorithms may estimate operations grounded on certain criteria. AI driving systems may overlook the biographies and resumes of job campaigners to give babe a better

understanding of the gift pool they must choose from. For bettered functional performance, business analysis, and route planning, Uber and numerous logistics companies heavily calculate on AI (Chan, 2019).

Forms of Artificial Intelligence

Artificial Narrow Intelligence (ANI)

Artificial Narrow Intelligence (ANI) machines are designed to do certain tasks. Computers mimic human behaviour using advanced algorithms.

Artificial General Intelligence (AGI)

Artificial General Intelligence (AGI), also known as Strong Artificial Intelligence, allows machines to mimic human behaviour.

Artificial Super Intelligence (ASI)

Computers are more efficient than humans. ASI are suitable to perform any task.

Applications of Artificial Intelligence in Biochemistry

Prophetic ways for lncRNAs and mRNAs have been created lately due to the limits of bio sequencing technology and ineluctable sequencing miscalculations. One aspect of these technologies in common is the use of machine literacy to train the identification model for lncRNAs and mRNAs. The lncRNA and mRNA parcels were recaptured using the Rendering Implicit Calculator (CPC) identification fashion, which also trained a support vector machine (SVM) to produce the model (Huang et al ,2015). The open reading frame (ORF) of the sample sequence and the homology of the protein sequence discovered by comparing the sample sequence with the protein library serve as the two main sources of features for the SVM model training via Rendering Implicit Calculator(CPC). Since CPC must be compared to the protein library, it is fated to achieve successful species identification. The Coding and Non-Coding Index (CNCI) is a system that has been proposed throughout time (Kinch et al, 2011). The score matrix of the CNCI, which is connected to several species with massive computations and poor portability, and the bracket model are the two main factors of the CNCI algorithm's general frame. Within a species, CNCI may achieve excellent identification delicacy. still, because the effectiveness of this approach depends on the quality of the sequences, the recognition delicacy will be dropped when the sequence is incorrect. PLEK is a recognition system that achieves an delicacy rate of over 90 in several datasets (King, 1995). Other approaches, including the codon

substitution frequence (CSF) algorithm and the PhyloCSF algorithm, were created grounded on the known protein library and the natural parcels of sequences, and they classify the sequence grounded on the frequence of codon reserves (Kong, 2007). The maturity of these ways calculates on face literacy-grounded machine literacy, which primarily benefits from simplicity and ease still, several complicated lncRNA features cannot be duly recovered due to the simplicity of face literacy, and the vaticination performance cannot be further enhanced (Kumar et al, 2012).

Artificial Intelligence in Protein Secondary Structure vaticination

The alignment of sequences using styles developed from dynamic programming is the stylish vaticination model for protein's secondary structure with an unknown 3D structure that is similar to a protein with a known 3D structure. In the absence of similar sequences, secondary structure vaticination ways are used. Unreasonably high success rates are attained when testing the perfection of a vaticination using training data. There are around 500 distinct proteins with unique sequences and understood three dimensional structures that may be used to gauge how well technology predicts the secondary structure of proteins still, numerous of moments vaticination models are grounded on a set of 126 protein chains used by Rost and Sander (Minsky, 1961). Cuff J A and Barton G J developed a newnon-redundant set of 396 protein disciplines which include proteins from RS126 protein chains (Pian et al, 2016). Another study used developed algorithm using CB513 data set and expanded operation interface API- EPE2 with better performances in comparing to API- EPE(Pereira,2016).

Artificial Intelligence in Drug Discovery

Absence of recent techniqueslimits the drug development process, making it a time-consuming and expensive task, which can be addressed by using AI. AI can optimize the drug design. QSAR-based models also face problems such as small training sets, experimental data fault in training sets, and lack of experimental proofs. To solve these problems, recently developed AI approaches, such as DL and relevant modelling studies, can be implemented for safety and efficacy assessments of drug molecules based on big data modelling. Numerous IN SILICO methods to virtual screen compounds from virtual chemical spaces along with structure and ligand-based approaches, provide a better profile analysis, faster elimination of nonlead compounds and choice of drug molecules, with reduced expense. QSAR modelling tools have been applied for the identification of potential drug. This was evolved to AI-

based QSAR methods, such as linear discriminant analysis (LDA), support vector machines (SVMs), random forest (RF) and decision trees, which can be applied to speed up QSAR analysis (Zhang,2017). Evolving DL methods followed to replace the traditional DE NOVO drug design.

Chematica program was developed which has the ability to encode a set of rules into the machine and propose possible synthesizing routes for eight medicinally essential targets. This method can improve the yield and cost effective (Grzybowski, 2018). Integrated Expert Systems (ES) and ANN developed to create a hybrid system for the development of direct-filling hard gelatin capsules of piroxicam in accordance with the specifications of its dissolution profile. CFD DEM, and the Finite Element Method have been used with AI could prove to be of immense help in the rapid production of pharmaceutical products (Chen, 2016).

