Dr. Preethi Kathirvel received her Ph.D degree from Bharathiar University, Coimbatore, India for the work on “Studies on Antioxidant and Pharmacological Activities of Muntingia calabura Linn. (Elaeocarpaceae) Fruits”. She is also SET qualified in Life Sciences. She is a Life Member in Association of Microbiologists of India and Society of Biological Chemists (India). She joined as an Assistant Professor in the Department of Microbial Biotechnology, School of Biotechnology and Genetic Engineering in March, 2011. Her thrust area of research includes screening of secondary metabolites and antioxidant compounds from diverse sources, waste management, bioplastic and biosurfactant production. She is engaged in teaching and research for the past 12 years. Despite teaching the students, she established Biopharmacy Laboratory. She has published one edited book, two book chapters and has more than 20 research publications in international and national journals.

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Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. The secondary metabolites are produced majorly by plants and are called phytochemicals, also by microbes such as bacteria, fungi, algae and so on. These secondary metabolites play a major role in defensive mechanism in plants, as well as its components are used in food industry, pharmaceuticals and so on. The applications and sources of each secondary metabolite is clearly discussed. We are very much thankful for the publisher who readily accepts and publish this subject. Also the author is very much thankful to her research team Mridul Umesh, Thazeem Basheer, Poorna Chandrika Sabapathy, Sabarinathan Devaraj and Sathishkumar Swamiappan for contributing their help and support for this work. The next edition of this book will more precisely discuss on the extraction and purification of the secondary metabolites.

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CONTENTS

Preface

Contributors

1. Introduction 1
   - Preethi Kathirvel

2. Alkaloids 8
   - Poorna Chandrika Sabapathy and Sabarinathan Devaraj

3. Flavonoids 30
   - Preethi Kathirvel

4. Steroids 48
   - Mridul Umesh and Thazeem Basheer

5. Terpenoids 69
   - Thazeem Basheer and Mridul Umesh

6. Quinones 88
   - Mridul Umesh and Thazeem Basheer

7. Saponins 102
   - Sabarinathan Devaraj and Poorna Chandrika Sabapathy

8. Polyketides 131
   - Thazeem Basheer and Mridul Umesh
1. INTRODUCTION

In general the natural products are the organic compounds which are formed by the living systems. These naturally occurring compounds can be classified broadly into three categories.

1. The compounds which occur in all cells and play a central role in the metabolism and reproduction of those cells. These include Nucleic acids, common amino acids and sugars. These compounds are known as primary metabolites.
2. There are high molecular weight compounds or polymeric materials such as cellulose, the lignins and the proteins which form the cellular structures.
3. There are those compounds that are very much limited range of species called the secondary metabolites.

Primary metabolites are a kind of metabolite (intermediates and products of metabolism), which directly involved in normal growth, development and reproduction. For example: aminoacids, proteins, enzymes, sugars, nucleic acids are considered as primary metabolite. These are much important for the intrinsic functions of the organism, which performs physiological functions. These are present in many cells and organisms. Hence referred to as central metabolite. Some common examples of primary metabolites are ethanol, lactic acid and certain amino acids.

Secondary metabolites are often created by modified primary metabolite synthases, or its getting substrates from primary metabolites. (Primary metabolites are considered to be essential for proper growth of the organisms) But there are exceptional compounds which do not depend on the primary metabolites. These secondary metabolites are formed during the end or near the stationary phase of growth. These are organic compounds, which are not directly involved in the normal growth, development or reproduction of an organism. But these secondary metabolites plays an important role in plant defense against herbivorous and other interspecies defences. There are many different uses of secondary metabolites. For example: Humans uses these as medicines, flavourings and recreational drugs, because these have different biological and chemical properties.
Unlike primary metabolites, secondary metabolites do not involve directly in intrinsic functions of the organism but they have certain important ecological functions – relational function. But the absence of secondary metabolites does not result in immediate death of the organism, but in long term impairment of the organism’s survivability, fecundity, or aesthetics or sometimes no significant change at all. These secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Some common examples: Ergot alkaloids, antibiotics, naphthalenes, phenazines, growth factors and so on. The secondary metabolites are produced majorly by plants and also by microorganisms such as bacteria, fungi, algae and actinobacteria.

It has been estimated that 40% of medicines have their origins in these natural compounds that too secondary metabolites. These compounds often have an ecological role in regulating the interaction between plants, microorganisms, insects and animals. They can be defensive substances, anti-feedants, attractants and pheromones. These compounds are hence considered as “Markers” for botanical and evolutionary relationships.

The secondary metabolites were originally investigated on the basis of their property or values as medicinal, perfumery or culinary value to man. During the latter part of 20th century, much and increasing attention towards the secondary metabolites and their applications. Developments in the instrumental methods arise for the screening, isolation, purification and characterization of these secondary metabolites.

These secondary metabolites often attracted interest because of their biological effect on other organisms. We need not expect the secondary metabolites are to be only useful and safe, but there are compounds which are toxic or poisonous too. But these are naturally occurring compounds.

**Sources of Secondary Metabolites:**

Majorly secondary metabolites are first produced and identified in plants. And they are also named as phytochemicals. Phyto- means plants, so plant derived chemicals- referred to as phytochemicals. These phytochemicals do not participate in plant metabolism, not needed by plants too since they do not perform any physiological functions. They may include pharmaceuticals, flavours, fragrances, cosmetics, food additives, feed stocks, and antimicrobials.
But why plants produce these chemicals?

These secondary metabolites/ phytochemicals even though do not contain any nutritive value but these are helping to protect the plants themselves from infections by producing some antimicrobial compounds called phytoalexins. It is also believed that production of secondary metabolites are linked to the introduction of morphological differentiation. More oftenly it is seen that some of the differentiated tissues get specialized to produce specific secondary metabolites, and in comparison with in vitro cultures, which are the masses of undifferentiated cells, produces high levels of secondary metabolites.

So where these secondary metabolites are present in plants?

These secondary metabolites/phytochemicals are present all over the parts of the plants. It is present in leaves, fruits, flowers, stem, bark and even in roots too.

Many secondary metabolites are produced in roots:

Scientists found the production of secondary metabolites in roots as well. Roots often secrete the metabolites into the surrounding medium, making it easy for collection. The precursors/starting material for secondary metabolites are obtained from the soil through roots and also from nearby roots of plants.

Some plants exert its dominance by the production of compounds which inhibit the germination of seeds and prevent the growth of seedlings. This harmful effect one plant on another is manually caused by the production of secondary metabolites, also known as allelopathy. One example for reference: Production of quinine juglone by the walnut tree, Juglans nigra. This compound inhibits seed germination and is toxic to other plants. Others such as monoterpenes – 1,8- cineole and camphor which are produced by the sagebrush (Salvia leucophylla), act as seed germination inhibitors. The presence of these monoterpenes allows this plant to dominate the vegetation in some of the drier parts of California until if it destroyed by bush fires. Similarly fungi attack plants by producing phytotoxins along with enzyme systems that digest plant tissues. When these fungal compounds attack the plants, plants produces phytoalexins, which act as natural antifungal agents. Examples: Rishitin, produced by potato Solanum tuberosum and phaseolin, produced by the bean Phaseolus vulgaris. Many such compounds produced by plants give protection against microbial attack.
Many plant – insect relationships are determined by the presence of secondary metabolites. These compounds may be either deferrents or attractants. Most of the plants have developed antifeedants and insecticides as defensive agents against insect attack. Example: Nicotine, the pyrethrins from *Chrysanthemum cinearifolium* and rotenone from derris root, have formed the basis of commercial insecticides.

There are some insects ‘borrowing’ secondary metabolites from plants and modifying the compounds to make insect trail substances for feeding and mating purposes. Example: The bark beetle, *Dendroctanus brevicomis* is attracted to a pine tree, *Pinus ponderosa*, by its volatile terpenes. At the same time female produces exo-brevicomin and the related frontalin as attractants, but when the population has reached a particular level the beetles begin to modify the alpha-pinene produced by the plant to verbenol, which acts as a deterrent.

Insects may be attracted to particular plants by secondary metabolites in order to lay their eggs. For example, allylisothiocyanate, produced from sinigrin in the cabbage, acts as an attractant for the cabbage white butterfly.

The volatile monoterpenes produced by flowers acts as attractants for bees, butterflies and moths for pollination. These components often have a synergistic action as attractant. Insects that are involved in the pollination of a particular species respond to a specific combination of monoterpenes and to the colour of the flower.

Some secondary metabolites are involved in the hormonal regulation of insect development. Many insects undergo metamorphosis through various juvenile forms before attaining the adult stage. The insect juvenile hormones that maintain this status are related to the sesquiterpenes, but the hormone scdysone is a steroid regulates the development of the adult insect from a cocoon. Fortunately plants also produce these steroid substances as protection against excessive insect populations.

Some secondary metabolites serve other important functions as well, such as providing structural support, as in the case of lignin, or acting as pigments, as in the case of anthocyanins.
Plants as important sources of phytochemicals

Over the centuries humans have relied upon plants for basic needs such as food, clothing and shelter. Plants have also been utilized for additional purposes, such as for ritualistic purposes, stimulants for endurance and hunger suppression, as well as inebriants and medicines. The plant chemical used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites and are not directly involved in the growth, development of reproduction of plants.

Nearly 80% of the world’s population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. The study of plants continues principally for the discovery of novel secondary metabolites.

Secondary Metabolites are not made through metabolic pathways common to all plants. In plant kingdom they are limited to occurrence and may be restricted to a particular taxonomic group genus, species or family. These secondary metabolites are accumulated by plant cells in smaller quantities than primary metabolites. These secondary metabolites are synthesized in specialized cells at particular developmental stages making extraction and purification much difficult.

Based on their biosynthetic origins, plant secondary metabolites can be divided into 3 major groups:

1. Terpenoids
2. Flavonoids and allied phenolic and polyphenolic compounds
3. Nitrogen containing alkaloids and sulphur containing compounds

1. Terpenoids: These are the largest and most diverse family of natural products, ranging in structure from linear to polycyclic molecules and in size from 5-carbon hemiterpenes to natural rubber, comprising thousands of isoprene units. All terpenoids are synthesized through the condensation of isoprene units (c5) and are classified by the number of 5-carbon units present in the core structure. Many flavoured and aromatic molecules are terpenoid compounds.

2. Phenolic Compounds: Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. Phenolics range from simple, low molecular
weight, single aromatic ringed compounds to large and complex tannins and derived polyphenols. These can be classified into 2 groups: The flavonoids and the non-flavonoids.

3. **Flavonoids:** These are polyphenolic compounds comprising 15 carbons, with two aromatic rings connected by a 3-carbon bridge.

Examples: Kaempferol, Quercetin, Isohamnetin, Myricetin.

4. **Non-flavonoids:** The main non-flavonoids of dietary significance are the C6-C1 phenolic acids, most notably gallic acid, the C6-C3 hydroxycinammates and their conjugated derivatives, and the polyphenolic C6-C2-C3 stilbenes.

5. **Alkaloid:** The alkaloids are a large and structurally diverse group of compounds, some of them are not entirely distinguishable from amines (eg: ephedrine). Many are derived from amino acids, but others result from modification of various classes of molecules including polyphenols, terpenes or steroids. Alkaloids are most soluble in hydroethanolic media and they generally occur as salts (eg: chlorides or sulphates) and or as N-oxides in the plants. Most of them have a heterocyclic nitrogenous ring or ring system and a basic (alkaline) character. Among the alkaloids we can find potent medicinal molecules as well as toxic or even potentially fatal ones.

**Examples:** Caffeine, Phenethylamines

**Microbial sources of secondary metabolites:**

Micro organisms are also capable of producing the secondary metabolites. These are low molecular mass products, they are not essential for growth of the producing cultures, but are very important for human health, nutrition and economics of our society. They include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptor antagonists and agonists, pesticides, growth promoters of animals and plants, anti tumor agents, cholesterol- lowering drugs and so on. They have unusual structures and are usually formed during the late growth phase of the producing organisms. The production of microbial metabolites often depends upon the nutrient source or medium on which the micro organism is growing and also on the fermentation conditions. The fungal metabolites are majorly retained in the fungal mycelium, whilst others are excreted into the growth/broth medium.
Isolation of secondary metabolites:

The secondary metabolites may be obtained from its respective sources by crushing the biological material and extracting the compounds by the process of extraction using various polar and non polar solvents such as water, petroleum ether, chloroform, methanol, ethyl acetate, petroleum ether, DMSO and so on. The extraction process plays a major role in identifying the compounds from different sources. More importantly solvents also very important in the case of compound extraction. Successful extraction begins with careful selection and preparation of source samples, during the extraction, it is important to minimize the interference compounds and impurities must be removed which may involve in destroying the purity of the target compounds. After the extraction process, the solvent extracts are subjected to a series of steps for purification and identification of the particular secondary metabolite using various analytical methods.

In this book we shall see the different types of metabolites, its structure, synthesis, sources and its importance.
2. ALKALOIDS

Poorna Chandrika Sabapathy and Sabarinathan Devaraj

1.0. INTRODUCTION

Alkaloids are a group of molecules with a relatively large occurrence in nature around the Globe. They are very diverse chemicals and biomolecules, but they are all secondary compounds and they are derived from amino acids or from the transamination process. Alkaloids are classified according to the amino acids that provide their nitrogen atom and part of their skeleton. Similar alkaloids can have quite different biosynthetic pathways and different bioimpacts. Alkaloids are derived from L-lysine, L-ornithine, L-tyrosine, L-tryptophan, L-histidine, L-phenylalanine, nicotinic acid, anthranilic acid or acetate. The terpenoid, steroid and purine alkaloids are also important. Millions of people around the Globe use purine alkaloids every day whether starting the day with a cup of coffee or drinking a cup of tea in the afternoon. Plants have been explored by chemist for their chemical compounds and their related medicinal values. Throughout this exploration many alkaloids and drugs were identified and studied. The studies have been aimed at compounds that are of the pharmaceutical interest in the scope of medicinal importance. Alkaloids also occur in the animal kingdom. Differently from plants, the source of these molecules in an animal’s body can be endogenous or exogenous. Alkaloids are molecules participating in both producer and consumer chains in nature. They are vital in feeding, and enjoy servations, agressivity and defence of the species. Homo sapiens are one of them.

The term “alkaloid” was first mentioned in 1819 by W. Meitner, an apothecary from Halle. He observed that these compounds appeared “like alkali”, and so named them alkaloids. The name "alkaloids" (German: Alkaloide) was introduced in 1819 by the German chemist Carl Friedrich Wilhelm Meitner, and is derived from late Latin root Latin: alkali (which, in turn, comes from the Arabic al-qalwī – "ashes of plants") and the suffix Greek: "like". However, the term came into wide use only after the publication of a review article by Oscar Jacobsen in the chemical dictionary of Albert Ladenburg in the 1880s. In another definition, Waller and Nowacki
Secondary Metabolites

mentioned many characteristics of alkaloids. They especially drew attention to the fact that alkaloids have nitrogen in the molecule and are connected to at least two carbon atoms. Moreover, this compound has at least one ring in the molecule, and its ring is not necessarily heterocyclic. The authors also stated that alkaloids could not be structural units of macromolecular cellular substances, vitamins or hormones. More recently, Sengbush simply stressed that alkaloids are a group of nitrogen-containing bases and that most of them are drugs.

Studies of alkaloids began in the 19th century. In 1804, the German chemist Friedrich Sertürner isolated from opium a "soporific principle" (Latin: *principium somniferum*), which he called "morphium" in honor of Morpheus, the Greek god of dreams; in German and some other Central-European languages, this is still the name of the drug. The term "morphine", used in English and French, was given by the French physicist Joseph Louis Gay-Lussac.

There is no unique method of naming alkaloids. Many individual names are formed by adding the suffix "ine" to the species or genus name. For example, atropine is isolated from the plant *Atropa belladonna*, strychnine is obtained from the seed of Strychnine tree (*Strychnosnux-vomica* L.). If several alkaloids are extracted from one plant then their names often contain suffixes "idine", "anine", "aline", "inine" etc. There are also at least 86 alkaloids whose names contain the root "vin" because they are extracted from vinca plants such as *Vinca rosea* (*Catharanthus roseus*); these are called vinca alkaloids.

Alkaloid-containing plants have been used by humans since ancient times for therapeutic and recreational purposes. For example, medicinal plants have been known in the Mesopotamia at least around 2000 BC. The *Odyssey* of Homer referred to a gift given to Helen by the Egyptian queen, a drug bringing oblivion. It is believed that the gift was an opium-containing drug. A Chinese book on houseplants written in 1st–3rd centuries BC mentioned a medical use of Ephedra and opium poppies. Also, coca leaves have been used by South American Indians since ancient times. Extracts from plants containing toxic alkaloids, such as aconitine and tubocurarine, were used since antiquity for poisoning arrows.

A significant contribution to the chemistry of alkaloids in the early years of its development was made by the French researchers Pierre Joseph Pelletier and Joseph Bienaimé Caventou, who discovered quinine (1820) and strychnine (1818). Several other alkaloids were
Secondary Metabolites

discovered around that time, including xanthine (1817), atropine (1819), caffeine (1820), coniine (1827), nicotine (1828), colchicine (1833), sparteine (1851) and cocaine (1860).

The first complete synthesis of an alkaloid was achieved in 1886 by the German chemist Albert Ladenburg. He produced coniine by reacting 2-methylpyridine with acetaldehyde and reducing the resulting 2-propenyl pyridine with sodium. The development of the chemistry of alkaloids was accelerated by the emergence of spectroscopic and chromatographic methods in the 20th century, so that by 2008 more than 12,000 alkaloids had been identified.

2.0. OCCURRENCE IN PLANTS

Alkaloids are substances very well known for their biological activity at the beginning of world civilization. They were used in shamanism, in traditional herbal medicine for the cure of diseases and in weapons as toxins during tribal wars and during hunting. They also had, and still have, socio-cultural and personal significance in ethnobotany. Moreover, they have been and continue to be the object of human interest concerning new possibilities for their safe utilization and ensuing health benefits. Of all secondary compounds, historically and contemporaneously, only alkaloids are molecules of natural origin with highly important benefits and diagnostic uses. They can be characterized as the most useful and also the most dangerous products of nature.

Alkaloids are most abundant in higher plants. At least 25% of higher plants contain these molecules. In effect this means that on average, at least one in fourth plant contains some alkaloids. In reality, it is not impossible that alkaloids occur more commonly. Using the latest equipment and technology, such slight traces of alkaloids may be detected (e.g., less than 10 giga grams per kg of plant mass) that these have no real influence on biological receptors and activity. Generally these species are not considered as alkaloid species.

Hegnauer has defined alkaloid plants as those species which contain more than 0.01% of alkaloids. This is right from the point of view of the classification. From the genetic point of view, and the genetic mechanism of alkaloid synthesis, it is a real limitation. Paying attention to slight traces of alkaloids in plants, we see the members of the plant family which are relatives. They have a genetically determined alkaloid mechanism with a species expression. Moreover, this expression is also on the hybrid level.

Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals. In plants alkaloids are common in the Angiosperms (Mono- and Dicotyledons), but
rare in lower plants, although there are exceptions, for example pacletaxel from yew (a Gymnosperm), lycopodine from Lycopodium and palustrine from Equisetum (both Pteridophytes), and even fungi, e.g. ergometrine (Claviceps). The distribution of alkaloids in the plant kingdom is listed in Table 1.

Table 1: Distribution of alkaloids in the plant kingdom

<table>
<thead>
<tr>
<th>Family</th>
<th>Alkaloid</th>
<th>Plant genus</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricaceae</td>
<td>Bufotenine</td>
<td><em>Amanita</em> (m)</td>
<td>Hallucinogen</td>
</tr>
<tr>
<td></td>
<td>Muscarine</td>
<td><em>Amanita</em> (m)</td>
<td>Acetylcholine-like</td>
</tr>
<tr>
<td></td>
<td>Psilocybine</td>
<td><em>Psilocybe</em> (m)</td>
<td>Hallucinogen</td>
</tr>
<tr>
<td>Amaryllidaceae</td>
<td>Lycorine</td>
<td><em>Amaryllis</em> (b)</td>
<td>Alzheimer disease</td>
</tr>
<tr>
<td></td>
<td>Galanthamine</td>
<td><em>Galanthus</em> (b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Narcissus</em> (b)</td>
<td></td>
</tr>
<tr>
<td>Apocynaceae</td>
<td>Alstonine</td>
<td><em>Alstonia</em> (bk)</td>
<td>Antimarial</td>
</tr>
<tr>
<td></td>
<td>Aspidospermine</td>
<td><em>Aspidosperma</em> (bk)</td>
<td>Respiratory stimulant</td>
</tr>
<tr>
<td></td>
<td>Yohimbine</td>
<td><em>Yohimbe</em> (bk)</td>
<td>Aphrodisiac</td>
</tr>
<tr>
<td></td>
<td>Conessine</td>
<td><em>Holarrhena</em> (bk)</td>
<td>Antidysenteric</td>
</tr>
<tr>
<td></td>
<td>Ellipticine</td>
<td><em>Ochrosia</em> (bk)</td>
<td>Anticancer</td>
</tr>
<tr>
<td></td>
<td>Akuammigine</td>
<td><em>Picralima</em> (s)</td>
<td>Antimalarial</td>
</tr>
<tr>
<td></td>
<td>Reserpine</td>
<td><em>Rauwolfia</em> (rh)</td>
<td>Tranquillizer</td>
</tr>
<tr>
<td></td>
<td>Serpentine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vinblastine</td>
<td><em>Catharanthus</em> (l)</td>
<td>Anticancer</td>
</tr>
<tr>
<td></td>
<td>Vincristine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aristolochiaceae</td>
<td>Aristolochic acid</td>
<td><em>Aristolochia</em> (rh)</td>
<td>Tumour-inducing</td>
</tr>
<tr>
<td>Berberidaceae</td>
<td>Berberine</td>
<td><em>Berberis</em> (bk)</td>
<td>Antibacterial</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Mahonia</em> (bk)</td>
<td>Antimalarial</td>
</tr>
<tr>
<td>Boraginaceae</td>
<td>Indicine N-oxide</td>
<td><em>Heliotropium</em> (l)</td>
<td>Anticancer</td>
</tr>
<tr>
<td>Cactaceae</td>
<td>Mescaline</td>
<td><em>Lophophora</em> (l)</td>
<td>Hallucinogen</td>
</tr>
<tr>
<td>Campanulaceae</td>
<td>Cathine</td>
<td><em>Catha</em> (l)</td>
<td>CNS stimulant</td>
</tr>
<tr>
<td></td>
<td>Ephedrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celastraceae</td>
<td>Maytansine</td>
<td><em>Maytenus</em></td>
<td>Anticancer</td>
</tr>
</tbody>
</table>
## Secondary Metabolites

<table>
<thead>
<tr>
<th>Family</th>
<th>Compound</th>
<th>Species</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chenopodiaceae</td>
<td>Anabasine</td>
<td><em>Anabasis</em> (l)</td>
<td>Insecticidal</td>
</tr>
<tr>
<td>Clavicepitateae</td>
<td>Ergometrine</td>
<td><em>Claviceps</em> (fb)</td>
<td>Postpartum haemorrhage, Migraine</td>
</tr>
<tr>
<td></td>
<td>Ergotamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Convolvulaceae</td>
<td>Calystegines</td>
<td><em>Calystegia</em> (r)</td>
<td>Antiviral, Hallucinogen</td>
</tr>
<tr>
<td></td>
<td>Agroclavine</td>
<td><em>Ipomoea</em> (l)</td>
<td></td>
</tr>
<tr>
<td>Ephedraceae</td>
<td>Ephedrine</td>
<td><em>Ephedra</em> (hb)</td>
<td>CNS stimulant</td>
</tr>
<tr>
<td>Equisitaceae</td>
<td>Palustrine</td>
<td><em>Equisetum</em> (l)</td>
<td></td>
</tr>
<tr>
<td>Erythroxylaceae</td>
<td>Cocaine</td>
<td><em>Coca</em> (l)</td>
<td>Local anaesthetic</td>
</tr>
<tr>
<td>Graminaceae</td>
<td>Loliine</td>
<td><em>Lolium</em> (l)</td>
<td></td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Castanospermine</td>
<td><em>Castanosperma</em> (s)</td>
<td>Antiviral, 'locoism' (stock)</td>
</tr>
<tr>
<td></td>
<td>Cytisine</td>
<td><em>Cytisus</em> (hb)</td>
<td>Very toxic, Teratogenic, Diuretic, Glycosidase inhibitor, 'locoism' (stock)</td>
</tr>
<tr>
<td></td>
<td>Sparteine</td>
<td><em>Anagyris</em> (hb)</td>
<td></td>
</tr>
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<td></td>
<td>Swainsonine</td>
<td><em>Swainsona</em></td>
<td></td>
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<tr>
<td></td>
<td>Monocrotaline</td>
<td><em>Crotalaria</em> (l)</td>
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<td></td>
<td>Physostigmine</td>
<td><em>Physostigma</em> (s)</td>
<td></td>
</tr>
<tr>
<td>Liliaceae</td>
<td>Colchicine</td>
<td><em>Colchicum</em> (c)</td>
<td>Induces polyploidy, Insecticidal, Antihypertension</td>
</tr>
<tr>
<td></td>
<td>Cevadine</td>
<td><em>Schoenocaulon</em> (s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rubijervine</td>
<td><em>Veratrum</em> (r)</td>
<td></td>
</tr>
<tr>
<td>Loganiaceae</td>
<td>Strychnine</td>
<td><em>Strychnos</em> (s)</td>
<td>Very poisonous</td>
</tr>
<tr>
<td>Lycopodiaceae</td>
<td>Lycopodine</td>
<td><em>Lycopodium</em> (l)</td>
<td></td>
</tr>
<tr>
<td>Menispermaceae</td>
<td>Tubocurarine</td>
<td><em>Chondrodendron</em> (bk)</td>
<td>Neuromuscular blocking agent, muscle relaxant</td>
</tr>
</tbody>
</table>

**Alkaloids**
### Secondary Metabolites

<table>
<thead>
<tr>
<th>Family</th>
<th>Compound 1</th>
<th>Compound 2</th>
<th>Species 1</th>
<th>Species 2</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraceae</td>
<td>Calystegines</td>
<td><em>Morus</em> (l)</td>
<td></td>
<td></td>
<td>Antiviral, oral hyperglycaemic Agent</td>
</tr>
<tr>
<td>Palmae</td>
<td>Arecoline</td>
<td><em>Areca</em> (s)</td>
<td></td>
<td></td>
<td>Anthelmintic</td>
</tr>
<tr>
<td>Papaveraceae</td>
<td>Chelerythrine</td>
<td><em>Dendrobia</em> (hb)</td>
<td></td>
<td></td>
<td>Diuretic</td>
</tr>
<tr>
<td></td>
<td>Codeine</td>
<td></td>
<td><em>Papaver</em> (lt)</td>
<td></td>
<td>Analgesic, narcotic</td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
<td></td>
<td></td>
<td></td>
<td>Cough suppressant</td>
</tr>
<tr>
<td></td>
<td>Narcotine</td>
<td></td>
<td></td>
<td></td>
<td>Anti-impotence</td>
</tr>
<tr>
<td></td>
<td>Papaverine</td>
<td></td>
<td></td>
<td></td>
<td>vasodilator</td>
</tr>
<tr>
<td>Ranunculaceae</td>
<td>Aconitine</td>
<td><em>Aconitum</em> (hb)</td>
<td></td>
<td></td>
<td>Diaphoretic, rheumatism, neuralgia (topical)</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td>Ajaconine</td>
<td><em>Delphinium</em> (hb)</td>
<td></td>
<td></td>
<td>Amoebic dysentery</td>
</tr>
<tr>
<td></td>
<td>Emetine</td>
<td><em>Cephaelis</em> (rh)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Psychotria</em> (r)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rutaceae</td>
<td>Quinine</td>
<td><em>Cinchona</em> (bk)</td>
<td></td>
<td></td>
<td>Antimalarial</td>
</tr>
<tr>
<td></td>
<td>Quinidine</td>
<td></td>
<td><em>Peganum</em> (sd)</td>
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<td>Antiarrhythmia</td>
</tr>
<tr>
<td></td>
<td>Harmaline</td>
<td></td>
<td><em>Pilocarpus</em> (l)</td>
<td></td>
<td>(heart)</td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td></td>
<td><em>Pentacerus</em></td>
<td></td>
<td>Anthelmintic</td>
</tr>
<tr>
<td></td>
<td>Canthin-6-one</td>
<td></td>
<td></td>
<td></td>
<td>Glaucoma, ophthalmology</td>
</tr>
<tr>
<td>Solanaceae</td>
<td>Capsaicin</td>
<td><em>Capsicum</em> (fr)</td>
<td></td>
<td></td>
<td>Antibacterial</td>
</tr>
<tr>
<td></td>
<td>Hyoscine</td>
<td><em>Atropa</em> (hb)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Hyoscyamine</td>
<td><em>Datura</em> (hb)</td>
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<tr>
<td></td>
<td></td>
<td><em>Hyoscyamus</em> (hb)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>Duboisia</em> (hb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Mandragara</em> (r)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
### Secondary Metabolites

<table>
<thead>
<tr>
<th>Family</th>
<th>Alkaloid</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxaceae</td>
<td>Tigloidine</td>
<td>Parkinson disease</td>
</tr>
<tr>
<td></td>
<td>Solanidine</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>Insecticidal, smoking</td>
</tr>
<tr>
<td></td>
<td>Pacletaxel</td>
<td>Anticancer drugs</td>
</tr>
<tr>
<td></td>
<td>Baccatin</td>
<td>Anticancer drugs</td>
</tr>
<tr>
<td></td>
<td><em>Duboisia</em> (hb)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Solanum</em> (tb)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Nicotiana</em> (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Taxus</em> (1)</td>
<td></td>
</tr>
</tbody>
</table>

*a* Part of plant used: (l), leaves; (s), seeds; (hb), herbs; (bk), bark; (rh), rhizome; (tb), tuber (green); (fr), fruits; (lt), latex; (r), roots; (c), corm; (fb), fruiting body; (b), bulb; (m), mushroom.

*b* Biological activity relates to the plant and not necessarily the alkaloids that are listed as representative examples of the families.

### 3.0. CLASSIFICATION

In spite of differences between the research fields of biology, medicine and chemistry, and the fact that there remain some differences of accentuation in alkaloid definitions, such definitions are very similar, indeed almost identical. Scientists are recognizing the vital importance of these products for biology, medicine and chemistry. What has been learnt about alkaloids from the last 200 years of studies? It is fascinating that alkaloids are just a product of nature, and a very small unit of global nature both in the material sense and in processes as they occur. They are just a product of living cells, for other living cells. The alkaloid is a product of chemical molecules for the production of other molecules. It is synthesized, playing its own role in the metabolism after that. The alkaloid represents perfection in much the same way as perfection appears in life and nature. This is the reason why alkaloids were and are a fascinating subject of study. This is also the reason why definitions of these groups of molecules, provided by scientists of biology, medicine and chemistry, are acceptably imperfect. However, alkaloids are recognized as a large group of compounds with biological, pharmacological or physiological and chemical activity. Without alkaloids, stupendous achievements in the battle against malaria, leukaemia and cancer as well as Parkinson disease would be not possible. The pharmaceutical drug industry has succeeded in the use of natural plant alkaloids for the development of...
antimalarial agents (quinine and chloroquine), anticancer agents (taxol, vinblastine and vincristine) and agents promoting blood circulation in the brain (vincamine). Many alkaloids can influence an animal’s nervous system, providing possible changes in the functionality of the organism. The activity of alkaloid molecules on a psychomental level (opium latex, papaverine, morphine, cocaine) is one of natural phenomena in the process of species self-protection, and the interactions between producers (plants) and consumers (herbivores). It is also a good example of natural selection mechanisms and results. Nowadays, there are more than 8000 natural compounds and their derivatives recognized as alkaloids.

Each year, scientists around the Globe discover at least 100 new molecules. They frequently occur as acid salts, but some also occur in combination with sugars whereas, others appear as amides or esters. Alkaloids can also be quaternary salts or tertiary amine oxides. Alkaloids can be classified in the terms of their (1) biological and ecological activity; (2) chemical structures and (3) biosynthetic pathway. From the point of view of biological activity, it is possible to divide alkaloids into (1) neutral or weakly basic molecules (e.g., lactams such as ricinine, certain N-oxides such as sindicine), (2) animal-derived alkaloids (e.g., anuran, mammalian and arthropodalkaloids), (3) marine alkaloids, (4) moss alkaloids, (5) fungal and bacterialalkaloids and (6) non-natural alkaloids (structurally modified or analogues).

Nowadays, the group of compounds mentioned as non-natural alkaloidsis is growing especially rapidly as a result of bio-organic and stereochemistry research. Pharmacological research and the drug industry rapidly advance and promote the most promising new molecules for possible production applications. This is necessary since the sources of infections (micro-organisms) are constantly changing their species and infection ability, becoming resistant to medicines and antibiotics. Alkaloids are generally classified by their common molecular precursors, based on the biological pathway used to construct the molecule. From a structural point of view, alkaloids are divided according to their shapes and origins. There are three main types of alkaloids: (1) true alkaloids, (2) protoalkaloids and (3) pseudoalkaloids. True alkaloids and protoalkaloids are derived from aminoacids, whereas pseudoalkaloids are not derived from these compounds.
Secondary Metabolites

- **True Alkaloid**
  The true alkaloids are compound in which the nitrogen-containing heterocyclic system is derived from a biogenetic amine, formed by decarboxylation from an amino acid. They are usually found as salts in plant such as liriodenine and morphine.

- **Pseudo Alkaloid**
  Pseudo alkaloids are apparently unrelated to amino acid. They are nitrogen containing molecules but they have carbon skeletons derived from monoterpenes and other acetate derivatives and aliphatic polyketoacids such as coniine and \( \beta \)-skytanthine.

- **Proto Alkaloid**
  These compound like true alkaloids, are derived from amino acid or biogenetic amines but they do not contain any heterocyclic system. They are represented in nature by biogenetic amine themselves and their methylated derivatives such as serotonin and mescaline.

### 4.0. SYNTHESIS AND METABOLISM

Each biomolecule of a chemical nature in living organisms has its own synthesizing, transformational and interconverting processes. Therefore, the formation of the ring of the alkaloid molecule, and the flow of the nitrogen atom into this molecule, is the basic point for understanding alkaloid synthesis and its metabolism. Alkaloid biosynthesis needs the substrate. Substrates are derivatives of the secondary metabolism building blocks: the acetyl coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid and 1-deoxyxylulose 5-phosphate. The synthesis of alkaloids starts from the acetate, shikimate, mevalonate and deoxyxylulose pathways. The acetyl coenzyme A pathway (acetate pathway) is the source of some alkaloids and their precursors (e.g., piperidine alkaloids oranthranilic acid as aromatized CoA ester (antraniloyl-CoA)). Shikimic acid is a product of the glycolytic and pentose phosphate pathways, a construction facilitated by parts of phosphoenolpyruvate and erythrose 4-phosphate. The shikimic acid pathway is the source of such alkaloids as quinazoline, quinoline and acridine. The mevalonate pathway is based on mevalonic acid (three molecules of acetyl-CoA) which is closely related to the acetate pathway, while the deoxyxylulosephosphate pathway is based on a combination of pyruvic acid and glyceraldehyde3-phosphate (both from the glycolytic pathway). Together, mevalonate and deoxyxylulose phosphate pathways produce terpenoid and steroid compounds. However, it is important to note that the Krebs cycle pathway is also key to many precursors of alkaloids.
Ornithine, a postcursor of l-arginine in animals and of l-glutamate in plants, and, for example, l-lysine, a principal protein amino acid, deriving from the Krebs cycle pathway compound, are useful examples of the role of the Krebs cycle for alkaloid precursors (Figure 21). Moreover, there are other sources of alkaloid substrates, particularly in purine alkaloids. Figure 23 represents the general scope of alkaloid synthesis in the metabolic system of organisms and their energy production. Enzymatic activity is very important in the primary metabolism of glycolysis and the Krebs cycle. Pyruvic acid and CoA are key compounds in the synthesis of alkaloid precursors. Moreover, these precursors (amino acids) can be derived from different points in the glycolysis and Krebs cycles. Consequently, the synthesis of alkaloids as a secondary metabolic activity is a very challenging research subject. Generally, it is recognized in the literature that alkaloid metabolism in animals, and especially in mammals, is closely related to that of plants210. However, some exceptions exist. In plants, this non-protein amino acid is derived from l-glutamate and in animals from l-arginine. Moreover, Figure 23 demonstrates that synthesis of alkaloids is complicated by the ability of the same amino acid to synthesize many different alkaloids.

4.1. Biogenesis of alkaloids

The synthesis and structural analysis of alkaloids leads to the following basic questions: why are alkaloids synthesized in an organism and on which mechanism is alkaloid formation and degradation dependent in the life cycle? It is known that alkaloids have a genetic nature and that alkaloid content is diverse inside and between the species. In nature the same species of plants may have both high and low alkaloid content. Natural hybridization has been successfully used in plant breeding for the development of the so-called “sweet cultivars” in crop production. “Sweet cultivars”, however, are not without alkaloids. The total removal of alkaloids is impossible. “Sweet cultivars” are therefore plants, in useful organs of which alkaloids are present at a very low level, the bioactivity of which is not of any significant or observable level. However, alkaloid decrease by hybridization is an undirect but strong argument for the case that alkaloids have an heredity nature and that their presence in plants is of an evolulational character. This is fundamental in answering the first question connected with the biogenesis of alkaloids. Alkaloids have a strong genetic–physiological function and background in the organisms which
produce them. The biogenesis of alkaloids is therefore a part of the total genetic-functional strategy of such metabolisms.

4.2. Methods of alkaloid analysis

These analytical dilemmas interfere with the methods of alkaloid analysis. Each group of alkaloids has its own methods of extraction, isolation and crystallization, as well as detection in structure, molecule and dynamicity. Not all these stages are still possible in the majority of alkaloids. In recent years, many techniques have been used in alkaloid detection. There are atomic and molecular electronic spectroscopy, vibration spectroscopy and electron and nuclear spin orientation in magnetic fields, mass spectroscopy, chromatography, radioisotope and electrochemical techniques.

4.2.1. Methods in history

The first method of alkaloid analysis was developed in 1805, in the case of morphine. This method of isolation, with minor and major variations, is still used today. By this method, the first quinolizidine alkaloids were also extracted: sparteine in 1851, lupinine in 1865 and lupanine 2 years later. At the beginning of the 20th century, the extraction and determination of total quinolizidine alkaloidsin the same analysis (common) was carried out by Jurkowski, Nowotnówna, Trier, Ivanov, Sengbusch, Łukaszewicz, Wuttke, Reiferand Niziołek and Wiewiórowski and Skolik initiated research in which the sum of the contents of the different and separate alkaloids is the total alkaloid content. The method of isolation of quinolizidine alkaloids was developed next by Wysocka et al. and Wysocka and Przyby.

4.2.2. Basic methods and instruments

The first step in the development of methods was the evidence that molecules synthesized and degradated, and that intermediate compounds existed. Initial methods have provided molecule isolation, and subsequently the place in the metabolic chains. The basic methods of alkaloid determination developed historically as follows: iodine, taster, seed colour, Dragendorf reagent, fluorescence, calorimetry, photometry, electrophotometry, spectrometry, paper chromatography, thin layer chromatography, high-performance liquid chromatography, gas chromatography, gas liquid chromatography-mass spectrometry, nuclear magnetic resonance, X-ray, enzyme-like immunosorbent assay, radioimmuno assay and scintillation proximity assay.
methods. The most effective method for establishing a metabolic pathway is the use of isotopes in radiotracing and mass spectrometry methods. The basic instruments that have been developed are photometers, calorimeters, analyzers, spectrometers, chromatographs and different mass spectrometers. These instruments have subsequently been improved to be more exact, and have been through many generations in their development by many different producers.

4.2.3. From iodine to enzyme

Alkaloid analytical methods were developed by applications based on different hypotheses, from the simple to the very complicated. Subsequently, corresponding instruments were developed. This development can be seen by considering the example of quinolizidine alkaloids. Iodine, Tester method, Seed colour method, Dragendorff reagent, Fluorescence method, Calorimetry method, Photometry method, Electrophotometry method, Paper chromatography, Thin layer (planar) chromatography, High performance liquid chromatography, Gas chromatography, Gas–liquid chromatography–mass spectrometry, Nuclear magnetic resonance, X-ray method, Enzyme-linked immunosorbent assay (ELISA), Radioimmunoassay, Scintillation proximity assay, Capillary zone electrophoresis.

5.0. ALKALOIDS AS DRUGS

Medical applications of alkaloids have led to the production of drugs and drug components. They can be based on pure natural alkaloids, as in the case of extracts. Purified alkaloids, partially and even totally synthesized compounds based on the natural alkaloid structure, are also used. Chemically modified alkaloids are yet another example. Chemically modifying the structure affects biological activity. The general trend in modern medicine is to develop compounds that are biologically more active than those found in nature. This is achieved in many cases by alkaloid modifications and synthesis. However, natural compounds themselves are very important because they are the basis for artificial drugs. Moreover, alkaloids used as natural products are important in phytomedicine, alternative medicine and homeopathy. Still today, folk- and ethnomedicine rely heavily on their use.

Aconitine-, ajmaline- and sanguinarine-based drugs have medicinal importance and are dosed clinically. The drugs are medical products developed by the pharmaceutical industry. Physicians have the right to determine the prescription, that is the use of these products and
determination of dosage. Pharmaceuticals based on these alkaloids are relatively strong. Several researched and patented drugs such as Aconitsat (aconitine) and Rauwopur (ajmaline) can be found on the pharmaceutical market today. Sanguinarine is generally used in toothpastes. Toothpaste generally has no side effects along with its anti-cavity properties. Atropine-, hyoscine- and hyoscyamine-based drugs are developed on a large scale and they also have a variety of clinical purposes. Atropinol, for example, is based on atropine. This drug contains atropine sulphate. Another example is Buscopan, based on hyoscine. Hyoscyamine is used in transdermal plasters.

Bella sanol also contains hyoscyamine. The therapeutic use is similar to that of atropine. At least 50 different products from these alkaloids have been developed and introduced on the pharmaceutical market. Drugs based on eserine, galanthamine, nicotine, lobeline and tubocurarine are also prominent. Two examples of drugs containing eserine are Anticholium and Pilo-Eserin. There are at least 20 different products based on this alkaloid. Nivalina is one drug that contains galanthamine. There are others as well, but less so than with the eserine drugs. Galanthamine-containing medicines have potential uses in the treatment of Alzheimer’s disease. Because of this, a greater number of products containing galanthamine is expected to reach the market. Nicotine is used in many products on the pharmaceutical market, for example Nicorette or Nicoderm. At least 20 different products are known to contain nicotine. These drugs are delivered in different forms. One of these is a transdermal plaster. Nicotine chewing gum and tablets are also available. These drugs are used especially to reduce nicotine cravings. The drugs that contain lobeline are, for example, Stopsmoke or Lobatox.

These products are used for similar purposes as drugs that contain nicotine. Tubocurarine-containing drugs such as Tubarine or Jexin are used in surgical procedures as muscle relaxants. Alkaloids such as boldine, codeine, narceine and morphine are also important in clinical practice. Boldosal and Oxyboldine are good examples of boldine based drugs with morphine-like properties. Codeine is a component of at least 250 pharmaceutical products on the market. Codicaps or Codipront can be mentioned as examples. All of these products are opium derivatives. Narceine-containing drugs are similar to those of codeine. They are used to treat coughs. Paneraj is an example of a typical trademark. There is a long list of morphine-containing drugs. These drugs are used in serious instances, for example in cases of surgical operations and post-operation treatments. Morphalgin and Spasmofen are examples.
The alkaloids ephedrine, ergotamine, ergometrine and yohimbine are used in many forms. Products like Dorex or Endrine contain ephedrine as their major component. They are used for many purposes, including treating nasal cold symptoms or in bronchial asthma. More than 25 different drugs containing ephedrine have been developed today. Ergotamine is an active alkaloid produced by fungi with many applications on the market. Pharmaceuticals such as Ergostat or Migral are typical examples of ergotamine-based products. Drugs containing this alkaloid are used widely in the treatment of migraines. Ergometrine is also an ergot alkaloid. It can be found in several products on the market, including Ergometron and Syntometrine. Yohimbine can be found in drugs such as Aphrodyne or Yohimex. About 20 different products containing this alkaloid have been developed. These drugs are aphrodisiacs used to treat impotency and impotency-related problems in men. Many other alkaloids are used with various applications. One of them is strychnine, an alkaloid known to be strongly toxic to animals. Drugs such as Dysurgal or Pasuma contain strychnine in clinical doses. Strychnine-containing drugs are used in many disorders, including those of the eye.

Vinblastine and vincristine are two further examples of important drug constituents. Vinblastine is found in such drugs as Periblastine or Velban, and vincristine in such drugs as Norcristine or Pericristine. These drugs are used in oncology as cancer treatments. Drugs with vinblastine are also used in the treatment of Hodgkin’s disease, and those containing vincristine in the treatment of Burkitt’s disease as well as brain and lung tumours. Many other alkaloid-based medicines exist. Drugs based on berberine are used in the treatment of infections as well as in the treatment of AIDS. Stimulant drugs are often based on caffeine, cathine, theobromine and theophylline. Caffeine is a component of more than 300 different drugs. It is also a minor component in many other pharmaceuticals. Analgen or Panax are examples of caffeine-based drugs.

Cathine is found in drugs such as Amorphan or Recatol. They have an anorectic influence of the liver. More than 25 different drugs have been developed from theobromine, for example Atrofed and Seominal. These drugs serve many clinical purposes including treating asthma and Angina pectoris. Moreover, 200 different drugs have been developed from theophylline. Theochron and Euphyllin are examples. Theophylline-containing drugs are used to treat bronchitis and asthma. The applications containing quinidine and quinine are very well known and relatively old. Drugs such as Quinidex or Quinalan are good examples of quinidine-containing drugs.
Secondary Metabolites

applications. Quinine can be found in drugs such as Adaquin or Biquinate. These drugs are important in the prevention and treatment of malaria.

5.1. ALKALOIDS IN FOOD

Alkaloidal plants used as food are small in number. The reason for this is the bioactivity and traditional use of alkaloids in medicines and drugs. Moreover, food is checked and controlled with the purposes of keeping alkaloid contaminated food off the market. However, cases where pyrrolizidine alkaloids were found in the honey produced by bees that had foraged on the flowers of *Echium* and *Senecio* species are documented. In these relatively old studies, bees had been feeding only on the pollen of one species, which is not typical of bee behaviour or natural honey production. More recent studies have reported that many alkaloids have been detected in the pollen of many species. However, it is known that nectar and pollen contain considerably lower levels of alkaloids in comparison to other plant parts. Therefore, the acute toxicity to the bees and the accumulation of alkaloids in honey should be not high, if not miniscule in natural environmental conditions. Moreover, a recent study by Wäckers criticizes studies on unsuitable nectars for insects that are based only on laboratory results, as they do not necessarily translate to ecosystems and natural environments. According to literature, alkaloids have also been detected in other animals and animal products. Pyrrolizidine alkaloids were found in the livers and kidneys of domesticated animals, as well as in milk and eggs. Food contaminated by alkaloids is generally considered to be a health risk.

However, some alkaloids are used as additional components of food. The most well known is the use of the quinine as a bitter in tonic water according to an established procedure. Theophylline is an important component of black tea. Caffeine is a well-known component of coffee. Theobromine is found in cacao plants but not in the final products based on cacao seeds, such as cacao drinks or chocolate. Theobromine is removed during fermentation and processing. Processing is very important for the production of the final alkaloid product consumed. In the case of coffee, a high-quality product is possible only from theripened berries. There are two methods of processing coffee. The first one is the so-called “dry method”, used especially in Brazil and in tropical Africa. It is based on the simple drying of berries in the sun. In humid areas this method cannot be used, as sun-drying can prove difficult. In these places, the so-called “wet process” is preferred. Berries are first crushed to a pulp in this method. Seed flesh and skin are
then separated. At this stage, a mucilaginous remnant from the fruit flesh is stuck to the skin. It can be removed via fermentation or by treatment with pectinase, an alkali, or mechanically. The coffee is washed, dried, hulled and polished. Next, the coffee is sorted according to size and colour. The green coffee can be stored for a long time. It contains caffeine as the main alkaloid. Raw coffee has no aroma. It is developed only during the roasting process.

Roasting is done just before the coffee is ready for the market. Roasting occurs at temperatures around 200-250°C. Instant coffee is produced from roasted coffee. Roasted coffee is grounded and extracted with water. After that it is powdered and dried. The drying process is crucial when considering the quality of instant coffee. Lower quality is achieved in the spray-drying procedure, and freeze drying produces the highest quality. Coffee quality depends on caffeine percentage, aroma and taste. Decaffeinated coffee and coffee surrogates are available on the market. One of these surrogates is roasted lupin beans containing nearly 200 micrograms of quinolizidine alkaloids per gram. Taste and caffeine content together with the catechins are also important quality parameters for tea. Moreover, a tea’s quality also depends on the chosen processing method. One such method is based on fresh leaves (water content of 75–80% and drying up to 58–64%). After rolling in heavy machines, the cell structure is destroyed. Polyphenoloxidases, enzymes from the plant, come into contact with catechins. Next, fermentation takes place in temperatures kept below 25°C. Here, the leaves change to a copper colour, which means that the catechins transform to theaflavin and thearubigin. These compounds are in the complex with caffeine and protein. In the case of the so-called “green tea” produced in Asia, the phenoloxidases in the fresh leaves are first inactivated by steaming or in heating pans. This means that composition of the catechinshas not changed. The rolling that takes place after inactivation is done as in the case of normal black tea. The colour of the tea is first olive green, but subtly turns to a golden colour. Green tea is more refreshing because there is more free caffeine, and is considered to be healthier because of the effect of catechins.

There are also other different teas such as red or yellow teas from China. Different colours are indicative of when fermentation ended. In earlier times, pure caffeine was extracted from coffee and tea. Caffeine was a product that could be used as a food additive. Nowadays, both caffeine and theophylline are chemically synthesized. They are used as additives to a large list of different products. Moreover, according to recent research data, consumption of green tea and coffee was linked to a decreased risk of type 2 diabetes in Japanese adults.
Theobromine is an alkaloid found in the raw material of the cacao. The processing of this product involves many steps beginning with the cutting open of harvested cacao fruits. The beans are then fermented in boxes together with the white, mucilaginous pulp. The rapid development of yeasts, acetic acid and lactic acid bacteria ensues. The temperature in this stage may reach 45°C. During fermentation the alcohol received is oxidized to acetic acid, which kills the embryos of the seeds. Phenoloxidases oxidizes catechins and other phenolic compounds. As a result of this, the brown chocolate colour appears. During the drying, the aroma and the bitter taste of cacao develop. Theobromine is extracted and cacao is then edible. In the case of chocolate production, cacao seeds are roasted at temperatures of 90–140°C for 10–45 minutes. Cacao butter is extracted and used directly in chocolate production. Theobromine is also extracted in this stage. Alkaloids are well known in food spices and herbs. The black, white, green (Piper nigrum L.) and long pepper (Piper longum L.) containing piperine are widely used in food. Other alkaloid plants used in food are Capsicum peppers such as chilli or red pepper (Capsicum annuum L.), Peruvian pepper (Capsicum baccatum L.), ají pepper (Capsicum chinense Jacq.), bird pepper ortabasco (Capsicum frutescens L.) and rocoto pepper (Capsicum pubescens).

According to recent studies, piperine is non-toxic and has a great deal of physiological activity. It has been recently documented that piperine interacts with a mammalian protein. This alkaloid is efficiently taken by calyx of bovine beta-lactoglobulin, which is the major whey protein in milk. Moreover, the piperine molecule can be also detected in the beta-barrel of human tearlipocali, human serum retinol–binding protein and human neutrophil gelatinase–associated lipocalin610. Capsaicin, from Capsicum spp., has similar effects. Some products containing ephedrine alkaloids are well-known dietary supplements.