AI in Disease Diagnosis

AI-based techniques such as machine and deep learning models to detect the diseases such as skin, liver, heart, Alzheimer, etc. that need to be diagnosed early. The methods like Boltzmann machine, K nearest neighbour (kNN), support vector machine (SVM), decision tree, logistic regression, fuzzy logic, and artificial neural network to diagnose the diseases are presented along with their accuracies. AI is used to predict brain metastasis. used a number of decision tree rules, fuzzy membership functions, and inference techniques for breast cancer survival analysis. AI may have a role in providing prognostic and predictive data for ovarian cancer patient. Researchers from MIT and MGH have developed a new AI tool, called Sybil, that can accurately predict whether a person will develop lung cancer in the next year 86% to 94% of the time. Sybil is a new ARTIFICIAL INTELLIGENCE tool that can DETECT lung CANCER up to six years in advance. Efficient early liver disease recognition through Multilayer Perceptron Neural Network calculation depends on different choice tree calculations, such as chi-square programmed communication indicator and characterization, and relapse tree with boosting strategy(Khaled et al,2018). AI can analyse and detect stroke signs in medical images.

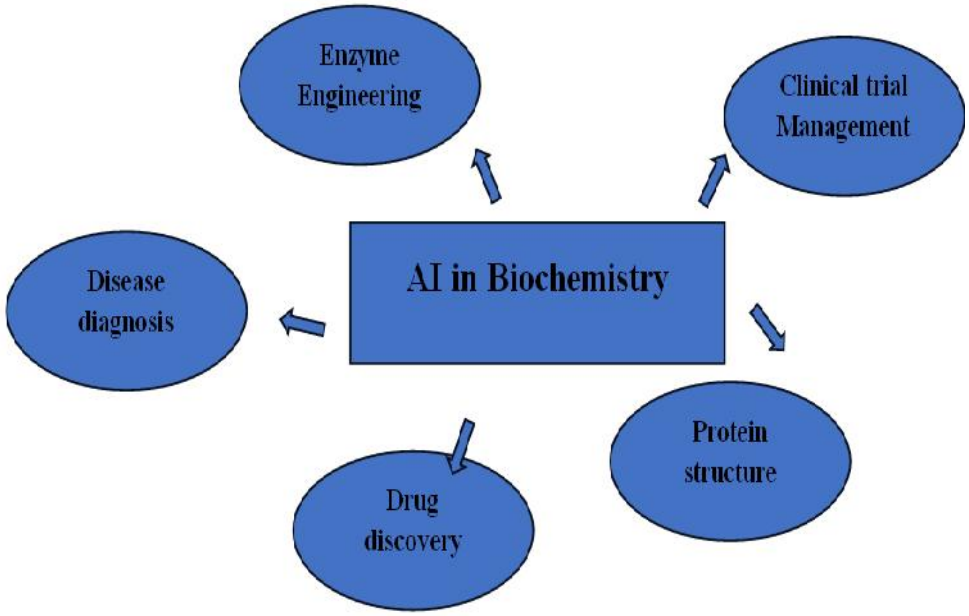
AI in Enzyme Engineering

Approaches are rational design and directed evolution. Thefirst approach is based on the structural analysis and in-depth computational modelling of enzymes by accounting of the physicochemical properties of

amino acids andtheir interactions with the environment.Over 7,500 promising enzymes, which were further experimentally tested and optimized using AI.

AI in clinical trial Management

Speed is one of the key advantages of using AI in clinical research and trials. Early detection of medicines is essential for the treatment of many serious illness.



Conclusion

Artificial intelligence can be used in processing data, diagnosis of diseases, to detect the protein structure within a short time. In recent time, AI has huge scope in the field of science. With the help of more innovative tools, health care sector become more advanced. Diagnosis of diseases ahead is a appreciate matter in medical field to start the treatment of serious illness and reduce the cost.AI-based programs and computer models have proven to be very efficient at optimizing the suitable conditions to obtain the maximum result. Scientists are relying on AI for research and discovery. Data analysis became easier. Study of amino acid sequences of proteins, machine learning algorithms can predict their three-dimensional structures, providing valuable perceptions into their functions and potential drug targets.

References

Avijeet B, 2021. Top 10 Artificial intelligence applications. Simplilearn. 21-27.

Chan, HS, 2019. Advancing drug discovery via artificial intelligence. Trends Pharmacological Science, 40(8):592–604.

Chen W., 2016. Mathematical model-based accelerated development of extended-release metformin hydrochloride tablet formulation. American Association of Pharmaceutical Scientists,17:1007–1013.

Ciallella HL and Zhu H, 2019. Advancing computational toxicology in the big data era by artificial intelligence: data-driven and mechanism-driven modelling for chemical toxicity. Chemical Research in Toxicology. 32:536–547.

Dabowsa N, Amaitik N, Maatuk A, and Shadi A, 2017. A hybrid intelligent system for skin disease diagnosis. In: Conference on engineering and technology, 1–6. 10.1109/ICEngTechnol.2017.8308157

Fan XN, and Zhang SW, 2015. Identification of human long non-coding RNAs by fusing multiple features and using deep learning. Molecular Biosystem. 11: 892– 897.