These alkaloids, namely epinephrine and norepinephrine, naturally occur in low concentrations in the human body. They have sympathomimetic effects and cause weight loss and enhanced athletic performance. The products are generally botanicals but ephedrine alkaloids may also be synthesized. There are risks connected with the use of these as supplements. Determining a risk-free dosage is currently a topic of discussion611. Dietary supplements containing ephedrine alkaloids are on the market.
5.2. BIOTECHNOLOGY

Alkaloids used as strongly bioactive molecules are vital in biotechnology for the development of new production methods. It is possible to produce more effective alkaloids via biotechnology, and also possible to produce them on a very large scale. Biotechnology can be defined generally as the application of organisms, biomaterials and systems or processes in manufacturing and production. In the case of alkaloids, biotechnology is a process of effective production of alkaloids in vitro and in vivo (Figure 95). Biotechnology can be divided into chemical and industrial biotechnology depending on the method used. Furthermore, it can be thought of in a biomedical and pharmaceutical sense when medical objectives are concerned.

Lastly, genome and proteomic biotechnology uses genetic and protein engineering. Biotechnology attempts to produce cells and molecules of animals, plants and micro-organisms. Alkaloids are one group of molecules which can be produced via biotechnological means. Plants are the basis for this type of production; however, alkaloids can also be produced in animal cells or in those of micro-organisms. Genetic or organ manipulation is needed in this production. Generally speaking, the best results in alkaloid production by biological methods are presently achieved using plant organs. However, it is also possible from micro-organisms as well as animal cells and tissues.

6.0. ALKALOID EXTRACTION PROTOCOL

One can find a number of methods of quinolizidine alkaloid extraction within existing literature. The most frequently applied methods are extraction by alcohols (50% methanol or 96% ethanol, or these alkaloids with a 1% addition of glacial acetic acid). Wysocka and Przybył presented an efficient extraction method of quinolizidine alkaloids. The following are the steps of extraction:

• maceration of the ground lupin seeds with 25% aq. KOH for about 36 hours in order to destroy the tissues and release the alkaloids from their salts;
• excess water present in the alkaline pulp is absorbed by diatomaceous earth;
• mass prepared in this way is poured into a linen sack and then placed in an extractor followed by elution with ethyl ether and then with methylenechloride;
• extracts are condensed to a volume of about 100cm3 and the alkaloids are eluted with 2N hydrochloric acid;
• in order to degrease, the acidic alkaloid solution is extracted with petroleum ether (bp40–60 °C) until the fats have been removed from the organic layers;
• after degreasing, the aqueous solution is made alkaline with 50% aq. KOH and then eluted with ethyl ether and methylene chloride. This method has since been modified for efficiency, resulting in the following steps:
  1. Grinding of seeds
  2. Degreasing of the ground seeds with petroleum ether
  3. Drying of the seeds in the air
  4. Maceration of the meal with 25% aq. KOH
  5. Mixing of the macerated meal with diatomaceous earth
  6. Elution of alkaloids with methylene chloride
  7. Purification of the extract by filtration through a column with aluminium oxide (activity grade II)
  8. Evaporation of the extract to a constant weight
  9. Yellow solidifying oil.
This method is more sensitive than other established methods. The amount of alkaloids obtained through this method is considerably higher than that through alcohol extraction.

7.0. CONCLUSION

The present chapter investigates the significance and structural elucidation of alkaloids as a prime secondary metabolite. The occurrence, nature and bioactive properties of various alkaloids highlight their therapeutic significance.

REFERENCES:


Secondary Metabolites


3. FLAVONOIDS

Preethi Kathirvel

1.0. INTRODUCTION

Flavonoids, an amazing array of over 6,000 different substances found in virtually all plants, are responsible for many of the plant colors that dazzle us with their brilliant shades of yellow, orange and red.

![Flavonoid Structure](image)

**Fig 1: Nuclear structure of flavonoids**

Dietary flavonoids are diverse and vary accordingly to hydroxylation pattern, conjugation between the aromatic rings, glycosidic moieties, and methoxy groups.

Flavonoids are the most diverse group of phytochemicals. Research suggests that flavonoids, in particular, may be important photochemical group that contributes to the reduced mortality rates observed in people consuming high of plant-based foods (Hertog et al., 1993). Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. Flavonoids belongs to the polyphenol group. Flavonoids can be visualized as two benzene rings which are
joined together with short three Carbon chains. One of the carbons of the chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. The flavonoids are one of the largest classes of phenolics. The basic carbon skeleton of a flavonoid contains 15 carbons arranged in 2 aromatic rings connected by a 3-carbon bridge:

![Flavonoid Structure](image)

The basic flavonoid carbon skeleton may have numerous substitutes. Hydroxyl groups are usually present at positions 3,5 and 7, but they may also be found at other positions. Sugars are very common as well, in fact the majority of flavonoids exist naturally as glycosides. Whereas both hydroxyl groups and sugars increase the water solubility of flavanoids, other substituent’s, such as methyl ethers or modified isopentyl units, make flavonoids lipophilic (hydrophobic). Different types of flavonoid perform very different functions in the plant, including pigmentation and defense.

### 2.0. DISTRIBUTION AND DIETARY SOURCES OF FLAVONOIDS

Flavonoids (specifically flavonoids such as the catechins) are “most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Flavonols, the original bioflavonoids such as quercetin, are also found ubiquitously, but in lesser quantities. The widespread dilution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans
ingest significant quantities in their diet. Foods with a high flavonoid content includes, onion, blueberries and other berries, black tea, green tea and oolong tea, bananas, all citrus fruits, Ginkgo biloba, red wine, sea-buckthorns, and dark chocolate (with a cocoa content of 70 per cent or greater). Further information on dietary sources of flavonoids can be obtained from the US Department of Agriculture flavonoid database. Flavonoids exist naturally in cocoa, but because they can be bitter, they are often removed from chocolate, even dark chocolate although flavonoids are present in milk chocolate, milk may interfere with their absorption.

Flavonoids are found in most plant material. The most important dietary sources are fruits, tea and soybean. Green and black tea contains about 25 per cent percent flavonoids. Other important sources of flavonoids are apple (quercetin), citrus fruits (rutin and hesperidin).

### Some examples of flavanoids and their food sources

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Common food source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonol</td>
<td>Quercetin</td>
<td>Apples, Onions</td>
</tr>
<tr>
<td>Flavanol</td>
<td>Carechin</td>
<td>Tea, coffee, chocolate</td>
</tr>
<tr>
<td>Isoflavone</td>
<td>Genistein</td>
<td>Soy</td>
</tr>
<tr>
<td>Flavonone</td>
<td>Hesperidin</td>
<td>Grapefruit</td>
</tr>
<tr>
<td>Anthocyanidin</td>
<td>Cyanidin</td>
<td>Berries</td>
</tr>
</tbody>
</table>

#### 2.1. Flavonoids in Plants

Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors. Flavonoids secreted by the root of their host plant help *Rhizobia* in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy. Rhizobia living in soil are able to sense the flavonoids and this triggers the secretion of Nod factors, which in turn are recognized by the host plant and can lead
to root hair deformation and several cellular responses such as ion fluxes and the formation of a root nodule. In addition some flavonoids have inhibitory activity against organisms that cause plant diseases, e.g. *Fusarium oxysporum* (Galeotti et al., 2008).

3.0. PHYSICAL AND CHEMICAL PROPERTIES OF FLAVONOIDS

Flavonoids are crystallic substances with certain melting point. Cathechins, leucoanthocyanidins, flavanes, isoflavanes, flavanones, flavanonoles are colorless crystals, flavones, flavonoles, chalcones and aurones are yellow or vividly yellow. Anthocyanes are sap pigments and the actual colour of the plant organ is determined by the pH of the sap. For example, the blue colour of the corn flower and the red of roses are due to the same glycosides and both of these plants on hydrolysis with hydrochloric acid yield cyanidin hydrochloride. Changes of anthocyanes colour depend upon pH: in acid medium their colour is red, in alkaline medium - blue. As a general rule, glycosides are water-soluble and soluble in alcohols. Flavonoid glycosides are soluble in diluted alcohols and hot water. Aglycones are, for the most part, soluble in a polar organic solvents: when they have at least one free phenolic group, they dissolve in alkaline hydroxide solutions. Flavonoid aglycones soluble indiethyl ether, acetone, alcohols, almost are insoluble in water.

Flavonoids (cathechins) are optically active. i.e., among four optical isomers (D-and L-cathechin_s, D- and L-epicatechins) only L-epicatechin, possess, P-vitaminic activity. Flavanones and flavononones are unstable compounds. Treated with oxidants, they turn into chalcones and leucocyanidins accordingly. Flavonoid O-glycosides may be treated with acid, alkaline or fermentative hydrolysis. Rutin occurs as a yellow crystalline powder, soluble in alkali but only slightly soluble in water. Rutin on hydrolysis yields quercetin, rhamnose and glucose, while hesperidin yield shesperetin (or methyl eriodictyol), rhamnose and glucose. C-linkage between a glycone and agar is very strong, therefore hydrolysis of C-glycosides is carried out with Killiani’s reagent (mixture of concentrated HCl and acetic acid) (Danylo Halytskyi).

4.0. BIOLOGICAL PROPERTIES OF FLAVONOIDS

As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capacity both in vivo and in vitro systems. Flavonoids have the ability to induce human protective enzyme systems. The number of studies has suggested protective
effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases. Flavonoids also act as a secondary antioxidant defense system in plant tissues exposed to different abiotic and biotic stresses. Flavonoids are located in the nucleus of mesophyll cells and within centers of ROS generation. They also regulate growth factors in plants such as auxin. Biosynthetic genes have been assembled in several bacteria and fungi for enhanced production of flavonoids.

5.0. TYPES OF FLAVONOIDS

Over 5000 naturally occurring flavonoids have been characterized from various plants. They have been classified according to their chemical structure, and are usually subdivided into the subgroups showing in Table 1.

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>• Anthoxanthins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavones</td>
</tr>
<tr>
<td></td>
<td>Flavonols</td>
</tr>
<tr>
<td></td>
<td>• Flavanones</td>
</tr>
<tr>
<td></td>
<td>• Flavanols</td>
</tr>
<tr>
<td></td>
<td>• Flavans</td>
</tr>
<tr>
<td></td>
<td>Flavan-3-ols</td>
</tr>
<tr>
<td></td>
<td>Proanthocyanidins</td>
</tr>
<tr>
<td></td>
<td>Flavan-4-ols</td>
</tr>
<tr>
<td></td>
<td>Flavan-3,4-diols</td>
</tr>
<tr>
<td>Isoflavonoids</td>
<td>• Anthocyanidins</td>
</tr>
<tr>
<td></td>
<td>• Isoflavones</td>
</tr>
<tr>
<td></td>
<td>• Isoflavans</td>
</tr>
<tr>
<td></td>
<td>• Pterocarpans</td>
</tr>
<tr>
<td>Neoflavonoids</td>
<td>• Dalbergichromene</td>
</tr>
<tr>
<td>Aurones</td>
<td>• Aureusidin</td>
</tr>
<tr>
<td></td>
<td>• Leptosidin</td>
</tr>
</tbody>
</table>
**Secondary Metabolites**

Other categories

- C-methylated flavanoids
- O- methylated flavanoids
- Flavonolignans
- Furanoflavonoids
- Pyranoflavonoids
- Prenylflavonoids
- Methylenedioxycaustavinsols

**Anthocyanins**: The colored pigments of plants provide visual cues that help to attract pollinators and seed dispersers. These pigments are of 2 principal types: carotenoids and flavonoids. Carotenoids are yellow, orange and red terpenoid compounds that also serve as accessory pigments in photosynthesis. The flavonoids also include a wide range of colored substances. The most widespread group of pigmented flavonoids is the anthocyanins, which are responsible for most of the red, pink, purple and blue colors observed in flowers and fruits.

Anthocyanins are glycosides that can have various sugars at position 3 and sometimes elsewhere. Without their sugars, anthocyanins are known as anthocyanidins. Anthocyanin color is influenced by many factors, including the number of hydroxyl and methoxyl groups, the presence of aromatic acids esterified to the main skeleton and the PH of the cell vacuole in which the anthocyanins are stored.

<table>
<thead>
<tr>
<th>ANTHOCYANIDIN</th>
<th>COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelargonidin</td>
<td>Orange red</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>Purplish red</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>Bluish purple</td>
</tr>
<tr>
<td>Peonidin</td>
<td>Rosy red</td>
</tr>
<tr>
<td>Petunidin</td>
<td>Purple</td>
</tr>
</tbody>
</table>
Flavones and Flavonols:

The flavonoids generally absorb light at shorter wave lengths than do anthocyanins, so they are not visible to human eye. But insects, such as bees, which see farther into the ultra violet range of the spectrum than humans do, may respond to flavones and flavonols as visual attractants cues.

Flavonols in a flower often form symmetric patterns of stripes, spots, or concentric circles called nectar guides. These patterns may be conspicuous to insects and are thought to help indicate the location of pollen and nectar. These flavones and flavonols are not restricted to flowers; they are also present in the leaves of all green plants. These two classes of flavonoids are thought to protect cells from excessive UV – B radiation because they accumulate in the epidermal layers of leaves and stems and absorb light strongly in the UV – B region while allowing the visible wave lengths to pass through uninterrupted. In addition, the exposure of plants to increased UV – B light has been demonstrated to increase the synthesis of flavones and flavonols. Plants which lack the synthesis of flavonoids are much more sensitive to UV – B radiation.

Isoflavonoids: Isoflavonoids are found mostly in legumes, have several different biological activities. Some have pesticidal action, some are responsible for the anticancer benefits of foods prepared from soybeans. Some serve as antimicrobial compounds which are synthesized in response to bacterial or fungal infection that help limit the spread of the invading pathogen.

5.1 Flavonoid Biosynthesis:

Flavonoids are synthesized by the phenylpropanoid metabolic pathway in which the amino acid phenylalanine is used to produce 4 – coumaroyl – coA. This can be combined with malonyl – coA to yield the true back bone of flavonoids, a group of compounds called chalcones, which contain two phenyl rings. Conjugate ring – closure of chalcones results in the familiar form of flavonoids, the three ringed structure of a flavones.
The metabolic pathway continues through a series of enzymatic modifications to yield

Flavanones $\xrightarrow{}$ dihydroflavonols $\xrightarrow{}$ anthocyanins

Along this pathway, many products can be formed, including the flavonols, flavan – 3 – ols, proanthocyanidins(tannins) and a host of other various polyphenolics.

The biosynthesis of flavonoids involves several enzymes – Anthocyanidinreductase, Chalconeisomerase, Dihydrokaempferol- 4-reductase, Flavone synthase, Flavonoid 3’-monooxygenase, Flavonol synthase, Flavanone 3 – dioxygenase, Flavanone 4 – reductase, Leucoanthocyanidinreductase, Leucocyanidinoxygenase, Naringenin – Chalcone synthase.

Several naturally occurring flavonoids have been synthesized following a new proposed method based on the use of the Heck reaction. The key steps involves the coupling of an aryl vinyl ketone with an aryl iodide. This procedure affords the flavonoid moiety in a single step.

5.2. Functions of Flavonoids

5.2.1. Protection of Cell Structures

Most flavonoids function in the human body as antioxidants. In this capacity, they help neutralize overly reactive oxygen-containing molecules and prevent these overly reactive molecules from damaging parts of cells. Particularly in oriental medicine, plant flavonoids have been used for centuries in conjunction with their antioxidant, protective properties. Scultellaria root, cornus fruit, licorice, and green tea are examples of flavonoid-containing foods widely used in oriental medicine. While flavonoids may exert their cell structure protection through a variety of mechanisms, one of their potent effects may be through their ability to increase levels of glutathione, a powerful antioxidant, as suggested by various research studies.
Fig 2: Flavonoid types and uses

5.2.2. Vitamin C Support

The relationship between flavonoids and vitamin C was actually discovered by mistake. Dr. Albert Szent-Gyorgyi, the Nobel Prize winning researcher who discovered flavonoids, was attempting to make a preparation of vitamin C for one of his patients with blood vessel problems. The preparation he gave the patient was not 100 per cent pure—it contained other substances along with the vitamin C. It worked amazingly well. Later, when Dr. Szent-Gyorgyi purchased a pure solution of vitamin C, he found it was not nearly so effective with his patient. He suspected flavonoids as the magic addition to vitamin C in his first impure preparation. Present-day research has clearly documented the synergistic (mutually beneficial) relationship between
flavonoids and vitamin C. Each substance improves the antioxidant activity of the other, and many of the vitamin-related functions of vitamin C also appear to require the presence of flavonoids.

5.2.3. Inflammation Control

Inflammation-the body's natural response to danger or damage-must always be carefully regulated to prevent over activation of the immune system and unwanted immune response. Many types of cells involved with the immune system-including T cells, B cells, NK cells, mast cells and neutrophils-have been shown to be altered in the presence of flavonoids. Prevention of excessive inflammation appear to be a key role played by many different chemical categories of flavonoids.

5.2.4. Antibiotic Activity

In some cases, flavonoids can act directly as antibiotics by disrupting the function of microorganisms like viruses or bacteria. The antiviral function of flavonoids has been demonstrated with the HIV virus, and also with HSV-1, a herpes simplex virus.

5.3. Health Benefits of Flavonoids

Flavonoids are becoming very popular because they have many health promoting effects. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral. The flavonoid Quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Epidemiological studies have illustrated that heart diseases are inversely related to the flavonoid intake (Groff et al., 1995) Apart from this flavonoids are doing many good things for us such as

* Help protect blood vessels from rupture or leakage

* Enhance the power of your vitamin C

* Protect cells from oxygen damage

* Prevent excessive inflammation throughout your body
The contribution of flavonoids to the total antioxidant activity of components in food can be very high because daily intake can vary between 50 to 500 mg. Red wine high levels of flavonoids, mainly quercetin and rutin. The high intake of red wine (and flavonoids) by the French might explain why they suffer less from heart coronary heart disease than other Europeans, although their consumption of cholesterol rich is higher (French paradox). Many studies have confirmed that one or two glasses of red wine daily can protect against heart disease. Tea flavonoids have many health benefits. Tea flavonoids reduce the oxidation of low-density lipoprotein lowers the blood levels, cholesterol and triglycerides. Soy flavonoids (isoflavones) can also reduce blood cholesterol and can help to prevent osteoporosis. Soy flavonoids are also used to ease menopausal symptoms (Yao et al., 2004).

Before any chemical compound can be approved as a pharmaceutical drug or any food can be labeled with a health claim, it must undergo extensive in vitro, in vivo, and clinical testing to confirm both safety and efficacy. National international regulatory authorities like the US Food and Drug Administration (FDA) and European Food safety Authority (EFSA) are responsible for assessing this evidence and granting such approval. At the current time, neither the FDA nor the EFSA has approved any health claim for flavonoids, or approved any flavonoids as pharmaceutical drugs. Moreover, several companies have been cautioned by the FDA over misleading health claims.

5.4 Therapeutical Potential of Flavonoids

5.4.1. Antioxidant Activity

Flavonoids possess many biochemical properties, but the best described property of almost every group of flavonoids is their capacity to act as antioxidants. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability. (Kelly et al., 2002; Pandey et al., 2012). Mechanisms of antioxidant action can include (1) suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; (2) scavenging ROS; and (3) up regulation or protection of antioxidant defenses. Flavonoid action involves most of the mechanisms mentioned above. Some
of the effects mediated by them may be the combined result of radical scavenging activity and the interaction with enzymes functions. Flavonoids inhibit the enzymes involved in ROS generation, that is, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, NADH oxidase, and so forth (Halliwell and Gutteridge, 1998).

Lipid peroxidation is a common consequence of oxidative stress. Flavonoid protects lipids against oxidative damage by various mechanisms. Free metal ions enhance ROS formation by the reduction of hydrogen peroxide with generation of the highly reactive hydroxyl radical. Due to their lower redox potentials flavonoids (F1-OH) are thermodynamically able to reduce highly oxidizing free radicals (redox potentials in the range 2.13-1.0v) such as superoxide, peroxyl, alkoxy, and hydroxyl radicals by hydrogen atom donation. Because of their capacity to chelate metal ions (iron, copper, etc.), flavonoids also inhibit free radical generation. Quercetin in particular is known for its iron-chelating and iron-stabilizing properties. Trace metals bind at specific positions of different rings of flavonoid structures (Kumar et al., 2013).

5.4.2. Hepatoprotective Activity

Several flavonoids such as catechin, apigenin, quercetin, naringenin, rutin and venoruton are reported for their hepatoprotective activities. Different chronic diseases such as diabetes may lead to development of hepatic clinical manifestations. Glutamate-cysteine ligase catalytic subunit (Gclc) expression glutathione and ROS levels are reported to be decreased in liver of diabetic mice. Anthocyanin’s have drawn increasing attention because of their preventive effect against various diseases (Tapas, 2008). Hepatoprotective activities were observed in flavonoids isolated from laggeraalata against carbon-tetrachloride (CCl-4) induced injury in primary cultured neonatal rat hepatocytes and in rats with hepatic damage. Flavonoids at a concentration range of 1-1.00µg/mL improved cell viability and inhibited cellular leakage of hepatocyte aspartate aminotransferase (AST) and alanine aminotransferase (ALT) caused by CCl4. Similarly in an in vivo experiment flavonoids at of 50, 100, and 200 mg/kg oral doses significantly reduced the levels of AST, ALT, total protein, and albumin in serum and the hydroxyproline and sialic acid levels in liver. Histopathological examinations also revealed the improvement in damaged liver with the treatment of flavonoid (Wu et al., 2006).
5.4.3. Antibacterial Activity

Flavonoids are known to be synthesized by plants in response to microbial infection; thus it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Flavonoid rich plant extracts from different species have been reported to possess antibacterial activity. Several flavonoids including apigenin, galangin, flavone and flavonolglycosides, isoflavones, flavanones, and chalcones have been shown to possess potent antibacterial activity (Mishra, 2013). Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action. One of their molecular actions is to form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and so forth. Lipophilic flavonoids may also disrupt microbial membranes (Cowan, 1999). Catechins, the most reduced form of the C3 unit inflavonoid compounds, have been extensively researched due to their antimicrobial activity. These compounds are reported for their in vitro antibacterial activity against Vibrio cholerae, Streptococcus mutans, Shigella and other bacteria. The catechins have been shown to inactivate cholera toxin in Vibrio cholerae and inhibit isolated bacterial glucosyltransferases in S. mutans, probably due to complexing activities. Robinetin, myricetin, and (‘‘)-epigallocatechin are known to inhibit DNA synthesis in Proteus vulgaris. It is suggested that the B ring of the flavonoids may intercalate or form hydrogen bond with the stacking of nucleic acid bases and further lead to inhibition of DNA and RNA synthesis in bacteria. Another study demonstrated inhibitory activity of quercetin, apigenin, and 3,6,7,32,42-pentahydroxyflavone against Escherichia coli DNA gyrase (Ohemenget al., 1993).

Naringenin and sophoraflavanone G have intensive antibacterial activity against methicillin resistant Staphylococcus aureus (MRSA) md, Streptococci. An alteration of membrane fluidity in hydrophilic and hydrophobic regions maybe attributed to this effect which suggests that these flavonoids might reduce the fluidity of outer and inner layers of membranes. The correlation between antibacterial activity and membrane interference supports the theory that flavonoids may demonstrate antibacterial activity by reducing membrane fluidity of bacterial cells. The 5,7- hydroxylation of the A ring Nrd22, 42-or 22,62-dihydroxylation of the B ring in
the flavanone structure is important for anti-MRSA actively. A hydroxyl group at position 5 in flavanones and flavones is important for their activity against MRSA. Substitution with C8 and C10 chains may also enhance the anti-staphylococcal activity of flavonoids belonging to the flavan-3-ol class. It is also shown that 5- hydroxyflavanones and 5-hydroxyisoflavanones with one, two, or three additional hydroxyl groups atthe7,22 and 42positions inhibited the growth of *s. mutans* and *Streptococcus sobrinus* (Osawa et al., 1992).

5.4.4. Anti-Inflammatory Activity

Inflammation is a normal biological process in response to tissue injury, microbial pathogen infection and chemical irritation. Inflammation is initiated by migration of immune cells from blood vessels and release of mediators at the site of damage. This process is followed by recruitment of inflammatory cells, release of ROS, RNS, and proinflammatory cytokines to eliminate foreign pathogens and repairing injured tissues. In general normal inflammation is rapid and self-limiting, but aberrant resolution and prolonged inflammation cause various chronic disorders (Pan et al., 2010).

The immune system can be modified by diet, pharmacologic agents, environmental pollutants and naturally occurring food chemicals. Certain members of flavonoids significantly affect the function of the immune system and inflammatory cells. A number of flavonoids such as hesperidin, apigenin, luteolin, and quercetinare reported to possess anti-inflammatory and analgesic effects. Flavonoids may affect specifically the function of enzyme systems critically involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinase. The inhibition of kinases is due to the competitive binding of flavonoids with ATP at catalytic sites on the enzymes. These enzymes are involved in signal transduction and cell activation processes involving cells of the immune system. It has been reported that flavonoids are able to inhibit expression of isoforms of inducible nitric oxide synthase, cyclooxygenase, and lipooxygenase, which are responsible for the production of great amount of nitric oxide, prostanoids, leukotrienes, and other mediators of the inflammatory process such as cytokines, chemokines, or adhesion molecules. Flavonoids also inhibit phosphodiesterases involved in cell activation. Much of the anti-inflammatory effect of flavonoid is on the biosynthesis of protein cytokines that mediate adhesion of circulating leukocytes to sites of
injury. Certain flavonoids are potent inhibitors of the production of prostaglandins, a group of powerful proinflammatory signaling molecules (Manthey, 2000).

Reversal of the carrageenan induced inflammatory changes has been observed with silymarin treatment. It has been found that quercetin inhibit mitogen stimulated immune globulin secretion of IgG, IgM, and IgAlsotypesinvitro. Several flavonoids are reported to inhibit platelet adhesion, aggregation, and secretion significantly at 1-10 mM concentration. The effect of flavonoid on platelets has been related to the inhibition of arachidonic acid metabolism by carbon monoxide. Alternatively certain flavonoids are potent inhibitors of cyclic AMP phosphodiesterase, and this may in part explain their ability to inhibit platelet function (Cumella et al., 1987).

### 5.4.5. Anticancer Activity

Dietary factors play an important role in the prevention of cancers' Fruits and vegetables having flavonoids hive been reported as cancer chemo preventive agents. Consumption of onions and/ or apples, two major sources of the flavonolquercetin, is inversely associated with the incidence of cancer of the prostate, lung, stomach, and breast. In addition, moderate wine drinkers also seems to have a lower risk to develop cancer of the lung, endometrium, esophagus, stomach and colon. The critical relationship of fruit and vegetable intake and cancer prevention been thoroughly documented. It has been suggested that major public health benefits could be achieved by substantially increasing consumption of the foods (Mishra et al., 2013).

Several mechanisms have been proposed for the effect of flavonoids on the initiation and promotion stages of the carcinogenicity including influences on development and hormonal activities. Major molecular mechanism of action of flavonoids are given as follows: (1) downregulation of mutant p53 protein (2) cell cycle arrest, (3) tyrosine kinase inhibition, (4) inhibition of heat shock proteins, (5) estrogen receptor binding capacity, (6) inhibition of expression of Rase proteins (Duthie et al., 2000).

Recently it has been shown that the flavanol epigallocatechin-3-gallate inhibited fatty acid synthase (FAS) activity and lipogenesis in prostate cancer cells, an effect that is strongly associated with growth arrest and cell death. In contrast to most normal tissues expression of
FAS is markedly increased in various human cancers. Upregulation of FAS occurs early in tumor development and is further enhanced in more advanced tumors (Koen et al., 2005).

Quercetin is known to produce cell cycle arrest in proliferating lymphoid cells. In addition to its antineoplastic activity, quercetin exerted growth-inhibitory effects on several malignant tumor cell lines in vitro. These included P-388 leukemia cells, gastric cancer cells (HGC-27, NUGC-2, NKN-7 and MKN-28), colon cancer cells (COLON 320 DM), human cells breast cancer cells, human squamous and gliosarcoma cells, and ovarian cancer cells. Markaverich et al. (1998) that tumor cells growth inhibition by quercetin may be due to its interaction with nuclear type II estrogen binding sites (EBS). It has been experimentally proved that increased signal transduction in human breast cancer cells is markedly reduced by quercetin acting as an anti-proliferative agent.

5.46. Antiviral Activity

Natural compounds are an important source for the discovery and the development of drugs because of their availability and expected low side effects. Naturally occurring flavonoids with antiviral activity have been recognized since the 1940s and many reports on the antiviral activity of various flavonoids are available. Search of effective drug against human immunodeficiency virus (HIV) is the need of hour. Most of the related with antiviral compounds revolves around inhibition of various enzymes associated with the life cycle of viruses. Structure function relationship between flavonoids and their enzyme inhibitory activity has been observed. Gerdin and Srensso demonstrated that flavan-3-ol was more effective than flavones and flavonones in selective inhibition of HIV-1, HIV-2, and similar immunodeficiency virus infections. Baicalin, a flavonoid isolated from Scutellarina baicalensis (Lamieaceae), inhibits HIV-1 and replication. Baicalein and other flavonoids such as robustaflavone and hinokiflavone have also been shown to inhibit HIV-1 reverse transcriptase. Another study revealed inhibition of HIV-1 entry into cells expressing CD4 and chemokine coreceptors and antagonism of HIV-1 reverse transcriptase by the flavone O-glycoside. Catechins are also known to inhibit DNA polymerases of HIV-1. Flavonoid such as demethylatedgardenin A and robinetin are known to inhibit HIV-1 proteinase. It has also been reported that the flavonoids chrysin, acacetin, and apigenin prevent HIV-1 activation via a novel mechanism that probably involves inhibition of viral transcription (Critchfield et al., 1996).
6.0. CONCLUSION

Prevention and cure of diseases using phytochemicals especially flavonoids are well known. Fruits and vegetables are natural sources of flavonoids. Variety of flavonoids found in the nature possesses their own physical, chemical, and physiological properties. Structure function relationship of flavonoids is epitome of major biological activities. Medicinal efficacy of many flavonoids as antibacterial, hepatoprotective, anti-inflammatory, anticancer and antiviral agents is well established. These substances are more commonly used in the developing countries. Therapeutic use of new compounds must be validated using specific biochemical tests. With the use of genetic modifications, it is now possible to produce flavonoids at large scale. Further achievements will provide newer insights and will certainly lead to a new era of flavonoid based pharmaceutical agents for the treatment of many infectious and degenerative diseases.

7.0. REFERENCES


4. STEROIDS

Mridul Umesh and Thazeem Basheer

1.0. INTRODUCTION

Plants and animals contain a lipid fraction that comprises of important group of biological regulators called as steroids. Steroids are tetracyclic natural products related to terpenes in structure and biosynthetic pathway. Steroid is a diverse class that includes dietary lipid cholesterol, sex hormones like estradiol and testosterone anti-inflammatory drugs like dexamethanasone. Hundreds of steroids are found in plants, animals and fungi. Synthesis of steroids irrespective of the type occurs from lanosterol in animals and fungi and cycloartenol in plants. Both these steroid precursors are derived from cyclization of the triterpene squalene. The principle biological functions of steroids basically include synthesis of cell membrane components which helps in altering cell fluidity and many play important role as signalling molecules.

2.0. STRUCTURE

Steroid means sterol-like. Sterols (Greek, stereos is solid, plus “ol”, the generic ending for alcohols) are solid alcohols having 27-29 carbons. The larger designation steroid covers those compounds containing the parent nucleus consisting of the A, B, C and D rings. Core structure of steroid is composed of seventeen carbon atoms, which forms four fused ring like structures and one five member cyclopentane ring. Chemically, these hydrocarbons are cyclopentanoperhydrophenanthrenes. Steroids vary based on the functional groups attached to the four ring core and by the oxidation of the rings. Steroids have same absolute configuration as cholesterol. The stereochemical designations of steroids are based on the angular methyl group placed above the plane of the steroid structure. The steroid nucleus is almost planar and is relatively rigid. The
Steroids are the compounds containing a cyclic steroid nucleus (or ring) namely cyclopentanoperhydrophenanthrene (CPPP). It consists of a phenanthrene nucleus (rings A, B and C) to which a cyclopentane ring (D) is attached. The main atomic sites of substitution in the steroid ring system are carbon-3 of ring A, carbon 11 of ring C and carbon 17 of ring D (figure 2.2). The fused rings do not allow rotation about C-C bonds. The steroid nucleus represents saturated carbons, unless specifically shown as double bonds. The methyl side chains attached to carbons 10 and 13 are shown as single bonds. At carbon 17, steroids usually contain a side chain. There are several steroids in the biological system. These include cholesterol, bile acids, vitamin D, sex hormones, adrenocortical hormones, sitosterols, cardiac glycosides and alkaloids. If the steroid contains one or more hydroxyl groups it is commonly known as sterol.

Fig 1: Structure of steroid
3.0. PHYSICAL AND CHEMICAL PROPERTIES OF STEROIDS

All the steroids give among other products, Diel’s hydrocarbon on dehydrogenation with selenium at 360°C. When the distillation with selenium is carried out at 420°C, steroids give mainly chrysene and a small amount of Picene. Sterols occur in animal and plant oils and fats, They are crystalline compound and contain an alcoholic group; they occur free or as esters of the higher fatty acids, and are isolated from the unsaponifiable portion of oils and fats. A large and important class of terpene-based lipids is the steroids. This molecular family, whose members affect an amazing array of cellular functions, is based on a common structural motif of three six-member rings and one five-member ring all fused together. Cholesterol is the most common steroid in animals and the precursor for all other animal steroids. The numbering system for cholesterol applies to all such molecules. Many steroids contain methyl groups at positions 10 and 13 and an 8- to 10- carbon alkyl side chain at position 17. The polyprenyl nature of this compound is particularly evident in the side chain. Many steroids contain oxygen at C-3, either a hydroxyl group in sterols or a carbonyl group in other steroids. It is also noted that the carbons at positions 10 and 13 and the alkyl group at position 17 are nearly always oriented on the same side of the steroid nucleus, the β - orientation. Alkyl groups that extend from the other side of the steroid backbone are α - orientation. Steroids derived from cholesterol in animal include five families of hormones (the androgens, estrogens, progestins, glucocorticoids and mineral corticoids) and bile acids. Androgens such as testosterone and estrogens such as estuarial, mediate the development of sexual characteristics and sexual function in animals. The progestins, such as progesterone, participate in the control of the menstrual cycle and pregnancy. Glucocorticoids (cortical, for example) participate in the control of carbohydrate, protein, and lipid metabolism, whereas the mineral corticoids regulate salt (Na, K, and Cl) balances in tissues. The bile acid (including cholic and dcoxycholic acid) are detergent molecules secreted in bile from the gallbladder that assist in the adsorption of dietary lipids in the intestine.
4.0. SOURCES OF STEROIDS

Sterols are hydroxylated derivatives of the perhydro-1, 2-cyclopentanophenanthrene nucleus and possess a hydroxyl group at C3, a side chain at C17 and a double bond, mostly at C5. They occur in plants, animals, and microorganisms, the best known animal sterol is cholesterol. Cholesterol is synthesized by the cell from simple molecules and serves as a precursor for the biosynthesis of bile acids, sex hormones, adrenocorticoids, and vitamin D. plants do not have cholesterol; instead of cholesterol they have a variety of closely related sterols, the phytosterols. Best known phytosterols are stigmasterol, from soy bean oil; β-sitosterol, from wheat germ; and ergosterol from yeast.

**Fig 2: Sources of steroids**

- **Zoosterols**: The steroids that are obtained from animal sources are often referred to as zoosterols.
- **Phytosterols**: The steroids that are obtained from plant sources as the phytosterols.
• **Mycosterols**: The steroids that are obtained from yeast and fungi are referred to as the mycosterols.

### 5.0. BIOSYNTHESIS OF STEROIDS

Steroid biosynthesis is an anabolic pathway which produces steroids from simple precursors. A unique biosynthetic pathway is followed in animals (compared to many other organisms). In humans and other animals the biosynthesis of steroids follows the mevalonate pathway, which uses acetyl-CoA as building blocks for dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). In subsequent steps DMAPP and IPP join to form geranyl pyrophosphate (GPP), which synthesizes the steroid lanosterol. Modifications of lanosterol into other steroids are classified as steroidogenesis transformations. Although steroids are not triterpenes since these possess C19-C29 skeletons, rather than a C30 skeleton found in the triterpenes but all steroids are derived from the same 30 carbon containing precursor, the squalene 8 from which the triterpenes are derived. The C30 skeleton of squalene 8 is formed by the union of two *trans*-farnesyl units joined in head to head fashion. It was first isolated from shark liver oil but latter on found to be present in almost all living organisms. The squalene 8 to steroid pathway was mainly explored by Bloch, Lynen, Cornforth and Popjak. The sequence of transformation of squalene 8 into steroid nucleus proceeds *via* acid catalyzed ring opening of monoepoxide derivative of squalene 8 and involves formation of a series of carbocationic intermediates. The steric structures of steroids can be rationalized from the possible folding (pseudochair or boat) of polyprenyl chain on the enzyme surface. In animals, squalene 8 is first converted into lanosterol 9 which is further transformed into cholesterol 11. From cholesterol 11, steroidal hormones are synthesized by various pathways. In plants on the other hand, squalene 8 is first converted into cycloartenol 10 from which different phytosterols and other plant steroids are obtained. In fungi and other lower organisms, squalene 8 appears to be first converted into lanosterol 9 but is then converted into ergosterol 12 that is also found in plants.
5.1. Mevalonate pathway

- Also called as HMG-CoA reductase pathway.
- Acetyl-CoA acts as precursor for the Mevalonate pathway.
- Dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) are the important end products.
- DMAPP and IPP donate isoprene units, which are assembled and modified to form terpenes and isoprenoids.
- The isoprene units are joined to make squalene and folded into a set of rings to make lanosterol.
- Lanosterol can then be converted into other steroids, such as cholesterol and ergosterol

5.2. Steroidogenesis

- Biological process of steroid formation from cholesterol.
- Major steroid hormones include progestogens, corticosteroids, androgens and estrogens.
6.0. BASIS OF STEROID CLASSIFICATION

Steroid can be grouped according to a number of criteria based on their chemical structure (side chain attached to the ring D), site of production (ovarian or adrenal steroids), biological function (a glucocorticoid or sex steroids) and molecular actions (an estrogen-receptor agonist), or on the basis of biochemical effects. On the basis of type of organism in which they are present, the steroids are classified into three broad categories.

1. Insect steroids
2. Vertebrate steroids
3. Plant steroids

6.1. Insect steroids or Ecdysteroids

- Ecdysteroids or the insect steroids are polyhydroxy steroids with cis-AB-ring junction.
These steroids are actually produced by certain plants (e.g., *Cyanotis vaga*) and are taken up by the insect during feeding on them; the 20-hydroxyecdysone (ecdysterone or 20-E) 13, is a naturally occurring ecdysteroid hormone.

Ecdysteroids have been found to play a key role in cell proliferation, growth and apoptosis by controlling gene expression involved in ecdysis and metamorphosis in the insects.

Ecdysone is one of the most common molting hormones in insects, crabs, and some worms that can disrupt their molting and reproduction.

These steroids have potential use in promoting muscle growth and fat loss, and have the advantage to lower frequency of side-effects usually associated with anabolic steroids. However their continuous use may cause increased testosterone and dihydrotestosterone (DHT) production in males, and androgenic changes in females such as increased growth of facial and body hairs and deepening of the voice, as well as gastrointestinal problems for both sexes, such as nausea, bloating, and diarrhea. Certain evidences are there which advocate their use as potential immunomodulator.

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**Fig 5: Ecdysteroid structure**
6.2. Vertebrate steroids
- Vertebrate steroids include steroid hormones, cholesterol and the neurosteroids.

6.2.1. Steroid hormones:
- The hormones are the chemical messengers responsible for specific biological functions.
- Depending upon the function performed and the site of action, the steroid hormones are categorized as: sex steroids, corticosteroids, anabolic steroids and vitamin D.
- They are involved in the metabolism of carbohydrates, proteins and fats; help to maintain homeostasis, immune system, maintain blood volume and also control renal excretion of electrolytes. Because of their anti-inflammatory activity (e.g., C60 glucocorticoid), they are effective remedy against arthritis, dermatitis and most importantly they are being used and have been found potent against autoimmune diseases.
- They act as post-transplantory immunosuppressants to prevent acute transplant rejection and graft versus host disease, in addition, they are effective against cancer and heart ailment (e.g., digitoxigenin).
- The gonadal hormones (estrogens, androgens and progesterones) interact with vertebrate androgen or estrogen receptors and play important roles in inducing the body changes known as primary and secondary sex characteristics.
- The tin containing steroid derivatives such as triphenyltincholestroyl ether 22 have been prepared and evaluated for use as insecticide, bactericide and fungicide.
6.2.2. Corticosteroids

- These are produced in the adrenal cortex and are involved in a wide range of physiologic systems such as stress response, immune response, regulation of inflammation, carbohydrate metabolism, protein catabolism, behavior and blood electrolyte levels mainly by promoting sodium retention in the kidney. Examples of corticosteroids include cortisol (a glucocorticoid) and aldosterone (a mineralocorticoid).

- Synthetic corticosteroids are used in a variety of conditions, ranging from brain tumors to skin diseases. These are used in the treatment of bronchopulmonary dysplasia (BPD) in infants, arthritis, temporal arthritis, dermatitis, allergic reactions, asthma, hepatitis, systemic lupus erythematosus, inflammatory bowel disease (ulcerative colitis and Crohn's disease), sarcoidosis and especially for the treatment of eye infections.

- Steroids used in combination with cyclosporine and azathioprene have been found effective in reduction of incidence of acute organ rejection and have shown a good safety profile in terms of infection and adverse effects in renal, liver, heart and pancreas transplant.
6.2.3. **Anabolic steroids:**

- Long term or excessive use of corticosteroids has been found to be associated with various health risks such as: hyperglycemia insulin resistance, diabetes mellitus, osteoporosis, anxiety, depression, gastritis, colitis, hypertension, ictus, hypogonadism, hypothyroidism, amenorrhoea and / or retinopathy.

- These are also known as anabolic-androgenic steroids (AAS) and are a class of steroid hormones related to testosterone. They increase protein synthesis within cells, which results in the buildup of cellular tissue (anabolism), especially in muscles.

- Anabolic steroids also have androgenic and virilizing properties, including the development and maintenance of masculine characteristics such as the growth of the vocal cords and body hair.

- These are being used therapeutically in medicine to stimulate bone growth, appetite, induce male puberty and treat chronic wasting conditions, such as cancer and AIDS.

- Anabolic steroids are also responsible for increased muscle mass and physical strength however; serious health risks can be produced by long-term use or excessive doses of anabolic steroids.
Longer steroid cycles are associated with more serious health effects which may include harmful changes in cholesterol levels, acne, high blood pressure, liver damage, dangerous changes in the structure of the left ventricle of the heart, fluid retention, weight gain, hirsutism, voice change and / or hair loss.

6.2.4. Sterols

These are the steroid alcohols and majority of them appear to be very long lived and are thus not metabolized. Sterols are essential nutrients for the eukaryotes since these plays a vital role in structural integrity of membrane structure of most organisms and control the permeability of ions through the membrane.

The cholesterol, a sterol, is found in the cell membranes of all tissues and it is transported in the blood plasma of all animals.

It is more abundant in the liver, spinal cord and brain and plays a central role in many biochemical processes, such as the composition of cell membranes and the synthesis of steroid hormones.

It is involved in the maintenance of biological membranes, formation of bile and acts as a precursor for different steroids and most importantly it is involved in metabolism of fat soluble vitamins. The cholesterol and its derivatives are being traced for their antioxidant properties and cell signaling.

Some examples of diseases treated with naturally occurring cholesterol derivatives are allergic reactions, arthritis, some malignancies and diseases resulting from deficiencies or abnormal production of hormones.
6.2.5. Neurosteroids:

- Neuroactive steroids (aka neurosteroids) are steroids synthesized in peripheral nervous system (PNS). This class of steroids is useful for treatment of various forms of peripheral neuropathy (e.g., aging, chemotherapy, diabetes, physical injury, etc).

- The neurosteroids alter neuronal excitability through interaction with neurotransmitter gated ion channels. These compounds can act as allosteric modulators of neurotransmitter receptors.

- The neurosteroids plays an important role in the development of nervous system and myelination, inhibition of neuronal toxicity, ischemia and have potential to be an effective therapy for Niemann Pick-type C disease (human childhood fatal neurodegenerative disease) and other lysosomal storage diseases.

- The neuroactive steroids are able to reduce aging associated morphological abnormalities of myelin and aging associated myelin fiber loss in sciatic nerves as well.

6.2.6. Vitamin D and seco-steroids:

- Vitamin D is classified as a seco-steroid in which the 9,10-C/C bond of ring B is broken. Several forms of vitamin D exists that include vitamin-D1, D2, D3, D4 and D5. The two major forms are vitamin-D2 (ergocalciferol) 40 and vitamin-D3 (cholecalciferol) 41.

- The vitamin D plays an important role in the maintenance of physiological system, e.g., regulates the Ca and P levels in the blood, which is necessary for healthy skeleton system and promotes immunosupression. These are currently being used for the treatment of cancer, tumor, hearing disorder, psoriasis, rickets, asthma, allergy, epilepsy, fancony syndrome, osteomalacia and osteoporosis. Physalins,
a group of vitamin D isolated from *Physalis angulata* have been found to have *in vitro* anti-inflammatory action.

![Vitamin D](image)

**Fig 10: Vitamin D**

### 6.3. Phytosterols:

- These are a group of steroid alcohol that occurs naturally in plants. They are white powders with mild, characteristic odor, insoluble in water and soluble in alcohols. They have many applications as food additives and in medicine and cosmetics.

- Phytosterols have cholesterol-lowering properties and can reduce cholesterol level in human subjects up to 15% and may act in cancer prevention and these are widely marketed as a dietary supplement.

### 6.4. Brassinosteroids:

- Brassinosteroids (BRs), such as brassinolide 49, have been shown to elicit a broad spectrum of responses including the promotion of cell elongation and cell division, inhibition of de-etiolation in the dark, repression of light-regulated genes in the dark and repression of stress-regulated genes.
In view of their structural similarities with animal steroids, it has been proposed that BRs might interact with a soluble receptor in order to regulate the expression of specific genes. The BRs tend to counter biotic as well as abiotic stress in plants.

Application of BRs to cucumbers results in increased metabolism and removal of pesticides; this property of BRs could be beneficial for reducing the human ingestion of residual pesticides from non-organically grown vegetables.

Fig 11: Brassinosteroids

6.4. Miscellaneous steroids:

Androstane derivatives have been screened for the treatment and prevention of allergy and its derivative are effective against bone loss, bone fracture, osteoporosis, metastatic bone disease, Paget’s disease, periodontal disease, cartilage degeneration, endometriosis, uterine fibroid disease, cardiovascular disease, cerebral degenerative disorder, retenosis, vascular smooth muscle cell proliferation, obesity, inflammatory bowel disease, hypertension, retinal degeneration and cancer especially of breast, uterus, and prostate.
Antenatal steroids reduce the chances of hyaline membrane disease and intraventricular hemorrhage and chronic lung disease or death in very low birth weight infants.

In addition, steroids exhibit antimitagenic (e.g., stigmasterol), anxiolytic, analgesic, anticonvulsant, sedative, hypnotic and anesthetic properties by enhancing GABA receptor function in non-genomic manner and some steroid derivatives have been synthesized as GABA receptor antagonist for the treatment of CNS abnormalities e.g., stress, anxiety, PMS seizures caused by epilepsy and to prevent muscle tension, depression and to induce anesthesia.

![Stigmasterol](image)

**Fig 12: Stigmasterol**

Cardiac glycosides are steroid based drugs used in the treatment of congestive heart failure and cardiac arrhythmia by increasing the force of contraction of heart muscles. In addition to their role in cardiac health, cardiac glycosides have anticancer properties as well.

Bufagins are also cardiotoxins just like cardenolides but these do have some local anesthetic action and some also have *anti*-cancer properties against leukemia, melanoma and prostate cancer cells. Certain bufodienolides such as cinobufagin isolated from Chusan island toad (*Bufo gargarizans*) are used in low doses in traditional Chinese medicines for treatment of atrial fibrillation.
The steroidal alkaloids represent an important class of alkaloids that contain a perhydro-1,2-cyclopentanophenenthrene nucleus. This class of alkaloids invariably occurs in plant kingdom as glycosidal combination with carbohydrate moieties. The steroidal alkaloids like dihydroplakinamine K from marine sponge Corocium niger have been screened for cytotoxic activity.

Samandarin isolated from the skin glands of fire salamander (Salamandra salamandra) causes muscle convulsions, high blood pressure and hyperventilation in vertebrates.

The bile acids are the steroid acids found predominantly in the bile of mammals. These bile acids are made by the cytochrome P450 mediated oxidation of cholesterol in liver from where these are stored in gallbladder conjugated with sulfates or amino acid glycine. Bile acids and their salts are mainly responsible for the emulsification of fats but in addition to this these also regulate the level of cholesterol in body and regulate the population of bacteria in small intestine and in biliary tract.

### 6.5. BIOLOGICAL SIGNIFICANCE OF SOME STEROLS

#### 6.5.1. Cholesterol

It is an integral component of animal cell membrane and is the reason for membrane fluidity and integrity. Cholesterol serves as the precursor for the biosynthesis of other steroids. It is absent in prokaryotes except mycoplasma. François Poulletier de la Salle first identified cholesterol in solid form in gallstones in 1769. However, it was not until 1815 that chemist Michel Eugène Chevreul named the compound “cholesterine”. Plants make cholesterol in very small amounts. Plants manufacture phytosterols (substances chemically similar to cholesterol produced within plants), which can compete with cholesterol for reabsorption in the intestinal tract, thus potentially reducing cholesterol.
reabsorption. When intestinal lining cells absorb phytosterols, in place of cholesterol, they usually excrete the phytosterol molecules back into the GI tract, an important protective mechanism. Major functions of cholesterol include maintaining of integrity and fluidity of the animal cell membrane. Cholesterol also reduces the permeability of the plasma membrane to neutral solutes, hydrogen ions, and sodium ions, cholesterol also functions in intracellular transport, cell signaling and nerve conduction. Current studies show that cholesterol is also implicated in cell signaling processes, assisting in the formation of lipid rafts in the plasma membrane. Cholesterol & phospholipids, both electrical insulators, in multiple layers, can facilitate speed of transmission of electrical impulses along nerve tissue.

![Fig 13: Cholesterol](image)

6.5.2. Coprastanol

It is formed through biohydrogenation of cholesterol in the gut of animals and birds. Since the molecule has a hydroxyl (-OH) group, it is frequently bound to other lipids including fatty acids.
6.5.3. Ergosterol

It is majorly found in ergot fungi. Ergosterol is a component of yeast and other fungal cell membranes, serving many of the same functions that cholesterol serves in animal cells. It is the major target site for antifungal drugs. Ergosterol is a smaller molecule than lanosterol; it is synthesized by combining two molecules of farnesyl pyrophosphate, a 15-carbon-long terpenoid, into lanosterol, which has 30 carbons. Ergosterol is a biological precursor of vitamin D₂, the chemical name of which is ergocalciferol. Exposure to ultraviolet light causes a photochemical reaction that converts ergosterol to ergocalciferol. This happens naturally to a certain extent, and many mushrooms are irradiated after harvest to increase their Vitamin D content.