Firth NC,2015. An integrated workflow for multiobjective optimization:implementation, synthesis, and biological evaluation. Journal of Chemical Information and Modelling. 55:1169–1180.

Grzybowski B.A.,2018. Chematica: a story of computer code that started to think like a chemist. *Chem.* 4:390–398.

Huang G, Huang GB, Song S, You K, 2015. Trends in extreme learning machines: A Review Neural Networks.61:3248.

Igor Pantic,Jovana Paunovic , Snezana Pejic , Dunja Drakulic ' Ana Todorovic , Sanja Stankovic , Danijela Vucevic , Jelena Cumic , andTatjana Radosa vljevic,2022.Artificial intelligence approaches to the biochemistry of oxidative stress: Current state of the art. Chemico-Biological Interactions. 358.

Khaled E, Naseer S, and Metwally N.,2018. Diagnosis of hepatitis virus using artificial neural network. Journal of Academic Pedagogical Research,2:1–7.

Kinch LN, Shi S, Cheng H, Cong Q, Pei J, Mariani V, Schwede T, and Grishin, NV, 2011. CASP9 Target Classification. Proteins: Structural and Functional Genetics.79:21– 36.

King RD, 1995. Comparison of artificial intelligence methods for modelling pharmaceutical QSARS. Application Artificial Intelligence. 9:213–233

Kong L, Zhang Y, Ye ZQ, Liu XQ, Zhao SQ, Wei L,and Gao G, 2007. Assess the proteincoding potential of transcripts using sequence features and support vector machine. Nucleic Acids Research.35: W345.

Kumar N, and Skolnick J, 2012. Application of a highprecision enzyme function predictor to 396 proteomes. Bioinformatics.28: 2687–2688.

Li Y, Wang S, Umarov R, Xie B, Fan M, Li L, and Gao X, 2018. DEEPre: Sequence-based enzyme ec number prediction by deep learning. Bioinformatics.34:769.

Li A, Zhang J, and Zhou Z, 2014. A tool for predicting long non-coding RNAs and messenger RNAs based on an improved k-mer scheme. BMC Bioinformatics.15:31.

Mak KK, and Pichika MR, 2019. Artificial intelligence in drug development: present status and future prospects. Drug Discovery Today. 24:773–780.

Mariner PD, Walters RD, Espinoza CA, Drullinger LF, Wagner SD, Kugel J.F, and Goodrich JA, 2008. Human Alu RNA Is a Modular Transacting Repressor of mRNA Transcription during Heat Shock. Molecular Cell.29: 499-509.

Minsky M, 1961. Steps toward artificial intelligence.49:(1)8-30.

Pian C, Zhang G, Chen Z, Chen Y, Zhang J, Yang T, and Zhang L, 2016. Classification of long non-coding RNAs and protein-coding transcripts by the ensemble algorithm with a new hybrid feature. PLoS ONE.11.

Pereira JC, 2016. Boosting docking-based virtual screening with deep learning. Journal of Chemical Information and Modelling.56:2495–2506.

Sellwood MA, 2018. Artificial intelligence in drug discovery. Future Science. 10:2025– 2028.

Sizilio GR, Leite CR, and Guerreiro AM, 2012. Fuzzy method for pre diagnosis of breast cancer from the fine needle aspirate analysis. Biomedical Engineering, 11(1):83.

Sun L, Luo H, Bu D, Zhao G, Yu K, Zhang C, Liu Y, Chen R, and Zhao Y, 2013. Utilizing sequence intrinsic composition to classify protein-coding and long non-coding transcripts. Nucleic Acids Research. 41.

Wang Y, 2015. A comparative study on familyspecific protein–ligand complex affinity prediction based on random forest approach. Journal of Computer -Aided Molecular Design. 29:349

Weng J, McClelland J, Pentland A, Sporns O, and Stockman IM,2001. Autonomous mental development by robots and animals. Science.291(5504): 599-600.

Wooldridge M, and Jennings NR, 1995. Intelligent agents: theory and practice.10:(2) 115-152.

Xulvi-Brunet R, and Li H.,2010. Co-expression networks: Graph properties and topological comparisons. Bioinformatics, ;26: 205–214.

Yu N, Yu Z, and Pan Y, 2017. A deep learning method for lincRNA detection using autoencoder algorithm. BMC Bioinformatics. 18:511.

Yang Y, Urolagin S, Niroula A, Ding X, Shen B, Vihinen M.,2018. PON-: Protein variant stability predictor. importance of training data quality. International Journalof Molecular Sciences;19: 1009. Zhang L., 2017. From Machine learning to Deep learning: progress in machine intelligence for rational drug discovery. Drug Discovery Today,22:1680–1685.

Zhang C, Freddolino PL, and Zhang Y, 2017. COFACTOR: Improved protein function prediction by combining structure, sequence and protein–protein interaction information. Nucleic Acids Research.45: 10:1093.

Zhu H, 2020. Big data and artificial intelligence modelling for drug discovery. Annual Review of Pharmacology and Toxicology.60:573–589.

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