6.5.4. Stigmasterols

Stigmasterol is an unsaturated phytosterol occurring in the plant fats or oils of soybean, calabar bean, and rape seed, and in a number of medicinal herbs, including
the Chinese herbs *Ophiopogon japonicus* (Mai men dong), in *Mirabilis jalapa* and American Ginseng. Stigmasterol is also found in various vegetables, legumes, nuts, seeds, and unpasteurized milk. Pasteurization will inactivate stigmasterol. Edible oils contains higher amount than vegetables. Phytosterols normally are broken down in the bile.

![Fig 15: Stigmasterol](image)

Stigmasterol is used as a precursor in the manufacture of semisynthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D₃.

### 7.0. CONCLUSION

Steroids are a class of potent secondary metabolites abundantly distributed in plants, animals and microbes. This chapter encompasses the mile stones regarding structure, physical, chemical and biological activity of steroids. It further extends a birds view on key application of steroids in various fields of medicine.

### 8.0. REFERENCE

Secondary Metabolites

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5. TERPENOIDS

Thazeem Basheer and Mridul Umesh

1.0. INTRODUCTION

Compounds obtained from turpentine (pine trees extracts – *Balsamum terebinthinae*) are named as terpenes. Turpentine, the so-called "resin of pine trees", is the pleasantly smelling viscous balsam which flows upon cutting or carving the bark. It contains the "resin acids" and some hydrocarbons, which were originally referred to as terpenes. Traditionally, all natural compounds built up from isoprene subunits and for the most part originating from plants are denoted as terpenes. Compounds with molecular formula C$_{10}$H$_{16}$ are referred to as terpenes and terpenes containing oxygen are known as terpenoids. Terpenoids are lipids that have played a central role in growth and development of living systems. They are volatile substances which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus. The functional diversity of chemicals within plants is best demonstrated by terpenoids. More than 30,000 terpenoids have been identified. The terpenes havea simple unifying feature by which they are defined and by which they may be easily classified. This generality, referred to as the isoprene rule, was postulated by Otto Wallach in 1887. This rule describes all terpenes as having fundamental repeating5-carbon isoprene units. Thus, terpenes are defined as a unique group of hydrocarbon-based natural products that possess a structure that may be hypothetically derived from isoprene, giving rise to structures that may be divided into isopentane (2-methylbutane) units.

By the modern definition: “Terpenoids are the hydrocarbons of plant origin of the general formula (C$_5$H$_8$)$_n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives.” The biological and eco-chemical functions of terpenes have not yet been fully investigated. Many plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to
expel certain animals using these plants as food. Lessvolatile but strongly bitter-tasting or toxic terpenes also protect some plants from being eaten by animals (antifeedants). Terpenes also play an important role as signal compounds and growth regulators (phytohormones) of plants. Many insects metabolize terpenes that they have received with their plant food to growth hormones and pheromones. The simpler mono and sesquiterpenes are chief constituent of the essential oils obtained from sap and tissues of certain plant and trees. The di and tri terpenoids are not steam volatile. They are obtained from plant and tree gums and resins. Tertraterpenoids form a separate group of compounds called ‘Carotenoids’.

20,000 naturally occurring terpenoids have been isolated and characterized. Chemical ecology rests heavily on the occurrence of profusely distributed plant terpenoids, hence they play a broad spectrum of highly specific and characteristic roles in the plant kingdom, as: insect propellants, antifeedants, phytoalexins, attractants for pollinates, pheromones, defensive substances against herbivorous animals, allelochemicals, signal molecules, and essentially as plant growth hormones. They engage in a variety of probable interactions, plant and plant, plant and microorganism and plant and animal. The International Union of Pure and Applied Chemistry (IUPAC) recommends a systematic mode of nomenclature of terpenoids as geraniol-3,7-Dimethyl-2,6-octadien-1-ol; Limonene - 1-Methyl – 4 – (1-methylethynyl)- cyclohexene and β-Myrecene-7-Methyl-3-methylene-1, 6-octadiene.

2.0. NATURAL OCCURRENCE

Isoprene is produced and emitted by many species of trees into the atmosphere (major producers are oaks, poplars, eucalyptus, and some legumes). The yearly production of isoprene emissions by vegetation is around 600 Tg. This is about equivalent to methane emission into the atmosphere and accounts for ~1/3 of all hydrocarbons released into the atmosphere. After release, isoprene is converted by free radicals (like the hydroxyl (OH) radical) and to a lesser extent by ozone into various species that mix into water droplets and help creates aerosols and haze.
3.0. INDUSTRIAL PRODUCTION

Isoprene is most readily available industrially as a byproduct of the thermal cracking of naphtha or oil, as a side product in the production of ethylene. About 800,000 tonnes are produced annually. About 95% of isoprene production is used to produce cis-1,4-polyisoprene—a synthetic version of natural rubber.

4.0. STRUCTURE

About 30,000 terpenes are known till now. Their basic structure follows a general principle: 2-Methylbutane residues, also referred to as isoprene units, \( (C5)_n \), build up the carbon skeleton of terpenes; this is the isoprene rule. Therefore, terpenes are also denoted as isoprenoids. In nature, terpenes occur predominantly as hydrocarbons, alcohols and their glycosides, ethers, aldehydes, ketones, carboxylic acids and esters.

4.1. Isoprene rule: Thermal decomposition of terpenoids give isoprene as one of the product. Otto Wallach pointed out that terpenoids can be built up of isoprene unit. Isoprene rule states that the terpenoid molecules are constructed from two or more isoprene unit. Further Ingold suggested that isoprene units are joined in the terpenoid via ‘head to tail’ fashion.

5.0. GENERAL PROPERTIES OF TERPENOIDS

5.1. Most of the terpenoids are colourless, fragrant liquids which are lighter than water and volatile with steam. A few of them are solids e.g. camphor. All are soluble in organic solvent and usually insoluble in water. Most of them are optically active.

5.2. They are open chain or cyclic unsaturated compounds having one or more double bonds. Consequently they undergo addition reaction with hydrogen, halogen, acids, etc. A number of addition products have antiseptic properties.

5.3. They undergo polymerization and dehydrogenation

5.4. They are easily oxidized nearly by all the oxidizing agents. On thermal decomposition, most of the terpenoids yield isoprene as one of the product.
6.0. BIOSYNTHESIS OF TERPENOIDS

Two different biosynthetic pathways produce the main terpene building block, isopentenyldiphosphate (IPP). The first classical biosynthetic route is known as the MVA (mevalonic acid) pathway. This takes place in the cytosol. The actual 5-carbon building blocks in vivo are the interconvertible isomers isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These two building blocks are condensed together in a sequential fashion by the action of enzymes called prenyltransferases. The products include geranyl, farnesyl, and geranylgeranyl pyrophosphate, squalene and phytoene, which are the direct precursors of the major families of terpenes.

The key intermediate in the process was mevalonic acid (MVA), a 6-carbon compound. MVA is formed by the enzymatic reduction of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), which in turn is formed by the head-to-tail condensation of three molecules of acetate. MVA is enzymatically converted to IPP with the loss of carbon dioxide, and subsequently IPP and DMAPP are incorporated directly into cholesterol.

The second biosynthesis route to terpenes is referred to as either the MEP (methylerythritol-4-phosphate) or DOX (1-deoxy-D-xylulose) pathway. When first discovered, this new plastid-bound pathway was distinct biochemically and was identical to that found in bacteria and probably is a legacy of prokaryotic endosymbiotic ancestors. In this case, IPP is derived, not from MVA, but from 1-deoxyxylulose 5-phosphate (1-DXP), formed from the glycolytic intermediates glyceraldehyde 3-phosphate and pyruvate.

The key step in the biosynthesis is the skeletal rearrangement and reduction of 1-DXP to form 2C-methylerythritol4-phosphate (MEP) using the biological reducing agent NADPH as cofactor. MEP is converted to IPP via a chemical sequence involving the removal of three molecules of water.

Thus, in higher plants, there are two pathways for generating terpenes. Here, IPP is formed in the chloroplast, mainly for the synthesis of more volatile mono- and diterpenes. The evidence indicates that there may be sharing of intermediates across these pathways, a sort of
biosynthetic crosstalk. In plants, the MEP pathway leads to monoterpenes, diterpenes, the prenyl side chain of chlorophylls and carotenoids as well as to the phytohormones such as abscisic acid, gibberellins and trans-cytokinins.

Fig 1: Biosynthesis of Terpenoids

7.0. CLASSIFICATION OF TERPENOIDS

Terpenoids are classified on the basis of the number of isoprene units incorporated into a specific unsaturated hydrocarbon terpenoid molecule, such as: Monoterpenoids: made up of two isoprene units formula; Sesquiterpenoids: composed of three isoprene units; Diterpenoids: composed of four isoprene units; Triterpenoids: composed of six isoprene units and Tetraterpenoids: composed of eight isoprene units.

Table 1: Classification of Terpenoids

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. of carbon atoms</th>
<th>Value of n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoterpenoids(C_{10}H_{16})</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Sesquiterpenoids(C_{15}H_{24})</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Diterpenoids(C_{20}H_{32})</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>
Each class can be further subdivided into subclasses according to the number of rings present in the structure.

i) Acyclic Terpenoids: They contain open structure.
ii) Monocyclic Terpenoids: They contain one ring in the structure.
iii) Bicyclic Terpenoids: They contain two rings in the structure.
iv) Tricyclic Terpenoids: They contain three rings in the structure.
v) Tetracyclic Terpenoids: They contain four rings in the structure.

7.1. Monoterpenoids

Monoterpenoids derivatives occur naturally in the purest optically active form. A few typical examples of monoterpenoids found in naturally occurring plant species are camphor, eucalyptol, menthol and thymol. Most commonly occurring structural variations are – acyclic (myrene), monocyclic (p-menthane) and bicyclic (α-pinene).

7.1.1. Camphor: It is a bicyclic terpenoid ketone. It occurs in all parts of the camphor tree, Cinnamomum camphora, belonging to family Lauraceae. It is used as a topical antipyretic in concentrations ranging between 0.1% to 0.3%, counterirritant (11%) for fibrositis and neuralgia, antiseptic, antifungal and carminative agent, reduces cough, acts as stimulant, rubefacient, antispasmodic and an analgesic, stimulates nerve endings in the skin and causes substantial relief of pain due to the masking of deeper visceral pain with a milder pain arising from the skin at the same level of innervations.

![Fig 2: Structure of camphor](image)
7.1.2. **Eucalyptol**: It is obtained from leaves of *Eucalyptus globus Labill*, family *Myrtaceae*. It is an epoxy or oxido derivative of p-methane, known as 1,8-epoxy-p-methane or 1,8-oxido-p-menthane. Eucalyptol is used internally as a stimulating expectorant to relieve severe cough and in bronchitis in the form of inhalations and externally as a mild anaesthetic and antiseptic for treatment of inflammatory conditions. It is used as decongestant nasal drops. They are profusely used in room sprays, lotions and all types of cosmetic preparations, and as flavouring agent in pharmaceutical preparations – mouth washes and gargles.

![Fig 3: Structure of eucalyptol](image)

7.1.3. **Menthol**: It is found in the peppermint oil obtained from the fresh flowering tops of the plants commonly known as *Mentha piperta* Linn., or other allied species of *Mentha*, family *Labiatae*. They are used in various types of mouth washes, toothpastes and similar oral formulations, and as flavouring agent for chewing gums, candies, throat lozenges and mentholated cigarettes. It is used on mucous membranes or on skin to serve as a counterirritant, antiseptic and as a mild stimulant (1 to 6%). They are employed in conjunction with other allied substances-camphor, eucalyptus oil in various pharmaceutical preparations such as expectorants, nasal sprays, and inhalants to get immediate relief from symptoms of nasal congestion, sinusitis and bronchitis. At a lower concentration (0.1 to 1%) when applied on skin, it helps in dilatation of blood vessels affording a cold feeling followed usually by a depression of the sensory cutaneous receptors.

![Fig 4: Structure of menthol](image)
7.1.4. Thymol: It is obtained from the essential oil of *Thymus vulgaris* L., *Monarda punctat* L., and *Monarda didyma* L., belonging to family *Labiatae*. It is also derived from *Carum capticum* of family *Umbelli ferae*; several species of *Ocium*, for instance: *Ocium gratissimum* L. belonging to family *Labiate*, available in various forms such as horsemint oil, oswego tea oil, ajowan oil and tulsi oil. It is used as an antifungal and antibacterial agent; vital component in several analgesic and topical ointments and used in preparations of mouthwashes, gargles, oral preparations and as a local anaesthetic in toothache.

![Structure of thymol](image)

**Fig 4: Structure of thymol**

7.2. Sesquiterpenoids:

Sesquiterpenoids are further classified into four major categories namely acyclic, monocyclic, bicyclic and tricyclic sesquiterpenoids. Few examples of sesquiterpenoids are β-cadinene, β caryophyllene and abscisic acid that are used for scenting soaps and as an antitoothache, antiseptic, as a spice, as a stimulant, aromatic carminative, perfumery and as a plant growth and development hormone. Examples of acyclic sesquiterpenoids are α-Farnesene and β-Farnesene that are used as perfume in toiletaries; monocyclic sesquiterpenoids (zingiberene) that act as stomachic carminative and bicyclic sesquiterpenoids (naphthalene derivatives) used as scenting soaps, (azulene derivatives) used as guaiazulene and have anti-inflammatory role; and tricyclic sesquiterpenoids (α-santalene) that act as scenting soaps.

**Sesquiterpenoid Lactones** – are another class of compounds essentially bearing few characteristic features chemically distinct from the sesquiterpenoids. The specific and vital biological nucleophiles – thiol and amino moieties present in the enzymes help in the augmentation of faster and reactive approach to receptor sites by these sesquiterpenoid lactones.
Their overall effect is evidenced by marked and pronounced biological activities, for instance: modified antimicrobial activity, enhanced antitumour properties.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Class</th>
<th>Name</th>
<th>Biological Source</th>
<th>Geographical Source</th>
<th>Special Features</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Germacranolides</td>
<td>Germacranolide</td>
<td>Leaf of Labrador Tea</td>
<td>Europe,</td>
<td>Has a ten membered carbon skeleton ring</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Geranium macrorrhizum (Geraniaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Eudesmanolides</td>
<td>Eudesmanolide</td>
<td>Magnolia obvata (Magnoliaceae)</td>
<td>Europe, North America</td>
<td>Has two fused six membered carbon skeleton ring</td>
<td>Neurotrophic activity</td>
</tr>
<tr>
<td>3.</td>
<td>Guaianolides</td>
<td>Guaianolide</td>
<td>Guaiacum officinale Linn., (Zygophyllaceae)</td>
<td>Tropical America</td>
<td>Has a five membered ring fused to a seven membered ring</td>
<td>Antioxidant</td>
</tr>
</tbody>
</table>

Fig 5: Examples of Sesquiterpenoid Lactones and their significance

Natural products that have sesquiterpenoid lactones functions are -

7.2.1. *Artemisinin*: It is obtained from the leaves and the closed, unexpanded flower heads of *Artemisia annuana* Linn., belonging to family *Asteraceae*. It is used in the Chinese system of medicine for the treatment of malaria since more than one thousand years. The drug and its derivatives are used as fast acting blood schizontocides in the control and management of malarial fever caused by *Plasmodium vivax* strain. It is active against both chloroquine resistant and chloroquine sensitive strains of *Plasmodium falciparum*. It shows extremely encouraging therapeutic effects specifically in the treatment of cerebral malaria by virtue of their significant rapid clearance of the prevailing parasites.
7.2.2. Parthenolide: It is obtained from the leaves of *Tanacetum parthenium*, belonging to family *Asteraceae*. It is commonly known as feverfew and has been employed for centuries as an effective febrifuge (antipyretic). It is found to act as a serotonin antagonist, thereby causing an inhibition of the release of serotonin from blood platelets. It is effective in the prophylaxis of migraine.

7.2.3. Matricarin: It is obtained from dried flower heads of *Matricaria chamomilla* L., and *Artemisia tulesii* Ledeb, belonging to family *Compositae*. Chamomile is a most popular herbal tea in the United States because of its anti-inflammatory and antiplasmodic therapeutic properties. Volatile oil of *Matricaria chamomilla* contains the sesquiterpenoid (-)-α- Bisabolol (Bisabolane) which exerts anti-inflammatory activity.
Secondary Metabolites

7.3. Diterpenoids:

Diterpenoids are a broad class of non-volatile C20 compounds that have been essentially obtained from geranyl pyrophosphate.

Sources: Plants and fungi are the main sources and they are invariably formed by certain insects as well as marine organisms. Few examples are -

7.3.1. Colforsin: It is obtained from the root of Coleus forskohlii, Briq., belonging to family Labiatae. It is also known as forskolin, due to the honour bestowed upon the Finnish botanist Forskal. It founds enormous use in the treatment of glaucoma and hypertension and possesses significant therapeutic potential in diseases like congestive cardiomypathy, bronchial asthma.

7.3.2. Taxol: It is obtained from the bark of the Pacific Yew tree, Taxus brevifolia Nutt belonging to the family Taxaceae. It is used in the treatment and management of metastatic carcinoma of
the ovarian glands after the failure of follow up chemotherapy. It is also used in the treatment of breast cancer, usually after the observed failure of combination chemotherapy for metastatic disease.

7.3.3. **Ginkgolide-B**: It is obtained from the root bark and leaves of *Ginkgo biloba* L., belonging to the family *Ginkgoaceae*. The standardized dehydrated acetone-water extract of the dried leaves equivalent to 6% terpenoids and 24% flavones glycosides is sold commercially in Europe as an approved drug to enhance blood fluidity and circulation. Ginkgolide-B(40mg) is sold as a dietary supplement. Ginkgolide-A, B, C and M have been shown to check the platelet activating factor, thereby preventing the bronchoconstriction, hypotension, cutaneous vasodilatation and finally the release of inflammatory compounds.

7.3.4. **Antioxidant property of some diterpenoids:**

Role of oxygen is crucial in aerobic life regarding production of energy, synthesis of several essential biomolecules and so on. However, when oxygen is available in its singlet form it can react with many biological materials. This reaction, called photosensitized oxidation, can cause damage to or death of an organism. Nature has evolved a complex network of endogenous enzymes and antioxidants to control the adverse effects. Some diterpenoids have been reported to participate by acting as primary or synergistic antioxidants (carnosic acid, carnosol and rosamanol). The phenol hydroxyl groups of diterpenoids can trap a radical and the phenol radical thus formed is resonance stabilized. The food preservative BHT is a synthetic phenol. A naturally
occurring preservative found in vegetable oils especially wheat germ oil is α-tocopherol (vitamin E). Ascorbic acid is also a naturally occurring antioxidant.

**7.4. Triterpenoids:**

It is obtained by biogenesis from six isoprene units. They are found to share commonly the acyclic precursor squalene (C$_{30}$). More than 4000 naturally occurring triterpenoids have been isolated and identified. They are classified into two major groups – tetracyclic and pentacyclic compounds.

**7.4.1. Cucurbitacin-B:**

A group of tetracyclic triterpenoids, usually termed as bitter principles of cucurbits have been found that essentially possess distinct antineoplastic and anti-gibberellin activity. It belongs to *Cucurbitaceae* family. It is used as vermifuges, narcotics, emetics and antimalarials.

![Structure of Cucurbitacin-B](image)

**Fig 10: Structure of Cucurbitacin-B**

**7.4.2. Quassin:** It is one of the bitter constituents of the wood of *Quassia amara* L., belonging to family *Simaroubaceae*. It’s a bitter tonic and is used as insecticides and anthelmintic for the expulsion of threadworms. It is also used as a febrifuge.
7.4.3. **Azadirachitin**: It is a tetranontriterpenoid obtained from the seeds of the Neem tree and the Chinaberry tree. It is a highly active feeding detergent and growth regulator, insect control agent and helps in insect ecdysis and growth inhibition.

7.4.4. **Triterpenoids as anticancer agents:**

A renewed interest in the cytotoxicity activity of triterpenoids has emerged from the reports that betulonic acid induces apoptosis in melanoma tumor cells and in leukemia cells (1995). *In vitro* findings showed that betulic acid is effective against small and non-small cell lung, ovarian, cervical, head and neck carcinomas (2005). Uncarinic acid A and the isomeric acid B, from *Uncaria rhynchphylla*, are under study as potential anticancer agents.

7.5. **Tetraterpenoids and Carotenoids:**

A plethora of natures yellow, orange, red and purple colours are mostly by virtue of the presence of carotenoids. They consist of important group of C_{40} tetraterpenoids. Biogenesis of carotenoids occurs in two specific regions-chloroplasts and chromatophores of bacteria and
fungi. 600 carotenoids have been duly isolated and identified from natural sources like plants, 
bacteria, fungi and marine organisms. Their three major roles are – as photosynthetic pigments, 
photoprotective agents, and as membrane stabilization substances in plants. In animals they serve 
as a precursor of vitamin A and other retinoids. They are cancer preventive agents. This may be 
due to the easy accessibility to various single oxygen atoms and ample free radicals, collectively 
checking the oxidation damage to cells and catering as antioxidants.

7.5.1. Vitamin A:

Vitamin A (retinol) is a classical example of tetraterpenoids. It mostly occurs in animals (not in 
plants) in the form of milk fat and fish liver oil. It gets easily absorbed from the normal intestinal 
tract to the extent of 80-90% and is subsequently stored in body tissues, mostly in the liver. 
Dietary sources are fish liver oils, dairy products-butter cream, whole milk powder, cheese and 
animal organs like liver, kidney and heart. It is useful in proper maintenance of vision, growth 
and tissue differentiation. It is a prophylactic and helps in the synthesis of specific glycoproteins.

![Fig 13: Structure of retinol](image)

8.0. ISOLATION OF MONO AND SESQUITERPENOIDS:

Both mono and sesquiterpenoids have common sources - essential oils. Their isolation is carried 
out in two steps:

i) Isolation of essential oils from plant parts

ii) Separation of Terpenoids from essential oils.

i) Isolation of essential oils from plant parts: The plants having essential oils generally have the 
highest concentration at some particular time. Therefore better yield of essential oil plant 
material have to be collected at this particular time. e.g. from jasmine at sunset. There are four 
methods of extractions of oils.
a) Expression method
b) Water / Steam distillation method
c) Extraction by means of volatile solvents
d) Adsorption in purified fats

Steam distillation is most widely used method. In this method, macerated plant material is steam distilled to get essential oils into the distillate form. These are extracted by using pure organic volatile solvents. If compound decomposes during steam distillation, it may be extracted with petrol at 50°C. After extraction, solvent is removed under reduced pressure.

ii) Separation of Terpenoids from essential oil: A number of terpenoids are present in essential oil obtained from the extraction. Definite physical and chemical methods (for instance, use of nitrosyl chloride and silver nitrate) can be used for the separation of terpenoids. They are separated by fractional distillation. The terpenoid hydrocarbons distill over first followed by the oxygenated derivatives.

More recently different chromatographic techniques have been used both for isolation and separation of terpenoids (for example, column chromatography, gas chromatography, high performance liquid chromatography).

9.0. GENERAL METHODS OF STRUCTURE ELUCIDATION OF TERPENOIDS:

i) Molecular formula: molecular formula is determined by usual quantitative analysis and molecular weight determination methods and by means of mass spectrometry. If terpenoid is optically active, its specific rotation can be measured.

ii) Nature of oxygen atom present: If oxygen is present in terpenoids its functional nature is generally as alcohol, aldehyde, ketone or carboxylic groups.

a) Presence of oxygen atom: presence of –OH group can be determined by the formation of acetates with acetic anhydride and benzoylate with 3,5-dinitrobenzoyl chloride. Primary alcoholic group undergo esterification more readily than secondary and tertiary alcohols.

b) Presence of >C=O group: Terpenoids containing carbonyl function form crystalline addition products like oxime, phenyl hydrazone and bisulphite etc. If carbonyl function is
in the form of aldehyde it gives carboxylic acid on oxidation without loss of any carbon atom whereas the ketone on oxidation yields a mixture of lesser number of carbon atoms.

iii) Unsaturation: The presence of olefinic double bond is confirmed by means of bromine, and number of double bond determination by analysis of the bromide or by quantitative hydrogenation or by titration with monoperphthalic acid. Presence of double bond also confirmed by means of catalytic hydrogenation or addition of halogen acids. Number of moles of HX absorbed by one molecule is equal to number of double bonds present.

iv) Dehydrogenation: On dehydrogenation with sulphur, selenium, polonium or palladium terpenoids converted to aromatic compounds. Examination of these products the skelton structure and position of side chain in the original terpenoids can be determined. For example α-terpenol on Se-dehydrogenation yields p-cymene.

v) Oxidative degradation: Oxidative degradation has been the parallel tool for elucidating the structure of terpenoids. Reagents for degradative oxidation are ozone, acid, neutral or alkaline potassium permanganate, chromic acid, sodium hypobromide, osmium tetroxide, nitric acid, lead tetra acetate and peroxy acids. Since oxidizing agents are selective, depending on a particular group to be oxidized, the oxidizing agent is chosen with the help of structure of degradation products.

vi) Number of the rings present: With the help of general formula of corresponding parent saturated hydrocarbon, number of rings present in that molecule can be determined.

vii) Relation between general formula of compound and type of compounds: For example limonene (mol. formula. C\textsubscript{10}H\textsubscript{16}) absorbs 2 moles of hydrogen to give tetrahydro limonene (mol. Formula C\textsubscript{10}H\textsubscript{20}) corresponding to the general formula. C\textsubscript{n}H\textsubscript{2n}. It means limonoene has monocyclic structure.

viii) Spectroscopic studies: All the spectroscopic methods are very helpful for the confirmation of structure of natural terpenoids and also structure of degradation products. The various methods for elucidating the structure of terpenoids are;

a) UV Spectroscopy: In terpenes containing conjugated dienes or α,β-unsaturated ketones, UV spectroscopy is very useful tool. The values of λ\textsubscript{max} for various types of terpenoids have been calculated by applying Woodward’s empirical rules. There is generally good agreement
between calculation and observed values. Isolated double bonds, \( \alpha,\beta \)-unsaturated esters, acids, lactones also have characteristic maxima.

b) **IR Spectroscopy:** IR spectroscopy is useful in detecting group such as hydroxyl group (\( \sim 3400\text{ cm}^{-1} \)) or an oxo group (saturated 1750-1700\text{ cm}^{-1} ). Isopropyl group, cis and trans also have characteristic absorption peaks in IR region.

c) **NMR Spectroscopy:** This technique is useful to detect and identify double bonds, to determine the nature of end group and also the number of rings present, and also to reveal the orientation of methyl group in the relative position of double bonds.

d) **Mass Spectroscopy:** It is now being widely used as a means of elucidating structure of terpenoids. It is used for determining molecular weight, molecular formula, nature of functional groups present and relative positions of double bonds.

ix) **X-ray analysis:** This is very helpful technique for elucidating structure and stereochemistry of terpenoids.

x) **Synthesis:** Proposed structure is finally confirmed by synthesis. In terpenoid chemistry, many of the synthesis are ambiguous and in such cases analytical evidences are used in conjunction with the synthesis.

**10.0. CONCLUSION:**

Terpenoids perhaps are the most structurally varied class of plant natural products. With recent success in the cloning of genes that encode enzymes of terpenoid synthesis, the transgenic manipulation of plant terpenoid metabolism may present a suitable avenue for achieving a number of goals. The genetic engineering of terpenoid-based insect defenses is particularly appealing, given the array of available monoterpen, sesquiterpene, diterpene, and triterpene compounds that are toxic to insects not adapted to them.

**11.0. REFERENCES:**

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6. QUINONES

Mridul Umesh and Thazeem Basheer

1.0. INTRODUCTION

Naturally occurring quinones have captured attention of humans for thousands of years ago. Pigments of various colours are now characterized as quinones and have been isolated from plants animals and microorganisms. Crude preparation of plants containing plants as active ingredients has been used as an integral part of traditional medicine. Throughout history several medicinal properties were attributed to quinones. The discoveries of anti-cancer and antibiotic properties assigned to quinones made them target of attraction for many scientist to exploit their potential from pharmaceutical point of view.

Quinones are a huge class of compounds gifted with rich and attractive chemistry (Patai et al., 1988). Molecules with the quinoid structure constitute one of the most interesting classes of compounds in organic chemistry. They represent an important class of biological molecules possessing extensive properties, and take part in various bioenergetics processes as important transport agents (Batra et al., 2006). They are the major group of natural pigments, but they play a moderately small role in natural colouring and their major input is in biological redox processes (Thomson et al., 1971; 1997; 1957). Their significance and usefulness is not only limited to metabolic processes, but they also represent a clinically valuable unit for therapeutic agents with various applications (Fattorusso et al., 2005). They also have importance as intermediates in organic synthesis (Patai, 1974) and are among the most abundant substructures found in numerous naturally occurring bioactive molecules (Hu et al., 2009; Moody et al., 2008; Langer et al., 2006; Abe et al., 2006, Manguro et al., 2003; Steglich et al., 2002; Sun et al., 2002; Williams et al., 2002; Hostettmann et al., 2001; Miles et al., 2001; Correia et al., 1994; Thakur et al., 1984). Quinones represent a
large group of natural pigments, exhibit fascinating photochemistry, and function as intermediates in the biosynthesis of important antibiotics like tetracyclines, exhibit a broad spectrum of biological activities with antioxidative, cytotoxic, anticancer, antidiabetic (Zhang et al., 1999) and enzyme inhibitory activities (Bruce et al., 1974; Campbell, 1974; Yoo et al., 1996; Che et al., 1989; Kasai et al., 1996; Puder et al., 2005).

2.0. STRUCTURAL CHEMISTRY OF QUINONES

Quinones can be derived by the oxidation of appropriate phenolic compounds, with 1,2-dihydroxybenzenes and 1,4-dihydroxybenzenes, yielding ortho-quinones and para-quinones respectively. Therefore, quinones can be formed from phenolics compounds by either the acetate or shikimate pathways, affording a catechol or quinol system. A range of quinone derivatives and related structures that contain a terpenoid fragment or shikimate-derived portion are also wide spread. For example, ubiquinones (coenzyme Q) have important biochemical functions in electron transport systems for respiration.

Fig 1: Quinones

Quinones are widely distributed in nature. They are best known as lipid-soluble components of membrane bound electron-transport chains, but, in animal cells, ubiquinone is found not only in the inner mitochondrial membrane but also in endoplasmic reticulum, the Golgi apparatus, lysosomes, peroxisomes and the plasma membrane. This distribution strongly suggests roles other than in respiratory electron transport. Derivatives of quinones are common in biologically active molecules. Some
serve as electron acceptors in electron transport chains such as those in photosynthesis (plastoquinone, phylloquinone), and aerobic respiration (ubiquinone). Phylloquinone is also known as Vitamin K₁, as it is used by animals to help form certain proteins, which are involved in blood coagulation, bone formation, and other processes. Natural or synthetic quinones show a biological or pharmacological activity, and some of them show anti-tumoral activity. They embody some claims in herbal medicine. These applications include purgative (sennosides), antimicrobial and antiparasitic (rhein- and saprorthoquinone, atovaquone), anti-tumor (emodin and juglone), inhibition of PGE2 biosynthesis (arnebinone and arnebifuranone) and anti-cardiovascular disease (tanshinone).

The fundamental feature of quinone chemistry is its ease of reduction and, therefore, its ability to act as an oxidising or dehydrogenating agent. This redox property is driven by the formation of a fully aromatic system. In folk medicine, plants containing naphthoquinones are often employed for the treatment of various diseases, and several quinonoids isolated from traditional medicinal plants are being investigated for their anticancer properties. The redox cycling of quinones may be initiated by either a one- or two-electron reduction. The one electron reduction of quinones is catalysed by NADPH-cytochrome P450 reductase, and yields unstable semiquinones. Quinones transfer electrons to molecular oxygen (O₂), and return to their original quinoidal formation, thus generating a superoxide anion radical (·O₂⁻). Superoxide can be converted to hydrogen peroxide (H₂O₂) via a superoxide dismutase (SOD)-catalysed reaction, followed by the formation of a hydroxyl radical (·HO) by the iron-catalysed reduction of peroxide via the Fenton reaction. Quinones are oxidants and electrophiles, and the relative contribution of these properties to both their toxic and therapeutic activities is influenced by their chemical structure, particularly substituent effects and the characteristics of the quinone nucleus. Two major mechanisms of quinone cytotoxicity have been proposed: stimulation of oxidative stress and alkylation of cellular nucleophiles, which encompass a large range of biomolecules.
The bioactive quinones include lapachol, α-lapachone and β-lapachone originally isolated from the heartwood of trees of the Bignoniaceae family (*Tabebuia* sp.). They can also be found in other families such as Verbenaceae, Proteaceae, Leguminosae, Sapotaceae, Scrophulariaceae, and Malvaceae. β-Lapachone was found to be cytotoxic to a variety of human cancers. This naphthoquinone is now under investigation for the treatment of specific cancers associated with elevated NQO1 levels, such as breast, non-small cell lung, pancreatic, colon, and prostate cancers, and is currently in phase II clinical trials for the treatment of pancreatic cancer. Particularly promising is the synergistic lethality of β-lapachone with taxol and genistein on several tumour cell lines implanted into mice.

### 3.0. BIOSYNTHESIS

Anthraquinones are structurally built from an anthracene ring (tricyclic aromatic) with a keto group each on carbon atom nine and ten. In plants, two main biosynthetic pathways leading to anthraquinones have been described:

1. The shikimate or chorismate/o-succinylbenzoic acid pathway is used to produce anthraquinones with only one hydroxylated ring like 1,2 dihydroxylated anthraquinones
2. The polyketide pathway forming anthraquinones by folding of a polyketide chain with both rings hydroxylated

- **Using Manganese dioxide and sulfuric acid:** The most common benzoquinone was the first synthesized Quinone in the late 1830’s in Liebig’s laboratory as a result of the oxidation of Quinic acid with manganese dioxide and sulfuric acid (Pardasani et al., 2011).
Using sodium perborate on wet Montmorillonite K10: Reaction of Sodium perborate and wet Montmorillonite K10 were mixed thoroughly and then phenol was added and mixed thoroughly using a mortar and pestle (Hashemi et al., 2005).

Using fremy’s salt: Conversion of 2,6 dimethoxy phenol in to 2,6 dimethoxy quinones by using fremy’s Salt , this conversion Required higher reaction time and tedious workup procedure (Sato et al., 1984).
- **From 4-bromo phenol:** This is debromination of 4-bromophenol to quinone formation using perchloric acid in presence of lead oxide at r.t in acetone solvent (Khan et al., 2010).

\[
\begin{align*}
\text{OH} & \quad \text{PbO}_2, \text{aq. HClO}_4 \\
\text{Br} & \quad \text{Acetone, r.t}
\end{align*}
\]

\[
\begin{align*}
\text{p-bromophenol} & \quad \text{p-benzoquinone}
\end{align*}
\]

- **Using Metal oxides and H\textsubscript{2}O\textsubscript{2}:** Synthesis of quinones by using metal oxides and hydrogen peroxide (Maiti et al., 2008). This reaction system includes reaction of metal oxides in presence of hydrogen peroxide. This reaction mainly having some limitations these are required reaction time is high, tedious workup procedure and use of metal oxides.
4.0. MAJOR TYPE OF QUINONES

4.1. Anthraquinones

- Anthraquinone, also called anthracenedione or dioxoanthracene, is an aromatic organic compound.
- Several isomers are possible, each of which can be viewed as a quinone derivative. The term anthraquinone, however, almost invariably refers to one specific isomer, 9,10-anthraquinone (IUPAC: 9,10-dioxoanthracene) wherein the keto groups are located on the central ring.
- Anthraquinone is obtained industrially by the oxidation of anthracene, a reaction that is localized at the central ring. Chromium(VI) is the typical oxidant. It is also prepared by the Friedel-Crafts reaction of benzene and phthalic anhydride in presence of AlCl₃. The resulting o-benzoic acid then undergoes cyclization, forming anthraquinone. This reaction is useful for producing substituted anthraquinones. The Diels-Alder reaction of naphthoquinone and butadiene followed by oxidative dehydrogenation will also produce 9,10-anthraquinone.

Fig 2: Anthraquinones
4.2. Benzoquinones

- 1, 4-Benzoquinones are an important class of compounds, which serve as valuable building blocks in synthesis and are key moieties in the synthesis of biologically active compounds.

- Benzoquinone was the first synthesized quinone in the late 1830’s in Liebig’s laboratory as a result of the oxidation of quinic acid with manganese dioxide and sulfuric acid. This reaction involves dehydration, decarboxylation and oxidation.

- In general, quinones are being synthesized from phenols, 1,4-dihydroxybenzenes or hydroquinones and dimethoxybenzenes.

- Besides these traditional precursors some miscellaneous compounds also lead to benzoquinones. The commonly used oxidizing agents employed for quinone synthesis are silver oxide, manganese oxide, nitric acid, salcomine/O2, chromium oxidants, benzene selenic anhydride, ceric ammonium nitrate.

![Fig 3: Benzoquinone](image)

4.3. Napthoquinone

- 1,4-Naphthoquinone or para-naphthoquinone is an organic compound derived from naphthalene. Several isomeric naphthoquinones are known, notably 1,2-naphthoquinone. 1,4-Naphthoquinone forms volatile yellow triclinic crystals
and has a sharp odor similar to benzoquinone. It is almost insoluble in cold water, slightly soluble in petroleum ether, and more soluble in polar organic solvents. In alkaline solutions it produces a reddish-brown color. Vitamin K is a derivative of 1,4-naphthoquinone. It is a planar molecule with one aromatic ring fused to a quinone subunit.

- Naphthoquinone forms the central chemical structure of many natural compounds, most notably the K vitamins. 2-Methylnaphthoquinone is a more effective coagulant than vitamin K. Other natural naphtoquinones include juglone, plumbagin, droserone. Naphthoquinone derivatives have significant pharmacological properties.

![Naphthoquinone](image)

**Fig 4: Naphthoquinone**

- They are cytotoxic, they have significant antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory, and antipyretic properties. Plants with naphthoquinone content are widely used in China and the countries of South America, where they are used to treat malignant and parasitic diseases. Naphthoquinone functions as a ligand (through the electrophilic C=C).

### 4.4. Plastoquinone

- Plastoquinone (PQ) is a quinone molecule involved in the electron transport chain in the light-dependent reactions of photosynthesis.
- Plastoquinone is reduced when it accepts two electrons from photosystem II and two hydrogen cations (H⁺) from the stromal matrix of the chloroplast,
thereby forming plastoquinol. It transports the protons into the lumen of thylakoid discs, while the electrons continue further along the electron transport chain, into the cytochrome bf protein complex. The prefix *plasto-* means either plastid or chloroplast, alluding to its location within the cell. Structurally it is a 2,3-dimethyl-1,4-benzoquinone molecule with a side chain of nine isoprenyl units.

![Plastoquinone](image)

**Fig 5: Plastoquinone**

### 4.5. Pyrroloquinoline Quinone

It is capable of catalyzing continuous redox cycling (the ability to catalyze repeated oxidation and reduction reactions), as well as oxidative deaminations. Enzymes containing PQQ are sometimes designated quinoproteins. PQQ can also be thought of as a trophic factor important to the growth and metabolism of bacteria, particularly methylotrophic bacteria (bacteria capable of growing on simple carbon sources). PQQ may play an important role in pathways important to cell signaling. PQQ can also serve as an antioxidant. The importance of PQQ to mammalian health is evident when it is omitted from chemically defined diets, resulting in a wide range of systemic responses, including growth impairment, compromised immune responsiveness, and abnormal reproductive performance in mouse and rat experimental models. Nutritional studies indicate PQQ can serve as a growth
factor and improves neonatal survival. In human fibroblast cultures, PQQ enhances cell growth and proliferation when added to cell cultures. Signs of PQQ deprivation include friable skin, evidence of hemorrhage and diverticuli, and reduction in general fitness. PQQ confers resistance to acute oxidative stress in freshly isolated cardiomyocytes.

![Fig 6: Pyrroloquinoline Quinone](image)

### 5.0. APPLICATIONS

- They are used in the manufacture of dye fabrics especially red and violet dyes.
- It initiates transport of hydrogen atoms and Vitamin K.
- PQQ serves as co catalyst.
- Doxorubicin, a quinone containing drug is used in cancer eradication.
- Used for treatment of bacterial and HIV infection.

### 6.0. CONCLUSION

The comprehensive literature survey pertaining to multiple aspects of quinone chemistry unveiled the sustaining importance of quinonoid compounds in many fields of science. The isolation of different quinones from plants and micro-organisms are still being carried out ambitiously. With the advancement of computational methods in solving chemical problems, theoretical studies in various properties of quinones had also been started to report abundantly in the last decade. All these development
leads to a better understanding of this secondary metabolite to exploit its bioactive potential.

### 7.0. REFERENCES


7. SAPONINS

Sabarinathan Devaraj and Poorna Chandrika Sabapathy

1.0. INTRODUCTION:

Saponins are secondary metabolites of glycosidic nature widely distributed in higher plants but also found in some animal sources, like e.g. marine invertebrates. (Bruneton 1995; Rao and Gurfinkel 2000; Francis et al. 2002). The latter contributed to naming this group “saponins”, which is derived from Latin sapo meaning soap. Saponins are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins occur in a great number of non-related plant species (mainly Angiosperms and a few Pteridophytes), both wild plants and cultivated crops. Triterpenoid saponins are mostly found in dicotyledonous species, while many of the major steroidal saponins are synthesized by monocots, such as members of the Liliaceae, Dioscoraceae and Agavaceae families (Hostettmann & Marston, 1995; Osbourn & Lanzotti, 2009; Vincken et al., 2007). Despite the sometimes negative biological actions of saponins on animals and humans, they do occur in a wide variety of crops and edible plants: triterpenoid saponins have been detected in many Leguminosae (legumes), such as soybeans, beans, peas and alfalfa (Medicago), in Chenopodiaceae like sugar beet, spinach and quinoa, and in Theaceae (tea), while steroidal saponins can be found in grasses like oats, in Solanaceae like Capsicum peppers, aubergine, tomato and potato, in Alliaceae (alliums) such as leek, onion and garlic and in Asparagaceae (asparagus) (Francis et al., 2002). One plant often contains several kinds of saponins, depending on age, place of growth or variety, and of the location inside the plant (roots, leaves etc.). For a secondary plant metabolite, they occur in remarkably high concentrations: 5-10% in the roots of Primula, 2-12% in glycyrrhizine, 10% in de bark of...
Saponins

Quillaja and the seeds of Camellia, and up till 13% in the seeds of the horse chestnut (Hostettmann & Marston, 1995). There is no clear relationship between the plant origin and the type of saponin, nor is there evidence that specific saponins are associated with particular parts of plants (Vincken et al., 2007). Although the vast majority of sources are from plants, saponins have also been isolated from marine organisms (Riguera, 1997), especially in the Cuvierian tubules of sea cucumbers. It was suggested that they store them as a form of chemical defence (Van Dyck et al., 2010). Commercial formulations of plant-derived saponins – e.g., from the soapbark tree, *Quillaja saponaria*, and from other sources – are available via controlled manufacturing processes, which make them of use as chemical and biomedical reagents (Sigma-Aldrich, 2009). In the - 18 - Asian region (China), saponin powder and solutions from tea seeds are sold as natural insecticides; a good example is “Liquid Tea Saponin” from Hangzhou Choisun Tea Sci-Tech Co., Ltd. Saponins are also found in the botanical family Sapindaceae, with its defining genus *Sapindus* (soapberry or soapnut), and in the closely related families Aceraceae (maples) and Hippocastanaceae (horse chestnuts; ref. needed). It is also found heavily in *Gynostemma pentaphyllum* (*Gynostemma*, Cucurbitaceae) in a form called gypenosides, and ginseng or red ginseng (*Panax*, Araliaceae) in a form called ginsenosides. Haemolysis of red blood cells seems to result from saponin ability to form complexes with cell membrane cholesterol leading in consequence to pore formation and cell permeabilization, and also to cause alterations in the negatively charged carbohydrate portions on the cell surface (Abe et al. 1981; Melzig et al. 2001; Gauthier et al. 2009a). It should be mentioned however that the exact mechanism of haemolytic activity of saponins is not clearly understood and is the subject of discussions within the scientific community. Steroidal compounds are less common and usually found among the Liliopsida (former Monocotyledones) in members of such families as *Liliaceae, Dioscoreaceae, Agavaceae*, while triterpenoid saponins are more widely distributed and typical of the Magnoliopsida families (former Dicotyledones), e.g. *Primulaceae, Sapotaceae, Caryophyllaceae* and others. In rare cases both types of saponins may accumulate in a plant, like for example in *Avena sp.* (monocotyledonous *Poaceae*) (Osbourn 2003) or *Lysimachia paridiformis* (dicotyledonous *Myrsinaceae*) (Xu et al. 2007b). Steroidal sapogenins (27C) can have a 6ring spirostane or a 5ring furostane skeleton whereas in the case of triterpenoid sapogenins (30C), which are much more structurally diverse, the basic ring system is most often made of five or, more seldom, of four units. Pentacyclic triterpenoid saponins belong,
Secondary Metabolites

in a majority of cases, to oleananetype, other skeleton types include ursane, lupane, hopane, germanicane, dammarane (Vincken et al. 2007). The presence in the polycyclic sapogenin of different substituents, such as for example hydroxyls, hydroxymethyls, carboxyls and acyl groups, as well as differences in the composition, linkage and number of sugar chains account for significant structural diversity of saponins and also their diverse bioactivity. Some saponins are toxic and are known as sapotoxin. Saponins are phytochemicals which can be found in most vegetables, beans and herbs. The best known sources of saponins are peas, soybeans, and some herbs with names indicating foaming properties such as soapwort, soaproot, soapbark and soapberry. Within these families, this class of chemical compounds is found in various parts of the plant: leaves, stems, roots, bulbs, blossom and fruit. The ability of a saponin to foam is caused by the combination of the nonpolar sapogenin and the water soluble side chain. Saponins are bitter and reduce the palatability of livestock feeds. However if they have a triterpenoid aglycone they may instead have a licorice taste as glucuronic acid replaces sugar in triterpenoids. Some saponins reduce the feed intake and growth rate of non-ruminant animals while others are not very harmful. For example, the saponins found in oats and spinach increase and accelerate the body's ability to absorb calcium and silicon, thus assisting in digestion. Certain pasture weeds contain substantial quantities of dangerous saponins and result in life threatening toxicities for certain animal species. Commercial saponins are extracted mainly from Yucca schidigera and Quillaja saponaria.

Commercial applications ranging from their use as sources of raw materials for the production of steroid hormones in the pharmaceutical industry, to their use as food additives and as ingredients in photographic emulsions, fire extinguishers and other industrial applications which take advantage of their generally non-ionic surfactant properties [Leung AY et al.,1981, Hostettmann K et al.,2009, Leung AY et al.,1996]. They also exhibit a variety of biological activities, and have been investigated towards the development of new natural medicines and prove the efficacy of traditional herbal medicines [Waller GR et al.,1995]. Other interesting biological applications for various specific saponins include their uses as anti-inflammatory [Balandrin MF et al., 1996], hypocholesterolemic [Oakenfull D et al., 1996] and immune-stimulating [Klausner A et al., 1988] whose properties are widely recognized and commercially utilized. As to the application of saponins to foods and cosmetics, it is indispensable that sufficient amount of plant resources is available, and that the content of saponins must be high.
Furthermore, a plant must have a long history of human use as foodstuffs or ingredients of cosmetics, and their safety should be officially guaranteed. A large amount of Quillaja saponin is utilized in photosensitized film as a surfactant. It is used also in beverages, food ingredients, shampoos, liquid detergents, tooth-pastes and extinguishers as an emulsifier and long-lasting foaming agent. Recently, the saponin mixture possesses the immunoadjuvant property and has pharmaceutical application as suspension stabilizer [Setten DC et al., 1996].

Nearly 50,000 tons of licorice roots (*Glycyrrhiza* spp., Leguminosae) are consumed on a year basis. Licorice extract and its major saponin, glycyrrhizin (yield: more than 2.5%), are used as a medicine and as a sweetener and flavor enhancer in foods and cigarettes [9]. It is known that the deterioration of cooked foods is caused mainly by yeast, and that many skin diseases are due to infection by dermatophytic fungi and yeasts.

**2.0. STRUCTURE:**

The non-sugar or the aglycone unit of the saponin molecule is called the sapogenin or just the genin. The saponins can be divided into three major classes according to the structure of genin: fig (1)

1) Triterpene glycosides,

2) steroid glycosides and

3) steroid alkaloid glycosides.

The common denominator for all saponins is the attachment of one or more sugar chains to the sapogenin. They can either be monodesmosidic (have a single sugar chain, usually attached at C-3) or bidesmosidic (two sugar chains attached to C-3 and C-28). The basic structure of monodesmosidic and bidesmosidic saponins, after Hostettmann & Marston (1995).
All saponins have in common the attachment of one or more sugar chains to the glycone. Monodesmosidic saponins have a single sugar chain, normally attached at C-3. Bidesmosidic saponins have two sugar chains, often with one attached through an ether linkage at C-3 and one attached through an ester linkage (acyl glycoside) at C-28 (triptene saponins) or an ether linkage at C-26 (Furostanol saponins). Tridesmosidic saponins have three sugar chains and...
are seldom found. Bidesmosidic saponins are easily transformed into monodesmosidic saponins by, for example, hydrolysis of the esterified sugar at C-28 in triterpene saponins; they lack many of the characteristic properties and activities of monodesmosidic saponins.

The saccharide moiety may be linear or branched, with 11 being the highest number of monosaccharide units yet found in a saponin (Clematoside C from Clematis manshurica (Ranunculaceae); Khorlin et al. 1965). As a rule however, most of the saponins so far isolated tend to have relatively short (and often unbranched) sugar chains, containing 2-5 monosaccharide residues. Kochetkov and Khorlin (1966) have introduced the term oligoside for those glycosides containing more than 3-4 monosaccharides.

The most common monosaccharide moieties found, and the corresponding abbreviations used in this book (according to IUPAC recommendations; pure Appl chem. (1982) 54, 1517-1522), are: D-glucose, D-galactose, D-glucuronic acid, D-galacturonic acid, L-rhamnose, L-arabinose, D-xylose and D-fructose. Saponins from marine organisms often contain D-quinovose. It is possible that D-fructose is present more often than generally thought, in view of its very similar chromatographic behavior to L-rhamnose. Unlike the cardiac glycosides, unusual monosaccharides are seldom found, but it should be noted that uronic acids often occur in triterpene glycosides and that amino sugars may be present. Glucose, arabinose, glucuronic acid and xylose are the monosaccharides most frequently attached directly to the aglycone. Acylated sugar moieties are also encountered. In marine organisms, methylated and sulfated sugars are not uncommon. Configurations of the interglycosidic linkages are given by alpha and beta and the monosaccharides can be in the pyranose (p) or furanose (f) forms. By virtue of carboxyl groups in the aglycone or sugar parts of a saponin, it can be rendered acidic.

2.1. BIOSYNTESIS OF SAPONINS:

Most known saponins are found in angiosperms (Magnoliophyta), though some marine invertebrates such as sea cucumbers (Holothuroidea) and starfish (Asteroidea) also produce these molecules (Bordbar et al., 2011; Liu et al., 2008; Osbourn et al., 2011; Williams & Gong, 2007). While the synthesis of saponins is widespread in plants, the majority of the producing plant species is dicotyledonous and accumulates mainly triterpenoid-type saponins. The monocotyledonous angiosperms on the other hand mostly, but not exclusively, synthesize...
Secondary Metabolites

steroidal-type saponins. This broad classification of saponin types is based on the nature of the aglycone backbone from which the saponin molecule is derived. Both triterpenoid and steroidal aglycone backbones are derived from the 30-carbon linear precursor 2,3-oxidosqualene. During the synthesis of the committed precursor, the steroidal aglycone loses three methyl groups to result in a 27-carbon backbone, whereas the triterpenoid aglycone retains all 30 carbons in its backbone. The steroidal glycoalkaloids, which are also sometimes referred to as saponins, share their biosynthetic origin with the steroidal saponins and contain a characteristic nitrogen atom incorporated into the aglycone backbone (Augustin ET AL., 2011; Friedman, 2006; Ginzberg ET AL., 2009; Itkin ET AL., 2013).

The triterpenoid and steroidal aglycone backbones are isoprenoids that are synthesized from isopentenyl pyrophosphate (IPP) units generated by the mevalonate (MVA) pathway (fig. 2). The multistep MVA/3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) pathway catalyzes the conversion of acetyl-CoA to the five-carbon terpene precursor IPP, which is then isomerized to its allylic isomer dimethylallyl pyrophosphate (DMAPP) by the enzyme isopentenyl diphosphate isomerase (IDI). The subsequent condensation of two IPP units with one DMAPP unit results in the formation of 15-carbon farnesyl pyrophosphate (FPP), the immediate prenylated precursor of the saponins. Finally, the condensation of two FPP units by squalene synthase (SQS) generates the linear 30-carbon precursor squalene, which is further epoxidized to 2,3-oxidosqualene by the enzyme squalene epoxidase (SQE). The 2,3-oxidosqualene is typically cyclized by a variety of oxidosqualene cyclases (OSCs) to polycyclic structures, which in higher plants marks the branch point between primary and specialized triterpene metabolism. The tetracyclic, primary triterpene precursor cycloartenol is generated by the cyclization of 2,3-oxidosqualene by cycloartenol synthase (CAS). In angiosperms, a mixture of phytosterols are generated from cycloartenol, including the 27-carbon cholesterol, the 28-carbon campesterol and the 29-carbon sitosterol. The steroidal saponins are synthesized by a series of oxygenations and glycosylations of the cholesterol backbone to spirostanol or furostanol derivatives with a fused O-heterocycle in their core aglycone structure (Thakur ET AL., 2011). The steroidal glycoalkaloids also utilize cholesterol as the committed precursor, but incorporate an amine group through side chain modifications to generate aglycones such as solasodine, solanidine, demissidine and tomatidine (Ginzberg ET AL., 2009; Itkin ET AL., 2013). The aglycones are
then tailored with oxidoreductases before being glycosylated with multiple sugar moieties (Friedman, 2006).

All the remaining cyclization products of 2,3-oxidosqualene are committed precursors for the synthesis of specialized triterpenes. This cyclization reaction, involving multiple carbocation rearrangement steps, generates the first level of structural diversity inherent to the triterpenoid saponin aglycones, as the single substrate 2,3-oxidosqualene can be cyclized to an array of triterpene scaffolds. As such, nine main classes of triterpene backbones have been documented to be synthesized by plants (shown in orange in figure 2; Vincken ET AL., 2007). The OSCs catalyzing these reactions are either specific or multifunctional in nature, and cyclize 2,3-oxidosqualene to a single product or to multiple products derived from a particular cyclization pathway in a single reaction. The triterpene aglycones are then modified, mainly oxidized, by a series of cytochrome P450-dependent monoxygenases (P450s). These modifications add a second level of complexity and expand the structural diversity of the backbone. Consecutive oxidations at different positions of the triterpene backbone increase the polarity of the scaffold and introduce reactive functional groups which are subsequently modified by an array of transferases, including UDP-dependent glycosyltransferases (UGTs), acyltransferases, malonyltransferases and methyltransferases. A comprehensive overview of the biosynthesis of triterpenoid saponins can be found in Thimmappa ET AL. (2014).

The genes involved in the biosynthesis of all three types of saponins (triterpenoid, steroid and steroidal glycoalkaloid) can therefore be grouped into four main categories based on their characteristic reactions, and include the OSCs, P450s, UGTs and other tailoring enzymes (mostly encoding transferases). Recently, numerous examples of gene clusters for the biosynthesis of different classes of specialized metabolites have been discovered in a variety of plant species, including clusters for saponin biosynthesis (Boycheva ET AL., 2014; Nützmann & Osbourn, 2014). Biosynthetic gene clusters have been identified for triterpenoid saponins and steroidal glycoalkaloids. The first triterpene gene cluster to be identified was the avenacin cluster from AVENA STRIGOSA (oat), which contains five biosynthetic genes encoding for an OSC, a P450, a UGT and two other tailoring enzymes (a methyltransferase and a serine carboxypeptidase-like acyltransferase) (Haralampidis ET AL., 2001a; Mugford ET AL., 2013; Papadopoulou ET AL., 1999; Qi ET AL., 2004, 2006). Other closely linked genes that form
part of this cluster have been defined by genetics but not cloned (Mylona ET AL., 2008; Papadopoulou ET AL., 1999; Qi ET AL., 2004). In *Arabidopsis thaliana*, two triterpenoid gene clusters have been identified for the biosynthesis of thalianol- and marneral-derived triterpenes, containing four and three genes, respectively. The thalianol cluster is comprised of genes encoding an OSC, two P450s and an acyltransferase (Field & Osbourn, 2008). Likewise, the marneral cluster encodes an OSC and two P450s (Field ET AL., 2011). A triterpenoid gene cluster encoding an OSC and two P450s has also recently been identified in *Lotus japonicus* (Krokida ET AL., 2013). Gene clusters for the synthesis of the steroidal glycoalkaloids α-tomatine and α-chaconine/α-solanine have been discovered in *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato), respectively (Itkin ET AL., 2013). The tomato gene cluster consists of eight characterized biosynthetic genes encoding for two P450s, four UGTs and two other tailoring enzymes (a dioxygenase and a transaminase). Similarly, the potato cluster contains six characterized genes encoding for two P450s, two UGTs, a dioxygenase and a transaminase. Although once thought to be an exception, the phenomenon of physical clustering of biosynthetic pathway genes for different types of secondary metabolites in plants is becoming common (Nützmann & Osbourn, 2014). The physical clustering of genes in the genome together with co-ordinate expression of the clustered genes is proving to be a useful tool to identify biosynthetic genes involved in new pathways for the synthesis of specialized metabolites (Castillo ET AL., 2013; Field & Osbourn, 2008; Field ET AL., 2011; Nützmann & Osbourn, 2014). Enzymes encoded by multigene families for OSCs, P450s, UGTs and other specialized transferases are the key actors in the biosynthesis of plant triterpenoid, steroidal and steroidal glycoalkaloid saponins. Although the number of characterized enzymes belonging to these gene families has increased over the last decade, there is still a wide gap in our understanding of saponin biosynthesis, in how the synthesis of these molecules in different plant tissues and organs is regulated during development and in response to environmental stimuli, and in how these molecules are transported. Unraveling these molecular mechanisms will provide a better understanding of the role of saponins in plant processes, and will guide their exploitation in industrial applications.
Fig. 2 Overview of structural diversity in saponin aglycones.
3.0. SOURCES AND PROPERTIES:

The structural complexity of saponins results in a number of physical, chemical, and biological properties, only a few of which are common to all members of this diverse group. Properties of a few selected aglycones and saponins are summarized in Table 1. Due to the presence of a lipid-soluble aglycone and water-soluble sugar chain(s) in their structure (amphiphilic nature), saponins are surface active compounds with detergent, wetting, emulsifying, and foaming properties (Wang et al., 2005; Sarnthein-Graf and La Mesa, 2004; Mitra and Dungan, 1997; Ibanoglu and Ibanoglu, 2000). In aqueous solutions surfactants form micelles above a critical concentration called critical micelle concentration (cmc). Saponins, including soybean saponins, saponins from Saponaria officinalis, and Quillaja saponaria, form micelles in aqueous solutions, the size and structure of which are dependent on type of saponin (Oakenfull, 1986). The micelle forming properties (cmc and the aggregation number (number of monomers in a micelle)) of quillaja saponins were affected by temperature, salt concentration, and pH of the aqueous phase (Mitra and Dungan, 1997). At 25°C, the values of cmc of quillaja saponins were in the range of 0.5 and 0.8 g/L. It increased with temperature and pH but decreased with increasing salt concentration. The incorporation of cholesterol into the saponin micelles increased their cmc, size, viscosity, and the aggregation number (Mitra and Dungan, 2000) resulting in the solubility enhancement of cholesterol as much as a factor of 103 at room temperature (Mitra and Dungan, 2001). Quillaja saponins also had a solubilizing effect on phenantherene, and fluoranthene, which increases linearly with saponin concentration at values higher than cmc (Soeder et al., 1996). A similar linear relationship has been observed between the concentration of the saponin extract from Sapindus mukurossi and aqueous solubility of hexachlorobenzene and naphthalene up to a surfactant concentration of 10% (Kommalapati et al., 1997; Roy et al., 1997). Solubility enhancement has also been observed for Yellow OB (Nakayama et al., 1986), and progesterone (Nakayama et al., 1986) in the presence of bidesmoside saponins from Sapindus mukurossi, and for α-tocopherol, and oleanolic acid in the presence of glucoside and glucuronide esters of glycyrrhizic acid (Sasaki et al., 1988). Purified saponins and saponin mixtures resulted in both enhancements and reductions in water solubility of test compounds quercetin (Schöpke and Bartlakowski, 1997), digitoxin (Walthelm et al., 2001), rutin (Walthelm et al., 2001), and aesculin (Walthelm et al., 2001), the extent of which
was determined by concentration of saponin and the model compound. Solubility enhancement of quercetin obtained by pure saponins at concentrations > cmc values can be attributed to micellar solubilization, whereas solubilization effect of some saponin mixtures at concentrations < cmc points to an alternative mechanism (Sch¨opke and Bartlakowski, 1997). Purified saponins or saponin mixtures may also have a solubilizing effect on other saponins. Solubility enhancement of monodesmosides (such as monodesmosides of Sapindus mukurossi (Nakayama et al., 1986; Kimata et al., 1983), Bupleuri radix (saikosaponins) (Kimata et al., 1985; Morita et al., 1986; Watanabe et al., 1988) and soyasaponins Bb, Bb and B-G (Shimoyamada et al., 1993)), which have very low water solubility, in the presence of bidesmoside saponins is well documented. The extent of the enhancement is dependent on the structure of the monodesmoside saponin, and the composition/concentration of the saponin bidesmosides. Solubility of Sapindus mukurossi monodesmosides was enhanced in the presence of mukurossi bidesmoside saponins containing hederagenin (Y1, Y2, X) (Nakayama et al., 1986; Kimata et al., 1983). However, mukurossi bidesmosides did not affect the solubility of saikosaponins (Kimata et al., 1985), which was enhanced by oleanolic acid bidesmosides with a glucuronide moiety such as ginsenosides (chikusetsusaponin-V (ginsenoside Ro) and IV) (Kimata et al., 1985; Watanabe et al., 1988), Hemsleya macroasperma (cucurbitaceae) bidesmosides (Ma2 and Ma3) (Morita et al., 1986), and cyclic bidesmoside tubeimoside I isolated from tubers of Bolbostemma paniculatum Franquet (Kasai et al., 1986b). The solubility of saikosaponin-a in water at 37°C (0.14 mg/mL) increased with concentration of ginsenoside Ro reaching a value of 4.08 mg/mL at a bidesmoside concentration of 1.4 mg/mL (Kimata et al., 1985). A significant decrease in the solubilizing effect on saikosaponin-a was observed upon methylation or reduction of the glucuronide carboxyl group of ginsenoside Ro indicating the role of the glucuronide moiety in the observed effect (Tanaka, 1987). A greater extent of enhancement was obtained for Hemsleya macroasperma (cucurbitaceae) bidesmosides Ma2 and Ma3, which are structurally similar to ginsenoside Ro with similar cmc values, at a concentration of 0.1% resulting in saikosaponin-a solubilities of 5–8.7 mg/mL compared to 3.4 mg/mL for ginsenoside Ro (Morita et al., 1986). The solubility enhancement of saikosaponin-a became apparent near the cmc of these bidesmosides (Kimata et al., 1985; Morita et al., 1986; Nakayama et al., 1986). The solubility of diene saponin saikosaponin-b1 produced by heating or mild-acid treatment of saikosaponin-a was increased by malonyl-ginsenosides and to a lesser extent by ginsenoside Ro (Zhou et al.,
Secondary Metabolites

The effect of malonyl-ginsenosides on saikosaponin-a has also been demonstrated (Zhou et al., 1991). While neutral dammarane ginsenosides did not have a solubilizing effect on saikosaponins by themselves, they enhanced the solubilizing effect of ginsenoside Ro (Watanabe et al., 1988) and dammarane ginsenosides (Zhou et al., 1991). Solubility enhancement of saikosaponin-a has also been observed in the presence of glycyr rhizic acid, which is the glucuronide monodesmoside saponin of licorice (Sasaki et al., 1988). The decrease in the degree of enhancement observed at high glycyr rhizic acid concentrations was attributed to the increase in solution viscosity (Sasaki et al., 1988). A solubilizing effect was also observed for the 30-β-glucoside (isolated from licorice roots) and glucuronide esters of glycyr rhizic acid at higher concentrations (Sasaki et al., 1988). In addition to bidesmosides, co-occurring compounds such as acyclic sesquiterpene oligoglycosides have also been shown to have a solubilizing effect on monodesmosides of Sapindus mukurossi (Kasai et al., 1986a) and Sapindus delavayi (Wong et al., 1991). Solubility enhancement may have important implications for the bioactivity and processing of saponins. Monodesmosides, while poorly soluble in water in purified form, can be extracted readily due to the solubilizing effect of co-occurring compounds (Kimata et al., 1983). Micellar solubilization by saponins can be exploited for the development of micellar extraction processes or to affect the solubilization of ingredients in cosmetic, pharmaceutical or food formulations (Shirakawa et al., 1986). Solubility of saponins is also affected by the properties of the solvent (as affected by temperature, composition, and pH). While water, alcohols (methanol, ethanol) and aqueous alcohols are the most common extraction solvents for saponins, solubility of some saponins in ether, chloroform, benzene, ethyl acetate, or glacial acetic acid has also been reported (Hostettmann and Marston, 1995). In the ethanol concentration range of 30–100%, solubility of soyasaponin Bb (soyasaponin I) was maximum in 60% ethanol (Shimoyamada et al., 1993). Solubility of gypsophia saponin in water increased with temperature from 7.4 g/100 mL at 30°C to 18.0 g/100 mL at 70°C (Biran and Baykut, 1975). A sharp increase was observed in the solubility of soyasaponin Bb, which was very low in the acidic region, in the pH range 6.5–7.3 (Shimoyamada et al., 1993). The degree of partitioning of components of crude 70% ethanol extract of soybeans between water and butanol was dependent on the concentration of the extract and pH of the aqueous phase (Shimoyamada et al., 1995). The highest recovery of soyasaponin I in the butanol layer was obtained using 0.04 g/mL of crude extract in the acidic region (about pH 4) (Shimoyamada et al., 1995). While bitterness is the most common sensory
attribute associated with saponins (Price et al., 1985), the occurrence of sweet saponins is also well known (Kennelly et al., 1996). For example, the sweetness of licorice is attributed to its main saponin, glycyrrhizic acid, which is 50 times sweeter than sugar (Muller and Morris, 1966). The complex structure of saponins may undergo chemical transformations during storage or processing which in turn may modify their properties/activity. The glycosidic bond (between the sugar chain and the aglycone), and the interglycosidic bonds between the sugar residues can undergo hydrolysis in the presence of acids/alkali, due to hydrothermolysis (heating in presence of water) or enzymatic/microbial activity resulting in the formation of aglycones, prosapogenins, sugar residues or monosaccharides depending on the hydrolysis method and conditions (Hostettmann and Marston, 1995). Complete acid hydrolysis yields the constituent aglycone and monosaccharides, whereas under basic hydrolysis conditions, cleavage of O-acylglycosidic sugar chains results in the formation of prosapogenins (Hostettmann and Marston, 1995). The solubility behavior of the parent aglycone can be markedly different than the saponin due to its lipophilic nature (Table 1). DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) conjugated saponins, which were determined to be the genuine saponins in intact soybeans, are hydrolyzed into Group B and E saponins upon heating, in alkaline solutions, and in the presence of iron (Kudou et al., 1993; Okubo and Yoshiki, 1995). Soyasaponin βg, which was stable in acidic solution and at temperatures < 90°C, was converted into soyasaponin Bb at basic pH and upon heating at 90–100°C (Okubo and Yoshiki, 1995). In the presence of FeCl3, it was degraded into soyasaponin Be and Bb in a ratio of 3:2 (Okubo and Yoshiki, 1995). Deacylation of quillaja saponins was observed upon storage in aqueous solution at pH > 6 (Okubo and Yoshiki, 1995). The interaction of sterols (Gestetner et al., 1971, 1972; Walter et al., 1954; Shany et al., 1970), minerals (West et al., 1978), and proteins (Potter et al., 1993; Tanaka et al., 1995) with saponins may result in the modification of the physicochemical properties and biological activity of these compounds. Steroid saponins (such as digitonin (Gestetner et al., 1972), alfalfa saponins (Walter et al., 1954)), and triterpenoid saponins (such as lucerne (Gestetner et al., 1971, 1972; Shany et al., 1970)) form waterinsoluble addition products with cholesterol and phytosterols such as sitosterol and stigmasterol. Interaction of sterols and lucerne saponins was dependent on the structure of the saponin and sterols (Gestetner et al., 1971, 1972). While cholesterol and β-sitosterol formed complexes with lucerne saponins containing medicagenic acid, which possess carboxyl groups at C23 and C28 positions, saponins with soyasapogenol aglycones did not
precipitate (Gestetner et al., 1971). Insoluble complexes were also formed between ammoniated glycyrrhizic acid and alfalfa root saponins and the minerals zinc and iron (West et al., 1978). The nature and effect of the saponin-protein interaction were dependent on the type of protein (Potter et al., 1993) and the type of the saponin mixture (Tanaka et al., 1995). Upon heating at 78°C (upto 26 min) quillaja saponin interacted with casein to form high molecular weight complexes, whereas soybean proteins formed insoluble aggregates independent of saponin addition (Potter et al., 1993). Similarly, while heating salt soluble proteins from walleye pollack meat at 40–100°C for upto 10 min in the presence of quillaja saponins increased protein aggregation, tea seed saponins inhibited the aggregation of the protein (Tanaka et al., 1995). Complex formation between beet saponin and protein (as evidenced by turbidity and interfacial tension measurements) and destabilization of a model dispersion of sucrose, oil, saponin, and protein in acidic conditions point to the role of beet saponin and protein in the formation of acid beverage floc in sucrose-sweetened carbonated soft drinks and acidified syrups (Morton and Murray, 2001). The interaction of saponins and proteins also resulted in modifications of protein properties such as heat and enzyme stability (Ikedo et al., 1996; Shimoyamada et al., 1998), and surface properties (Chauhan et al., 1999). Heat stability of bovine serum albumin (BSA) (Ikedo et al., 1996), and resistance of BSA (Ikedo et al., 1996) and soybean protein (Shimoyamada et al., 1998) to chymotryptic hydrolysies improved upon addition of soybean saponins. The stability of whey proteins to chymotryptic hydrolysies however decreased upon addition of soybean saponins (Shimoyamada et al., 2000). Similarly, unlike soybean protein whose sensitivity to trypptic hydrolysis improved, whey proteins showed higher sensitivity in the presence of soya saponins (Shimoyamada et al., 2000). The influence of soybean saponin on the trypsin hydrolysis of bovine milk α-lactalbumin was attributed to the modification of the protein’s tertiary structure (Shimoyamada et al., 2005). Desaponization of quinoa protein increased water hydration capacity and lowered the fat binding and buffer capacity, and total nitrogen solubility (Chauhan et al., 1999). Removal of saponins reduced the emulsion and foaming capacity of the proteins but increased the stability of the foams and emulsions (Chauhan et al., 1999).
# Secondary Metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Solubility</th>
<th>Source</th>
<th>MW</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aglycone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>C$<em>{30}$H$</em>{48}$O$_{3}$</td>
<td>Insoluble in water, sol in 65 parts ether, 106 parts 95% alcohol, 35 parts boiling 95% alcohol, 118 parts chloroform, 180 parts acetone, 235 parts methanol.</td>
<td>Quinoa</td>
<td>457</td>
<td>310</td>
</tr>
<tr>
<td>Quillaic acid</td>
<td>C$<em>{30}$H$</em>{46}$O$_{5}$</td>
<td>Soluble in alcohol, ether, acetone, ethyl acetate, glacial acetic acid</td>
<td>Quillaja</td>
<td>487</td>
<td>292–293</td>
</tr>
<tr>
<td>Diosgenin</td>
<td>C$<em>{27}$H$</em>{42}$O$_{3}$</td>
<td>Soluble in the usual organic solvents, in acetic acid</td>
<td>Dioscorea, fenugreek, yam</td>
<td>415</td>
<td>204–207</td>
</tr>
<tr>
<td>Glycyrrhetic acid</td>
<td>C$<em>{30}$H$</em>{46}$O$_{4}$</td>
<td></td>
<td>Licorice</td>
<td>471</td>
<td>298–300</td>
</tr>
<tr>
<td><strong>Saponin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycyrrhizic acid (Glycyrrhizin)</td>
<td>C$<em>{42}$H$</em>{62}$O$_{16}$</td>
<td>Freely soluble in hot water, alcohol, practically insoluble in ether</td>
<td>Licorice</td>
<td>823</td>
<td></td>
</tr>
<tr>
<td>Escin</td>
<td></td>
<td></td>
<td>Horse chestnut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-escin</td>
<td></td>
<td>Very soluble in water and methanol, only slightly soluble in acetone, insoluble in ether and hydrocarbons</td>
<td></td>
<td>225–227</td>
<td></td>
</tr>
<tr>
<td>β-escin</td>
<td></td>
<td>Readily soluble in methanol, slightly soluble in acetone, practically insoluble (very little solubility) in water, insoluble in ether and hydrocarbons</td>
<td></td>
<td>222–223</td>
<td></td>
</tr>
<tr>
<td>Gypsophia saponin</td>
<td>C$<em>{35}$H$</em>{61}$O$_{24}$</td>
<td>Soluble in water (0.5147 g/100 mL at 25°C)</td>
<td>Gypsophia</td>
<td>863</td>
<td>221–227</td>
</tr>
</tbody>
</table>

Table 1 Properties of a few selected aglycones and saponins
4.0. COMMERCIAL APPLICATIONS:

The diverse physicochemical and biological properties of saponins have been successfully exploited in a number of commercial applications in food, cosmetics, agricultural and pharmaceutical sectors. Market trends towards the use of natural ingredients, and increasing evidence of their biological activity have increased the demand for saponins in recent years (Brown, 1998; Malcolm, 1995). As natural non-ionic surfactants, they find widespread use as emulsification and foaming agents, and detergents (San Martin and Briones, 1999; Balandrin, 1996). Other investigated/proposed applications of saponins and saponin containing plants include as feed additives (Cheeke, 1999; Zhan, 1999; Aoun et al., 2003; Jensen and Elgaard, 2001), as bacterial (Henderson, 2001) and vegetable growth regulators (Yamauchi et al., 2000), and for soil remediation (Roy et al., 1997). While the two major commercial sources of saponins are *Quillaja saponaria* and *Yucca schidigera* extracts (San Martin and Briones, 1999; Balandrin, 1996), a number of other plant materials such as horse chestnut (Indena, 2005), tea seed (Zhan, 1999), and soybeans (Organic Technologies, 2005) are being utilized/evaluated for use as commercial sources of saponins. Pharmaceutical applications of saponins include as raw materials for production of hormones (Blunden et al., 1975), immunological adjuvants (Kensil et al., 2004), and as drugs (Panagin Pharmaceuticals Inc., 2005; Panacos, 2005). Saponins have also been reported to be the active ingredients in various natural health products, such as herbal extracts (Balandrin, 1996)

4.1. Food Applications:

Yucca (*Mohave yucca, Yucca schidigera* Roezl Fla) and quillaja (quillaia, soap bark, *Quillaja saponaria* Mol Fla) are classified as food additives in the US under section 172.50 (Natural Flavoring Substances and Natural Substances Used in Conjunction with Flavors) (US Food and Drug Administration, 2003). The food additives from natural origins containing saponins used in Japan include enzymatically modified soybean saponin, Pfaf- fia paniculata extract, quillaja extract, tea seed saponins, and yucca foam extract (Japanese Ministry of Health and Welfare, 2005). Quillaja extract is classified by the European Union as a foaming agent for use in water-based, flavored non-alcoholic drinks (E 999; 200 mg/liter calculated as anhydrous extract) (Office for Official Publications of the European Communities, 1996). Although quillaja
Secondary Metabolites

and yucca are not considered Generally Recognized As Safe (GRAS) by the US Food and Drug Administration (FDA), they have been given GRAS designation by Flavor and Extract Manufacturers’ Association (FEMA) (FEMA #2973, and 3120 respectively) (Ash and Ash, 2002). There is a pending GRAS notice (GRN #165) received by FDA in 2005 from the American Beverage Association for quillaja extract (type 2) to be used as a foaming agent in semi-frozen carbonated and non-carbonated beverages at levels not exceeding 500 milligrams dry weight per kilogram beverage (US Food and Drug Administration, 2005a).

Quillaja extract (type 1) is used in foods and beverages mainly for its foaming properties at concentrations of 100 ppm (dry basis, undiluted extract) in soft drinks, and at concentrations up to 250 ppm in frozen carbonated beverages (Joint FAO/WHO Expert Committee on Food Additives, 2004). Quillaja extract, type 2 is used in Japan as an emulsifier for preparations containing lipophilic colors or flavors that are added to soft drinks, fermented vegetables, and dressing (at claimed concentrations < 100ppm) Saponins have also been proposed for use in foods as antimicrobial (Sogabe et al., 2003) and anti-yeast agents (Ashida and Matsuda, 1999). Other commercial saponin products for food applications include soybean concentrates marketed as functional food ingredients and nutraceuticals (Organic Technologies, 2005), and a Korean ginseng extract called saponia (Godwithus Co Ltd., 2005).

The presumed health benefits of oleanolic acid led to the development of methods to fortify food products (such as olive oil) with oleanolic acid (van Putte, 2002). Proposed applications for oleanolic acid include as a flavoring agent to modify the aftertaste/taste of the artificial sweetener (Kang et al., 1999) and in fat blends as crystal modifier (Bhaggan et al., 2001). The physicochemical properties of saponins can also be utilized in food processing applications. Thus, while complex formation of saponins with cholesterol has been used for the removal of cholesterol from dairy products such as butter oil (Micich et al., 1992; Richardson and Jimenez-Flores, 1994), the interaction of saponins with cell membranes has been considered for the selective precipitation of fat globule membranes from cheese whey (Hwang and Damodaran, 1994). In this last application, saponins are used to increase the hydrophobicity of the fat membrane to facilitate flocculation and precipitation of the formed complexes (Hwang and Damodaran, 1994).
4.2. Cosmetics:

Due to their surface active properties, saponins are being utilized as natural surfactants in cleansing products in the personal care sector such as shower gels, shampoos, foam baths, hair conditioners and lotions, bath/shower detergents, liquid soaps, baby care products, mouth washes, and toothpastes (Indena, 2005; Olmstead, 2002; Brand and Brand, 2004). Natural surfactants containing saponins available commercially include Juazarine from the bark of *Zizyphus joazeiro* tree (Anonymous, 2004), horse chestnut saponins (Indena, 2005) and mixture of plant saponins (Bio-Saponins, Bio-Botanica, Inc., 2005). Saponins and sapogenins are also marketed as bioactive ingredients in cosmetic formulations with claims to delay the aging process of the skin (Yoo et al., 2003; Bonte et al., 1998), and prevent acne (Bombardelli et al., 2001).

4.3. Pharmaceutical/Health Applications:

Steroid saponin-containing plant materials gained commercial significance in 1950s as raw materials for the production of steroid hormones and drugs. The synthesis of progesterone from the sapogenin diosgenin obtained from Mexican yam by Marker et al. in 1940s (Marker et al., 1947) was the beginning of a remarkable era in steroid research culminating in the synthesis of the first oral contraceptive in 1951. Diosgenin isolated from *Dioscorea* species and to a lesser extent structurally similar sapogenins such as hecogenin from Agave species have been widely used as raw materials by the steroid industry (Blunden et al., 1975). Saponins have been used as immunological adjuvants in veterinary vaccine formulations due to their immune enhancing properties since 1950s (Dalsgaard, 1974). Their use in human vaccines, however, has been limited by their complexity and toxicity. Purification of the quillaja extract to yield fractions with differing chemical and biological properties enabled the characterization and thus reproducible production of the fractions for optimal adjuvant activity and minimal haemolytic activity and toxicity (Cox et al., 2002; Kensil and Marciani, 1991). Consequently, there have been significant advances in the development of saponins as human vaccine adjuvants in the last decade leading to the development of a new generation of vaccines against cancer and infectious diseases which are at various phases of clinical trials (Kensil et al., 2004). The use of quillaja extracts (even at concentrations commonly used in foods) as oral adjuvants in human clinical tests requires
Saponins supporting toxicology and general safety data due to their non-GRAS status (Dirk and Webb, 2005). The wealth of information on the biological activity of saponins and aglycones from a variety of sources is providing leads for the development of drugs. The chemopreventive and chemotherapeutic activities of ginseng dammarane sapogenins have prompted the development of anticancer drugs which are at various stages of development (Panagin Pharmaceuticals Inc., 2005). A new class of HIV drugs called Maturation Inhibitors (PA-457, in Phase 2 clinical trials) are being developed using betulinic acid derivatives (Panacos, 2005). Pharmaceutical compositions or plant extracts containing saponins have been patented for the prevention and/or treatment of a variety of conditions such as inflammation (Forse and Chavali, 1997; Bombardelli et al., 2001), infection (Forse and Chavali, 1997), alcoholism (Bombardelli and Gabetta, 2001), pre- and post-menopausal symptoms (Bombardelli and Gabetta, 2001), cerebrovascular diseases such as coronary heart disease and hypertension (Yao et al., 2005; Hidvegi, 1994), prophylaxis and dementia (Ma et al., 2003), ultraviolet damage including cataract, and carcinoma cutaneum (Satoshi et al., 2004), gastritis, gastric ulcer, and duodenal ulcer (Kim et al., 2003a). The use of saponins in pharmaceutical preparations as adjuvants to enhance absorption of pharmacologically active substances or drugs has also been patented (Kensil et al., 1996; Tanaka and Yata, 1985). Saponin-containing plants such as ginseng, yucca, horse chestnut, sarsaparilla, and licorice have been used in traditional medicine by various cultures for centuries for the prevention/treatment of various ailments (Liu and Henkel, 2002; Hostettmann and Marston, 1995). Characterization of the medicinal plants and their extracts points to the role of saponins in conjunction with other bioactive components such as polyphenols in the observed health effects (Liu and Henkel, 2002; Alice et al., 1991). Over 85% of the herbs most commonly used in Traditional Chinese Medicine were observed to contain saponins (in addition to polyphenols) in significant detectable amounts, while the herbal products in the eight best known and most commonly used formulae were explicitly rich in these components (Liu and Henkel, 2002). It should be noted that while some of the health benefits associated with these plants have been supported by clinical data or described in pharmacopeias and in traditional systems of medicine, a variety of uses attributed to these medicinal plants have not been substantiated.
Secondary Metabolites

5.0. CONCLUSION:

Saponins include a diverse group of compounds characterized by their structure containing a steroid or triterpenoid aglycone and one or more sugar chains. Their physicochemical and biological properties, few of which are common to all members of this diverse group, are increasingly being exploited in food, cosmetics and pharmaceutical sectors. The full realization of their commercial potential, which is driven by consumer demand for natural products and increasing evidence of their health benefits, requires development of commercially feasible processes that can address processing challenges posed by their complex nature, including their stability. Information on the composition (qualitative and quantitative) and properties of the saponins present in the raw material, and the effects of processing on their composition and properties are key elements of successful process design. The abundance of saponins in nature and their presence in significant quantities in processing by-products (such as by-products of soybean processing) result in a wide range of natural materials that can be exploited for commercial production.

6.0. REFERENCES:


Secondary Metabolites

Secondary Metabolites

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Secondary Metabolites


8. POLYKETIDES

Thazeem Basheer and Mridul Umesh

1.0. INTRODUCTION:

Polyketides are a class of secondary metabolites produced by certain living organisms in order to impart to them some survival advantages. Many mycotoxins produced by fungi are polyketides. Structurally, polyketides are complex organic compounds that are often highly active biologically. Many pharmaceuticals are derived from or inspired by polyketides. Polyketides are the secondary metabolites having a large number of structures and few of them are veterinary or medicinal agents. Polyketides are found in plants, fungi, bacteria and include important clinical drugs like daunorubicin, tetracycline, erythromycin, and lovastatin and rapamycin. Biologically unparallel activities are included in modern search of polyketide which are commercially useful for modern drug discovery. Secondly, mechanism, catalytic reactivity and extraordinary structure of polyketides provide an opportunity for the investigation of enzyme catalysis mechanism, protein–protein interaction and molecular recognition. Thirdly, amenability and remarkable versatility of polyketides allow generation of the novel compounds.

Polyketides represent a highly diverse group of natural products having structurally intriguing carbon skeletons which comprises polyphenols, macrolides, polyenes, enediyynes, and polyethers. Although their exact roles in their original biological contexts are not known in all cases, it is believed that they function as pigments, virulence factors, infochemicals, or for defense. From a pharmacological point of view, polyketides are an important source of novel therapeutics. In particular, they are used in medicine mainly as antibiotics, immunosuppressants, antiparasitics, cholesterol-lowering, and antitumoral agents.

The highly complex structures and the strong pharmacological relevance of these compounds have triggered an immense endeavor to gain synthetic access to the natural products and derivatives thereof. Whereas the total chemical synthesis of polyketidesis highly
challenging, it is remarkable that their vast structural and functional diversity results from the controlled assembly of some of the simplest biosynthetic building blocks: acetate and propionate. With the advent of molecular techniques, it has now become possible to gain a better understanding of the biosynthetic logic of polyketide diversity. On the basis of this growing body of knowledge, polyketide biosynthesis pathways may be manipulated or redesigned for the production of novel drug candidates.

Figure 1 - Representative polyketide structures including prominent bioactives. Reproduced from Methods in Enzymology, 2009, Vol. 459, cap. 1, Pages 3-16 with permission of Elsevier Ltd. (Weissman, 2009).

**Fig 1: Prominent polyketides**
2.0. BIOSYNTHESIS OF POLYKETIDES:

Polyketides are usually biosynthesized through the decarboxylative condensation of malonyl-CoA derived extender units in a similar process to fatty acid synthesis (a Claisen condensation). The polyketide chains produced by a minimal polyketide synthase are often further derivitized and modified into bioactive natural products. Polyketides are formed due to stepwise condensation of the acetate units. Alternate C atoms are present in the resultant C chain due to CH3 and COOH groups of acetate. Polyketide biosynthesis has much in common with fatty acid biosynthesis. Not only are they alike in the chemical mechanisms involved in chain extension but also in the common pool of simple precursors employed, such as acetyl coenzyme A (CoA) and malonyl-CoA (MCoA) units. In general, both polyketides and fatty acids are constructed by repetitive decarboxylative Claisen thioester condensations of an activated acyl starter unit with malonyl-CoA-derived extender units. Typically, this process involves a β-ketoacyl synthase (KS), an optional (malonyl) acyl transferase (MAT/AT), and an phosphopantethenylated acyl carrier protein (ACP) or coenzyme A (CoA), which serves as an anchor for the growing chain. After every chain elongation, the β-oxo functionality is processed by a ketoreductase (KR), dehydratase (DH), and an enoyl reductase (ER), which yields a fully saturated acyl backbone.

However, polyketide biosynthesis deviates in many ways from fatty acid biosynthesis. Polyketidesynthases (PKSs) clearly differ from fatty acid synthases (FASs) not only in their ability to use a broader range of biosynthetic building blocks but also in the formation of various chain lengths. Most importantly, whereas FAS typically catalyze a full reductive cycle after each elongation, in polyketide biosynthesis the reductive steps are optional; they can be partly or fully omitted before the next round of elongation, thus giving rise to a more complex pattern of functionalization. Nonetheless, in both pathways, the elongation/reduction cycles are repeated until a defined chain length is obtained, and finally the thioester-bound substrates are released from the enzyme complex. The primary products may then be subjected to additional modifications.

Despite striking similarities in their enzymology in chain propagation, PKSs and FASs are different and constitute a metabolic branch point between primary and secondary metabolism.
Secondary Metabolites

Both pathways may have diverged at an early stage during evolution. Even so, in this context it may be interesting to note that PKSs may be involved in the biosynthesis of microbial polyunsaturated fatty acids as well as mycobacterial cell wall lipids. Differences between fatty acid and polyketides biosynthesis are due to different types and numbers of the acyl precursors, position, pattern of cyclization of products and extent of the keto-group reductions. So polyketide and fatty acid biosynthesis are related mechanistically and precursor molecules used in their biosynthesis are same.

Fig 2: Polyketide Biosynthesis

3.0. CLASSIFICATION:

Polyketides are structurally a very diverse family of natural products with diverse biological activities and pharmacological properties. They are broadly divided into three classes: type I polyketides (often macrolides produced by multimodular megasynthases), type II polyketides (often aromatic molecules produced by the iterative action of dissociated enzymes),
and type III polyketides (often small aromatic molecules produced by fungal species). Polyketide antibiotics, antifungals, cytostatics, anticholesteremic, antiparasitics, coccidiostats, animal growth promoters and natural insecticides are in commercial use.

3.1. Macrolides and lactone polyketides:

Macrolide antibiotics are metabolites of Streptomyces and Micromonospora spp. Many antibiotics classified as macrolides have been reported for which full structures are not described. The macrolides are a class of natural products that consist of a large macrocyclic lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. The lactone rings are usually 14-, 15-, or 16-membered. Macrolides belong to the polyketide class of natural products. Some macrolides have antibiotic or antifungal activity and are used as pharmaceutical drugs. Few examples are - Azithromycin, Clarithromycin, Erythromycin, Fidaxomicin and Telithromycin. Structurally, macrolides are a class of complex glycosidic lactones; the aglycone is normally a 12–16 membered macrocyclic ring and one to three neutral or aminosugar residues are linked to the aglycone via ether linkages. Many of the aglycones have also been isolated from the fermentation broths, often from mutant strains, but these are usually devoid of biological activity. Pikromycin was the first isolated macrolide. Erythromycin is a typical macrolide antibiotic.

3.1.1. Uses:

Antibiotic macrolides are used to treat infections caused by Gram-positive (e.g., Streptococcus pneumoniae) and limited Gram-negative (e.g., Bordetella pertussis, Haemophilus influenzae) bacteria, and some respiratory tract and soft-tissue infections. The antimicrobial spectrum of macrolides is slightly wider than that of penicillin, and, therefore, macrolides are a common substitute for patients with a penicillin allergy. Beta-hemolytic streptococci, pneumococci, staphylococci, and enterococci are usually susceptible to macrolides. Unlike penicillin, macrolides have been shown to be effective against Legionella pneumophila, mycoplasma, mycobacteria, some rickettsia, and chlamydia.

3.1.2. Mechanism of action:

Antibacterial: Macrolides are protein synthesis inhibitors. The mechanism of action of macrolides is inhibition of bacterial protein biosynthesis, and they are thought to do this by
preventing peptidyltransferase from adding the growing peptide attached to tRNA to the next amino acids as well as inhibiting ribosomal translation. Another potential mechanism is premature dissociation of the peptidyl-tRNA from the ribosome. Macrolide antibiotics do so by binding reversibly to the P site on the subunit 50S of the bacterial ribosome. This action is considered to be bacteriostatic. Macrolides are actively concentrated within leukocytes, and are, therefore, transported into the site of infection. The macrolide antibiotics erythromycin, clarithromycin, and roxithromycin have proven to be an effective long-term treatment for the idiopathic. With macrolide therapy in asian-prevalent lung disease diffuse panbronchiolitis (DPB), great reduction in bronchiolar inflammation and damage is achieved through suppression of not only neutrophil granulocyte proliferation but also lymphocyte activity and obstructive secretions in airways.

![Fig 3: Structure of Erythromycin](image)

3.2. Ansamycins and related polyketides:

Ansamycins are benzenoid or naphthalenoid aromatic compounds in which nonadjacent positions are bridged by an aliphatic chain to form a cyclic structure. One of the aliphatic-aromatic junctions is always an amide bond. They are reproduced by *Streptomyces*, *Nocardia* and *Micromonospora spp.* and have also been isolated from plant sources; although for the latter, the involvement of microorganisms has not been ruled out. The natural ansamycins may be subdivided according to the nature of the aromatic moiety and the length of the aliphatic chain. The major group contains a naphthalenoid moiety and a 17 carbon aliphatic chain. The differences in structure are not merely of chemical interest but indicate a profound difference in biological activity. Members of this group show selective antibacterial activity and inhibit RNA
Secondary Metabolites

polymerase. The benzenoid ansamycins with a 15-C chain include the Ansamitocins and the related Maytansine; these compounds show pronounced antitumour activity.

Ansamycins are a family of secondary metabolites that show antimicrobial activity against many gram-positive and some gram-negative bacteria and includes various compounds, among which: streptovaricins and rifamycins are more common. In addition, these compounds demonstrate antiviral activity towards bacteriophages and poxviruses. They are named ansamycins (from the Latin ansa, handle) because of their unique structure, which comprises an aromatic moiety bridged by an aliphatic chain. The main difference between various derivatives of ansamycins is the aromatic moiety, which can be anaphthalene ring or a naphthoquinone ring as in rifamycin and the naphthomycins. Another variation comprises benzene or a benzoquinone ring system as in geldanamycin or ansamitocin. Ansamycins were first discovered in 1959 by Sensi et al., from Amycolatopsis mediterranei, an actinomycete.

3.2.1.Classical example:

Rifamycin is a typical member of this group. Rifamycins are a subclass of ansamycins with high potency against mycobacteria. This resulted in their widespread use in the treatment of tuberculosis, leprosy, and AIDS-related mycobacterial infections. Since then various analogues have been isolated from other prokaryotes. Geldanamycin and Macbecin are potent antitumor agents.

![Fig 4: Structure of Geldanamycin](image)

Rifamycin is a typical member of this group. Rifamycins are a subclass of ansamycins

Polyketides

Page 137
3.3. Polyenes:

Polyenes are poly-unsaturated organic compounds that contain at least three alternating double and single carbon–carbon bonds. These carbon–carbon double bonds interact in a process known as conjugation. Related to polyenes are dienes, where there are only two alternating double and single bonds. Another related class of compounds have three or more double bonds, but they are not alternating with single bonds. The group of antibiotics known collectively as polyenes is characterised by a large lactone ring (20–44 membered) containing a series of conjugated double bonds. This leads to the subdivision of the group into trienes, tetraenes etc. Themacrolide ring is often linked by a hydroxyl group to an aminosugar unit and may have an aliphatic side chain possibly terminating with an aromatic residue. *Streptomyces* are the usual producing organisms, and to date over 200 polyenes have been claimed. However, only some of these have established structures. One reason for the paucity of structural information is that they are often mixtures of closely related compounds. The advent of HPLC has enabled better separation to be obtained and has indicated that many polyenes previously considered to be defined were in fact mixtures of the same components but in different proportions. The macrolide ring is probably derived from acetate and propionate, otherwise little is known about their detailed mechanism of biosynthesis.

Nystatin is a typical polyene antibiotic showing antifungal activity. Some representative polyenes are Amphotericin B (antimycotic antibiotic), Leukotriene A4 (immune response modulator), Retinal, Polyacetylenes, and Beta-carotene (red-orange pigment).

![Fig 5: Structure of Nystatin](image-url)
3.4. Linear tetracyclines:

Tetracycline is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system. It is marketed under the brand names Sumycin, Tetracyn, Lymecycline, and Panmycin, among others. Actisite is a thread-like fiber formulation used in dental applications. It is also used to produce several semisynthetic derivatives, which together are known as the tetracycline antibiotics. The term "tetracycline" is also used to denote the four-ring system of this compound; "tetracyclines" are related substances that contain the same four-ring system. Historically it was important in reducing the number of deaths from cholera. The tetracyclines, which contain a polyhydronaphthacene nucleus, form a small but very important group of antibiotics. Many of the Streptomyces metabolites have been used clinically since their discovery in the late 1940s. They are active against gram-positive and gram-negative bacteria, spirochaetes, mycoplasmas and rickettsiae. In addition, they display significant amoebicidal activity and have efficacy in some diseases caused by large viruses. They have veterinary applications in promoting growth and feed efficiency. They are second to the beta-lactam group in terms of clinical use and exhibit low toxicity and good oral absorption. Their mode of action is by the inhibition of protein biosynthesis. It is first-line therapy for rocky mountain spotted fever (Rickettsia), Lyme disease (B. burgdorferi), Q fever (Coxiella), psittacosis and lymphogranuloma venereum (Chlamydia), Mycoplasma pneumoniae and to eradicate nasal carriage of meningococci. Tetracycline tablets were used in the plague outbreak in India in 1994.

Tetracyclines have a broad spectrum of antibiotic action. Originally, they possessed some level of bacteriostatic activity against almost all medically relevant aerobic and anaerobic bacteria genera, both Gram-positive and Gram-negative, with a few exceptions, such as Pseudomonas aeruginosa and Proteus spp., which display intrinsic resistance. However, acquired (as opposed to inherent) resistance has proliferated in many pathogenic organisms and greatly eroded the formerly vast versatility of this group of antibiotics. Resistance amongst Staphylococcus spp., Streptococcus spp., Neisseria gonorrhoeae, anaerobes, members of the Enterobacteriaceae and several other previously sensitive organisms is now quite common. Tetracyclines remain especially useful in the management of infections by certain obligately intracellular bacterial pathogens such as Chlamydia, Mycoplasma and Rickettsia. They are also
of value in spirochaetal infections, such as syphilis, leptospiriosis and Lyme disease. Certain rare or exotic infections, including anthrax, plague and brucellosis, are also susceptible to tetracyclines. These agents also have activity against certain eukaryotic parasites, including those responsible for diseases such as malaria and balantidiasis.

3.4.1. Mechanism of Action

Tetracycline inhibits protein synthesis by blocking the attachment of charged aminoacyl-tRNA to the A site on the ribosome. Tetracycline binds to the 30S subunit of microbial ribosomes. Thus, it prevents introduction of new amino acids to the nascent peptide chain. The action is usually inhibitory and reversible upon withdrawal of the drug. Mammalian cells are less vulnerable to the effect of tetracyclines, despite the fact that tetracycline binds to the small ribosomal subunit of both prokaryotes and eukaryotes (30S and 40S respectively). This is because bacteria actively pump tetracycline into their cytoplasm, even against a concentration gradient, whereas mammalian cells do not. This accounts for the relatively small off-site effect of tetracycline on human cells.

![Fig 6: Structure of Tetracycline](image)

3.5. Angucyclines:

The angucycline antibiotics are related to the tetracyclines but they have an angular arrangement of the tetracyclic ring system as in Urdamycin A. Angucyclinones are defined as natural products with a benz[a]anthracenenucleus but no hydrolysable sugar moieties whereas the term angucycline includes those with hydrolysable sugars.
3.6. Polyether antibiotics:

The majority of polyethers are characterised by a linear series of tetrahydrofuran and tetrahydropyran residues, frequently linked by spiroketal systems. These compounds always terminate with a carboxylic acid residue or a simple ester function thereof. Some polyethers also carry a sugar unit linked to a hydroxyl group on one of the tetrahydropyran rings. The most common sugar residue is 4-O-methylamicetose. More than 1000 polyether antibiotics have been isolated so far, mostly as metabolites of *Streptomyces* spp., although some *Streptoverticillium*, *Actinomadura*, *Nocardia* and *Dactylosporangium* spp. are also reported to produce them. Polyethers are generally produced as a series of closely related compounds e.g. the major component may possess methyl substituents on each of the cyclic ether units, but in addition small amounts of ethyl homologues may also be present. Chemical subdivision is based on the number of spiroketal functionalities, and the presence or absence of a sugar residue.

Polyethers possess the ability to bind and transport certain ions, and each antibiotic has its own ion specificity. For this reason, they are important biochemical tools in studying the role of cations in biological systems. The antibiotics show a wide range of activities, being active against gram-positive organisms and mycobacteria, fungi and yeasts, but because of their toxicity, these properties have found little application. Their uses to date are mainly as feed additives. Biosynthetically, the polyethers are polyketide in origin. The major building blocks are acetate, propanoate, and butyrate. There is evidence to suggest the intermediacy of an epoxide in the formation of the tetrahydrofuran and tetrahydropyran systems. Monensin A is a typical polyether antibiotic.

![Structure of Monensin A](image)

*Fig 7: Structure of Monensin A*
3.7. Aflatoxins and related substances:

Structurally, aflatoxins consist of a hydrogenated difurano-moiety fused to a substituted coumarin. The naturally occurring aflatoxins are acutely toxic and extremely carcinogenic compounds produced by Aspergillus spp. Metabolism of these compounds by microbial and animal species or chemical transformation leads to a number of equally potent aflatoxin derivatives. Toxic effects centre primarily on the liver. The formation of the principal toxin, Aflatoxin B1, has been studied inconsiderable detail. The results are consistent with a pathway from a single decaketide chain via a series of intermediates e.g. Averufin and Sterigmatocystin. The other aflatoxins are formed from Aflatoxin B1.

![Fig 8: Structure of Aflatoxin B1](image)

3.8. Acetogenins:

Acetogenins are a class of polyketide natural products found in plants of the family Annonaceae. They are characterized by linear 32- or 34-carbon chains containing oxygenated functional groups including hydroxyls, ketones, epoxides, tetrahydrofurans and tetrahydropyrans. They are often terminated with a lactone or butenolide. Over 400 members of this family of compounds have been isolated from 51 different species of plants. Examples include - Annonacin, Annonins, Bullatacin and Uvaricin.

3.8.1. Biological effects:

Acetogenins have been investigated for their potential therapeutic use in treating cancer. Neither purified acetogenins nor crude extracts of the pawpaw or the Brazilian pawpaw (Asimina triloba, Annonaceae) have been approved by the FDA for cancer treatment, but they have exhibited antitumor efficacy both in animal models and in a limited number of clinical studies. There is a lack of rigorously controlled clinical trials, casting doubt of the efficacy of
acetogenins. Both the Pawpaw extract and acetogenins appear to inhibit HIF-1 activation by blocking the hypoxic induction of nuclear HIF-1α protein.

3.8.2. Marine halogenated Acetogenins:

Marine metabolites include a series of halogenated polyketides particularly from red algae \((Laurencia \text{ spp.})\). The metabolites contain, along with bromine and chlorine substituents, oxygen heterocycles, acetylenes and allenes. A typical example is Bermudynol.

3.8.3. Annonaceae Acetogenins:

The \textit{Annonaceae} are a large family of tropical and subtropical trees. Several species contain compounds of apparent polyketide origin typified by the first example of this class, Uvaricin. They contain from 35 to 38 carbons, one, two or less commonly three tetrahydrofuran rings, a \(\gamma\)-lactone and various other oxygen functions and are characterised by a three carbon unit joined onto a long aliphatic chain. The determination of the stereochemistry of this group is often very difficult since they are generally waxy, amorphous compounds unsuitable for X-ray analysis.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig9.png}
\caption{Structure of annonacin}
\end{figure}

4.0. CONCLUSION:

Polyketides are a large and diverse group of natural products including polyphenols, polyenes, and macrolides with a wide variety of biological activities with antibiotic, antifungal, and anticancer properties. They are chemically distinct group containing multiple \(-\text{CH}_2\text{-CO-}\) ketide group synthesized by repetitive condensation or polymerization reactions. Polyketides are among the most important microbial natural products used in medicine. Members of this diverse family of compounds are used as a wide variety of therapeutics, including antibiotics such as
Secondary Metabolites

erthromycin, anticancer epothilones, immunosuppressant rapamycin, and cholesterol-lowering lovastatin. This colourful spectrum of biological activities arises from their considerable structural diversity. Further insights in engineering the stereospecificity of polyketides’ biosynthesis shall become feasible for generating novel, desirable polyketide products.

5.0. REFERENCES: