PHARMACOGNOSY 2

Dr. Dima MUHAMMAD

1 -Trease and Evans Pharmacognosy, William C. Evans, Saunders Elsevier, 2009, sixteenth edition., ISBN 978-0 -7020 -2934 9

2- Textbook of pharmacognosy & phytochemistry, Biren Shah & A.K. Seth, Elsevier, 2010, 1st edition, ISBN: 978-81-312-2298-0

3-Medicinal Natural Products: A Biosynthetic Approach. Paul M Dewick, John Wiley & Sons, 2009,3rd edition, ISBN 978-0-470-74168-9.

4- Pharmacognosy.Phytochemistry, medicinal plants. Bruneton Jean, Lavoisier; 2009 4th edition; ISBN 978-2743011888.

5- Natural Products Isolation, Satyajit D. Sarker, Zahid Latif, and Alexander I. Gray, Humana Press Inc; New Jersey; 2005; 2nd edition; ISBN 1-59259-955-9.

INTRODUCTION

Natural Products: Present and Future

Nature has been a source of therapeutic agents for thousands of years, and an impressive number of modern drugs have been derived from natural sources, many based on their use in traditional medicine. Over the last century, a number of top selling drugs have been developed from natural products (vincristine from *Vinca rosea*, morphine from *Papaver somniferum*, Taxol from *T. brevifolia*, etc.). In recent years, a significant revival of interest in natural products as a potential source for new medicines has been observed among academia as well as pharmaceutical companies. Several modern drugs (~40% of the modern drugs in use) have been developed from natural products.

In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancers had natural origins. In 2001, eight (simvastatin, pravastatin, amoxycillin, clavulanic acid, azithromycin, ceftriaxone, cyclosporin, and paclitaxel) of the 30 top-selling medicines were natural products or their derivatives, and these eight drugs together totaled US $16 billion in sales.

Natural products can contribute to the search for new drugs in three different ways:

1- by acting as new drugs that can be used in an unmodified state (e.g., vincristine from *Catharanthus roseus*).
2- by providing chemical "building blocks" used to synthesize more complex molecules (e.g., diosgenin from *Dioscorea floribunda* for the synthesis of oral contraceptives).

3- by indicating new modes of pharmacological action that allow complete synthesis of novel analogs (e.g., synthetic analogs of penicillin from *Penicillium notatum*).

Natural products will certainly continue to be considered as one of the major sources of new drugs in the years to come because:

1- they offer incomparable structural diversity.
2- many of them are relatively small (<2000 Da).
3- they have "drug-like" properties (i.e., they can be absorbed and metabolized).

Advent, introduction, and development of several new and highly specific *in-vitro* bioassay techniques, chromatographic methods, and spectroscopic techniques, especially nuclear magnetic resonance (NMR), have made it much easier to screen, isolate, and identify potential drug lead compounds quickly and precisely. The substances from the plants were isolated, their structures elucidated and pharmacological active constituents studied.

Pharmacognosy is no longer a descriptive study of plants used in traditional medicine, but it is a substantial science based on a combination of multiple expertise in various fields like organic & analytic chemistry and pharmacology.

Nowadays, pharmacognosy is a multidisciplinary science which could be defined after the American society of pharmacognosy, as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources"

Strategies for research in the area of natural products have evolved quite significantly over the last few decades. These can be broadly divided into two categories:

I. Older strategies:
   a) Focus on chemistry of compounds from natural sources, but not on activity.
   b) Straightforward isolation and identification of compounds from natural sources followed by biological activity testing (mainly *in-vivo*).
   c) Chemotaxonomic investigation
   d) Selection of organisms primarily based on ethnopharmacological information, folkloric reputations, or traditional uses.

II. Modern strategies:
   a) Bioassay-guided (mainly *in-vitro*) isolation and identification of active "lead" compounds from natural sources.
   b) Production of natural products libraries.
   c) Production of active compounds in cell or tissue culture, genetic manipulation, natural combinatorial chemistry, and so on.
   d) More focused on bioactivity.
   e) Selection of organisms based on ethnopharmacological information, folkloric reputations, or traditional uses, and also those randomly selected.

1. GENERAL SCHEME OF PHYTOCHEMICAL STUDY

Plants are complex matrices, producing a range of secondary metabolites with different functional groups and polarities. Categories of natural products commonly encountered include waxes and fatty acids, polyacetylenes, terpenoids (e.g., monoterpenoids, iridoids, sesquiterpenoids, diterpenoids, triterpenoids), steroids, essential oils (lower terpenoids and phenylpropanoids), phenolics (simple phenolics, phenylpropanoids, flavonoids, tannins, anthocyanins, quinones, coumarins, lignans), alkaloids, and glycosidic derivatives (e.g., saponins, cardiac glycosides, flavonoid glycosides).

Several approaches can be employed to extract the plant material. Although water is used as an extractant in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various solubilities of plant constituents.

Many sequential Steps are needed to achieve the general scheme of phytochemical study:

1.1. Preparation of plant material

Plant preparation includes the following important steps:

- Authentication of plant material by botanist
- Selecting the right plant part, the age of plant, the time, season and place of collection.
- Drying, processing (fermentation or freezing) and storage
- Grinding methods and powdering techniques:

The aim of grinding (fragmentation of the plant into smaller particles) is to improve the subsequent extraction by:

- rendering the sample more homogenous
- increasing the surface area, and facilitating the penetration of solvent into the cells, so enhancing the mass transfer of active principle from plant material to the solvent.
- The rupture of plant tissue and cell structures so that its active principles are exposed to the extraction solvent.

Potential problems of grinding include the fact that some material (e.g., seeds and fruits rich in fats and volatile oils) may clog up the sieves and that the heat generated may degrade thermolabile metabolites.

1.2. Extraction:

The choice of extraction procedure depends on the nature of the source material and the compounds to be isolated. Prior to choosing a method, it is necessary to establish the target of the extraction. There can be a number of targets; some of these are mentioned here.

- A known compound present in an organism.
- A group of compounds within an organism that are structurally related.
- An unknown bioactive compound.
- Identification of all secondary metabolites present in an organism for chemical fingerprinting or metabolomics study.

The typical extraction process for plant materials, incorporates the following steps:

I. Choice of solvents:

- Polar extraction: water, ethanol, methanol (MeOH) and so on.
- Medium polarity extraction: ethyl acetate (EtOAc), dichloromethane (DCM) and so on.
- Nonpolar: n-hexane, petroleum ether, chloroform ($CHCl_3$) and so on.

II. Choice of extraction method:

Solvent extraction procedures applied to plant natural products include maceration, percolation, Soxhlet extraction, pressurized solvent extraction, ultrasound-assisted solvent extraction, extraction under reflux, and steam distillation, the fundamentals of these techniques are to be fully detailed later.

III. Choice of optimal parameters influencing the extraction procedures:

- Length of the extraction period
- Solvent used
- pH of the solvent
- Temperature
- Particle size of the plant tissues
- The solvent-to-sample ratio.

1.3. Fractionation:

A crude natural product extract is literally a cocktail of compounds. It is difficult to apply a single separation technique to isolate individual compounds from this crude

mixture. Hence, the crude extract is initially separated into various discrete fractions containing compounds of similar polarities or molecular sizes.

These fractions may be obvious such as the two phases of a liquid–liquid extraction or they may be the contiguous eluate from a chromatography column.

The aims of fractionation may be summarized as follow:

- enrichment of the sub-fractions with compounds of interest
- removal of interfering compounds
- minimization of the total masse of the crud extract
- facilitatation of the following purification processes

1.4. Isolation and purification:

The most important factor that has to be considered before designing an isolation protocol is the nature of the target compound present in the crude extracts or fractions.

The general features of the molecule that are helpful to ascertain the isolation process include solubility (hydrophobicity or hydrophilicity), acid–base properties, charge, stability, and molecular size.

In the situation where the types of compounds present are totally unknown, it is advisable to carry out qualitative tests for the presence of various types of compounds, e.g., phenolics, steroids, alkaloids, flavonoids, etc.

The chromatographic techniques are mainly used in the isolation of various types of natural products such as Thin-layer chromatography (TLC), open-column chromatography (CC) and high-performance liquid chromatography (HPLC), etc.

1.5. Quantification

The yield of compounds at the end of the isolation and purification process is important in natural product research. An estimate of the recovery at the isolation stage can be obtained using various routine analytical techniques.

1.6. Structure Elucidation:

In most cases of extraction and isolation of natural products, the end point is the identification of the compound or the conclusive structure elucidation of the isolated compound. However, structure elucidation of compounds isolated from plants, fungi, bacteria, or other organisms is generally time consuming, and sometimes can be the "bottleneck" in natural product research.

There are many useful spectroscopic methods of getting information about chemical structures, but the interpretation of these spectra normally requires specialists with detailed spectroscopic knowledge and wide experience in natural product chemistry.

The following spectroscopic techniques are generally used for the structure determination of natural products:

1. Ultraviolet-visible spectroscopy (UV-vis): provides information on chromophores present in the molecule.

2. Infraredspectroscopy (IR): determines different functional groups, e.g.,—CO, —OH, —NH2, aromaticity, and so on, present in a molecule.
3. mass spectrometry (MS): gives information about the molecular mass and the molecular formula.
4. Nuclear magnetic resonance (NMR): reveals information on the number and types of protons and carbons (and other elements like nitrogen, fluorine, etc.) present in the molecule, and the relationships among these atoms.
5. X-ray crystallographic techniques provide information on the crystal structure of the molecule.
6. polarimetry: offers information on the optical activity of chiral compounds.

1.7. Assays

Chemical and biological assays are necessary to pinpoint the target compound(s) from a complex natural product extract. At present, natural product research is more focused on isolating target compounds (assay-guided isolation) rather than trying to isolate all compounds present in any extract. Therefore, appropriate assays should be incorporated in the extraction and isolation protocol.

2. Range of Extraction Methods

A number of methods using organic and/or aqueous solvents are employed in the extraction of natural products. Solvent extraction relies on the principle of either "liquid–liquid" or "solid–liquid" extraction.

2.1. Liquid–liquid extraction

Liquid–liquid extraction, whereby two immiscible liquids are used to separate organic compounds based on their differential solubility in each solvent. The distribution of the solute molecules between the two phases is governed by the partition constant (K):

$$K = C_A / C_B$$

Where C_A and C_B represent the concentration of the solute in the two solvents.

K: is a constant at any given temperature, and theoretically solutes possessing differing partition constants can be separated, where solute molecules are separated on the basis of an equilibrium established between the two phases involved.

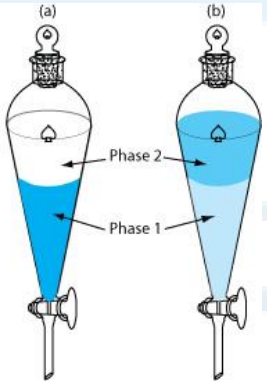


Figure (1): Separatory Funnel

2.2. Solid – liquid extraction:

In the first instance, the solvent has to diffuse into cells, in the following step it has to solubilize the metabolites, and finally it has to diffuse out of the cells enriched in the extracted metabolites.

In general, extractions can be facilitated by grinding (as the cells are largely destroyed, the extraction relies primarily on the solubilization of metabolites) and by increasing the temperature (to favor solubilization).

Evaporation of the organic solvents or freeze-drying (of aqueous solutions) yields dried crude extracts.

2.2.1. Maceration:

This simple, but still widely used, procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container at room temperature. The method is suitable for both initial and bulk extraction.

Occasional or constant stirring of the preparation (using mechanical shakers or mixers to guarantee homogenous mixing) can increase the speed of the extraction.

The extraction ultimately stops when an equilibrium is attained between the concentration of metabolites in the extract and that in the plant material.

After extraction, the residual plant material (marc) has to be separated from the solvent, first by decanting, which is usually followed by a filtration step. Centrifugation may be necessary if the powder is too fine to be filtered.

To ensure exhaustive extraction, it is common to carry out an initial maceration, followed by clarification, and an addition of fresh solvent to the marc.

The main disadvantage of maceration is that the process can be quite time-consuming, taking from a few hours up to several weeks (3). Exhaustive maceration can also consume

large volumes of solvent and can lead to the potential loss of metabolites and/or plant material. Furthermore, some compounds may not be extracted efficiently if they are poorly soluble at room temperature.

On the other hand, as the extraction is performed at room temperature, maceration is less likely to lead to the degradation of thermolabile metabolites.

2.2.2. Ultrasound-assisted solvent extraction:

This is a modified maceration method where the extraction is facilitated by the use of ultrasound (high-frequency pulses $\geq$20kHz). The plant powder is placed in a vial. The vial is placed in an ultrasonic bath.

Ultrasound is used to induce a mechanical stress on the cells through the production of cavitations in the sample. The cellular breakdown increases the solubilization of metabolites in the solvent and improves extraction yields.

The efficiency of the extraction depends on the instrument frequency, and length and temperature of sonication.

Ultrasonification is rarely applied to large-scale extraction; it is mostly used for the initial extraction of a small amount of material. It is commonly applied to facilitate the extraction of intracellular metabolites from plant cell culture

2.2.3. Percolation:

In percolation, the powdered plant material is soaked initially in a solvent in a percolator (a cylindrical or conical container with a tap at the bottom). Additional solvent is then

poured on top of the plant material and allowed to percolate slowly (dropwise) out of the bottom of the percolator.

Additional filtration of the extract is not required because there is a filter at the outlet of the percolator. Percolation is adequate for both initial and large-scale extraction. As for maceration, successive percolations can be performed to extract the plant material exhaustively by refilling the percolator with fresh solvent and pooling all extracts together.

To ensure that percolation is complete, the percolate can be tested for the presence of metabolites with specific reagents. Both the contact time between the solvent and the plant (i.e., the percolation rate) and the temperature of the solvent can also influence extraction yields.

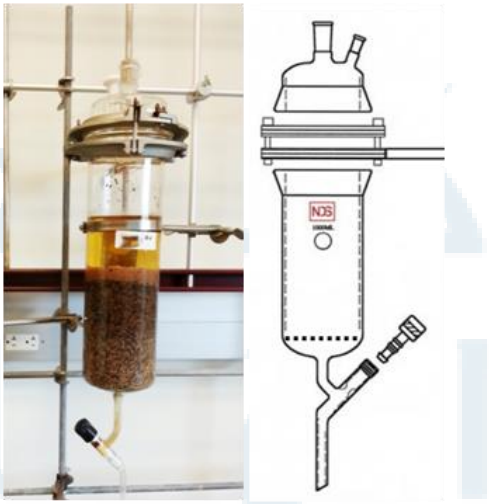


Figure (2): Laboratorial glass percolator.

There are several issues to consider when carrying out a percolation; The percolator can be clogged with very fine powders and materials such as resins and plants that swell excessively (e.g., those containing mucilage). Furthermore, if the material is not

distributed homogenously in the container (e.g., if it is packed too densely), the solvent may not reach all areas and the extraction will be incomplete.

A higher temperature will improve extraction but may lead to decomposition of labile metabolites. The other disadvantages of percolation are that large volumes of solvents are required and the process can be time-consuming.

2.2.4. Soxhlet Extraction: (Hot Continuous Extract)

Soxhlet extraction is used widely in the extraction of plant metabolites because of its convenience. The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the set up is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath.

The main advantage of Soxhlet extraction is that it is a continuous process. As the solvent (saturated in solubilized metabolites) empties into the flask, fresh solvent is re-condensed and extracts the material in the thimble continuously.

This makes Soxhlet extraction less time- and solvent-consuming than maceration or percolation. However, the main disadvantage of Soxhlet extraction is that the extract is constantly heated at the boiling point of the solvent used, and this can damage thermolabile compounds and/or initiate the formation of artifacts.

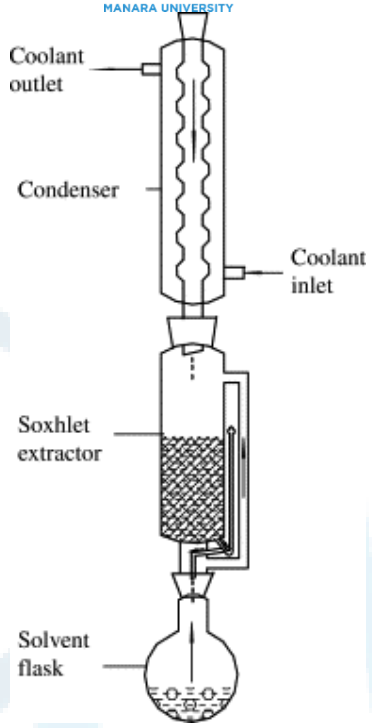


Figure (3): Schematic representation of a Soxhlet extractor.

2.2.5. Pressurized Solvent Extraction PSE

Pressurized solvent extraction, also called "accelerated solvent extraction," employs temperatures that are higher than those used in other methods of extraction, and requires high pressures to maintain the solvent in a liquid state at high temperatures. It is best suited for the rapid and reproducible initial extraction of a number of samples.

The powdered plant material is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilize the remaining components. A final purge with nitrogen gas is performed to dry the material.

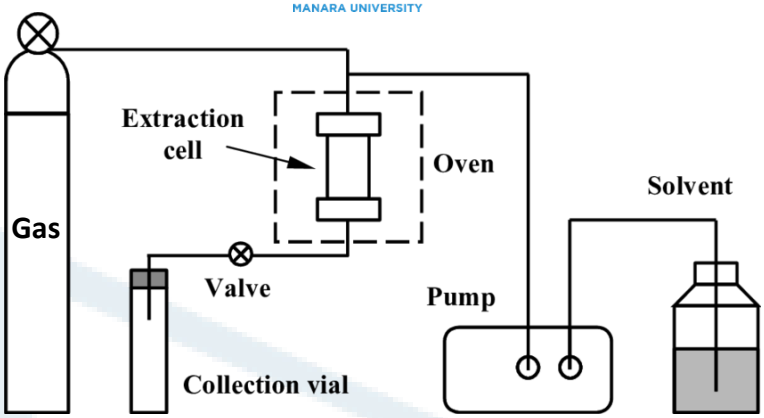


Figure (4): Schematic diagram of the pressurized liquid extraction PLE system

High temperatures and pressures increase the penetration of solvent into the material and improve metabolite solubilization, enhancing extraction speed and yield. Moreover, with low solvent requirements, pressurized solvent extraction offers a more economical and environment-friendly alternative to conventional approaches.

As the material is dried thoroughly after extraction, it is possible to perform repeated extractions with the same solvent or successive extractions with solvents of increasing polarity. An additional advantage is that the technique can be programmable, which will offer increased reproducibility. However, variable factors, e.g., the optimal extraction temperature, extraction time, and most suitable solvent, have to be determined for each sample.

2.2.6. Supercritical fluid extraction SFE:

Pressurized solvent extraction (PSE) is similar in theory to the SFE technique with one major difference; the solvent used in the PSE technique is typically hexane or some other hydrocarbon-based solvent.

The use of supercritical fluids extraction has become of increasing economic and research interest especially in of a range of materials including plant products of medicinal, flavoring and cosmetic interest.

In 1822, Cagniard de la Tour reported that above a certain temperature and pressure, single substances do not condense or evaporate but exist as a fluid. Under these conditions the gas and liquid phases both possess the same density and no division exists between the two phases. This is the critical state.

For water the critical conditions for temperature (t_c) and pressure (p_c.) are 374° C and 220 atmospheres respectively and for carbon dioxide t_c = 31° C and p_c 74 atm. In phytochemistry these properties can be exploited to maximize the extraction of plant constituents.

For industrial purposes supercritical fluid carbon dioxide has an environmental advantage over many common organic solvents and leaves no solvent residues in the product. It also allows a low temperature process and has proved of value for the extraction of labile expensive fragrances and medicinal phytochemicals. To render it more polar a small amount of modifier. e.g. methanol. may be added to the carbon dioxide. The high pressures and for some substances the high temperatures, involved in supercritical fluid extraction are the principal disadvantages of the technique.

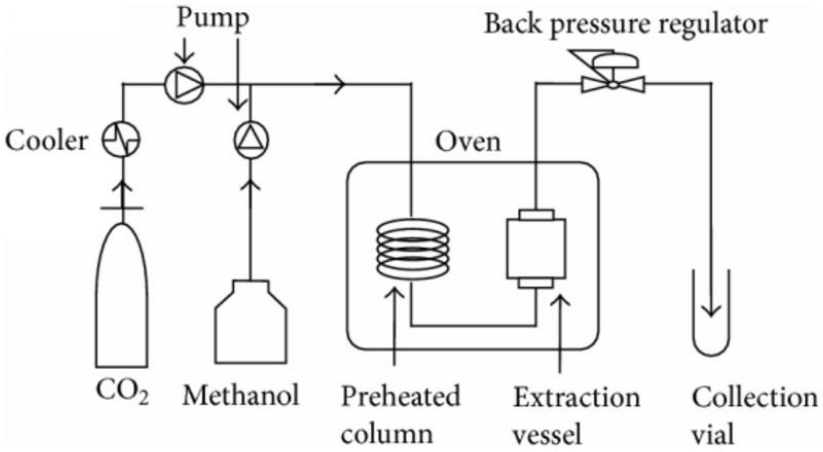


Figure (5): Schematic diagram of supercritical fluids extraction

2.2.7. Extraction under reflux and steam distillation

In extraction under reflux, plant material is immersed in a solvent in a round-bottomed flask, which is connected to a condenser. The solvent is heated until it reaches its boiling point. As the vapor is condensed, the solvent is recycled to the flask.

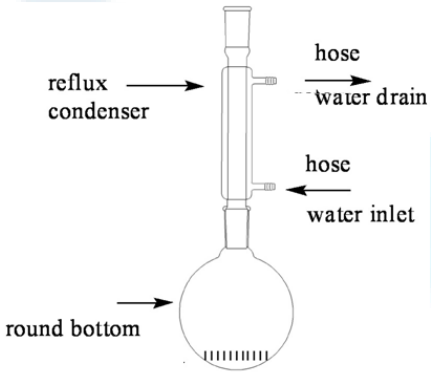


Figure (6): Extraction under reflux.

Steam distillation is a similar process and is commonly applied to the extraction of plant essential oils (a complex mixture of volatile constituents). The plant (dried or fresh) is covered with water in a flask connected to a condenser. Upon heating, the vapors (a mixture of essential oil and water) condense and the distillate (separated into two immiscible layers) is collected in a graduated tube connected to the condenser. The aqueous phase is re-circulated into the flask, while the volatile oil is collected separately.

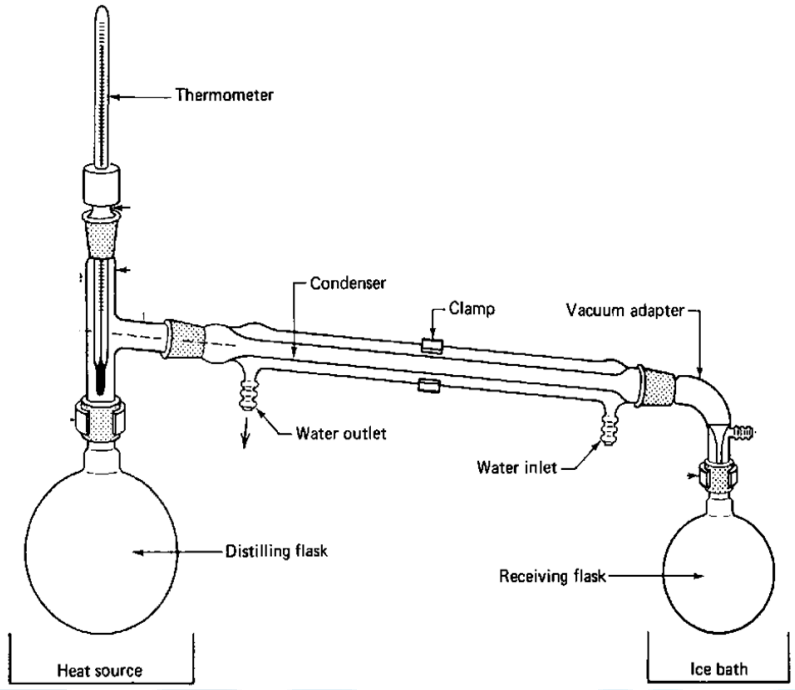


Figure (7): Steam distillation.

Optimum extraction conditions (e.g., distillation rate) have to be determined depending on the nature of the material being extracted. The main disadvantage of extraction under reflux and steam distillation is that thermolabile components risk being degraded.

3. Selection of an Extraction Method and Solvent

The ideal extraction procedure should be exhaustive (i.e., extract as much of the desired metabolites or as many compounds as possible). It should be fast, simple, and reproducible if it is to be performed repeatedly.

The solvent selected should have a low potential for artifact formation, a low toxicity, a low flammability, and a low risk of explosion. Additionally, it should be economical and easily recycled by evaporation.

Extractions can be either "selective" or "total." The initial choice of the most appropriate solvent is based on its selectivity for the substances to be extracted.

In a selective extraction, the plant material is extracted using a solvent of an appropriate polarity following the principle of "like dissolves like"; Thus, non-polar solvents are used to solubilize mostly lipophilic compounds (e.g., alkanes, fatty acids, pigments, waxes, sterols, some terpenoids, alkaloids, and coumarins). Medium polarity solvents are used to extract compounds of intermediate polarity (e.g., some alkaloids, flavonoids), while more polar ones are used for more polar compounds (e.g., flavonoid glycosides, tannins, some alkaloids). Water is not used often as an initial extractant, even if the aim is to extract water-soluble plant constituents (e.g., glycosides, quaternary alkaloids, tannins).

A selective extraction can also be performed sequentially with solvents of increasing polarity. This has the advantage of allowing a preliminary separation of the metabolites present in the material within distinct extracts and simplifies further isolation.

In an extraction referred to as "total" a polar organic solvent (e.g., ethanol, methanol, or an aqueous alcoholic mixture) is employed in an attempt to extract as many compounds as possible. This is based on the ability of alcoholic solvents to increase cell wall permeability, facilitating the efficient extraction of large amounts of polar and medium- to low-polarity constituents. The "total" extract is evaporated to dryness, re-dissolved in water, and the metabolites re-extracted based on their partition coefficient (i.e., relative affinity for either phase) by successive partitioning between water and immiscible organic solvents of varying polarity.

Specific protocols during which the pH of the extracting aqueous phase is altered to solubilize selectively groups of metabolites (such as acids or bases) can also be used.

For instance, these are applied to the extraction of alkaloids (which occur mostly as water-soluble salts in plants).

3.1. Traditional preparation of medicinal plants:

If the plant material has been selected from an ethnobotanical point of view, it may be worthwhile reproducing the extraction methods employed traditionally (if they are reported) to enhance the chances of isolating potential bioactive metabolites. Traditional methods rely principally on the use of cold/hot water, alcoholic, and/or aqueous alcoholic mixtures to obtain preparations that are used externally or administered internally as teas (e.g., infusions, decoctions). Boiling solvent can be poured on the plant material (infusion) or the plant can be immersed in boiling solvent (decoction).

- Decoction: In this process, the crude drug is boiled in a specified volume of water for a defined time (15-30 min); it is then cooled and strained or filtered.
- Infusion: Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.
- Maceration: In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of time (30min - 3days) with frequent agitation until the soluble matter has dissolved.
- Digestion: This is a form of maceration in which gentle heat is used during the process of extraction.

PRIMARY AND SECONDARY METABOLITES

All organisms need to transform and interconvert a vast number of organic compounds to enable them to live, grow, and reproduce. An integrated network of enzyme-mediated and carefully regulated chemical reactions is used for this purpose. Despite the extremely varied characteristics of living organisms, the pathways for generally modifying and synthesizing carbohydrates, proteins, fats, and nucleic acids are found to be essentially the same in all organisms, apart from minor variations. These processes demonstrate the fundamental unity of all living matter, and are collectively described as primary metabolism, with the compounds involved in the pathways being termed primary metabolites.

In contrast to these primary metabolic pathways, there also exists an area of metabolism concerned with compounds, called secondary metabolites. They are found in only specific organisms, and are an expression of the individuality of species.

Secondary metabolites are not necessarily produced under all conditions, and in the vast majority of cases the function of these compounds are not yet known.

Some are undoubtedly produced for easily appreciated reasons, un example: for survival purposes, e.g. as toxic materials providing defense against predators, as volatile attractants towards the same or other species, or as colouring agents to attract or warn other species, but it is logical to assume that all do play some vital role for the well-being of the producer. It is this area of secondary metabolism which provides most of the pharmacologically active natural products.

These secondary metabolites can be summarized as follows:

1- Carbohydrate and derived products;

2- Lipids;

3- Phenols (Phenolic acids, coumarines, Flavonoides, anthocyanes, tanins...);

4- Terpens (monoterpens, diterpens, triterpens.....);

5- Alkaloids;

6- Cardio-active Glycosides;

7- Volatile oils and resins.

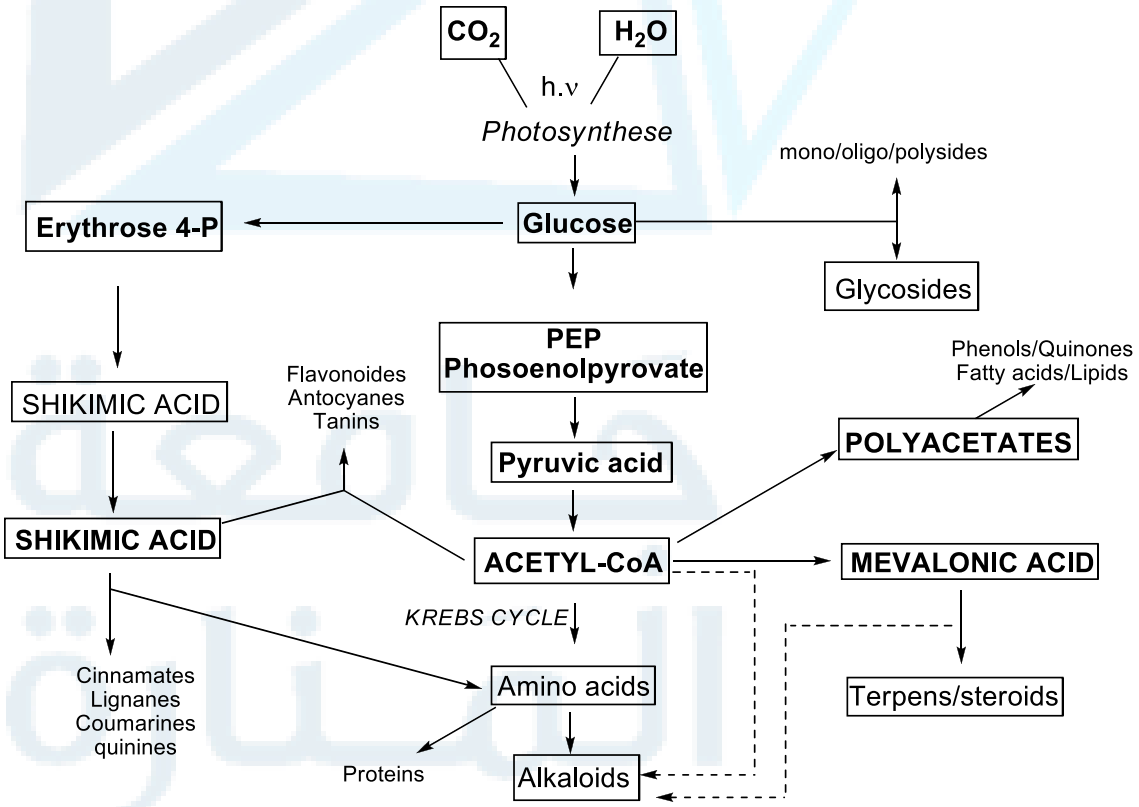


Figure (8): Origins of secondary metabolites in relation to the basic metabolic pathways of plants

1. CARBOHYDRATES

Carbohydrates are the first products formed in photosynthesis, and are the products from which plants synthesize their own food reserves and other chemical constituents. These materials then become the foodstuffs of other organisms.

Carbohydrates are among the most abundant constituents of plants, animals, and microorganisms. Polymeric carbohydrates function as important food reserves and as structural components in cell walls. Animals and most microorganisms are dependent upon the carbohydrates produced by plants for their very existence.

The name carbohydrate was introduced because many of the compounds had the general formula $C_x(H_2O)_y$, and thus appeared to be hydrates of carbon. But this definition has certain drawbacks as given below:

- There are some organic compounds present these proportion and they are not carbohydrates, for example, formaldehyde HCHO; acetic acid CH_3COOH; and lactic acid $CH_3CHOHCOOH$.
- Many carbohydrates such as rhamnose ($C_6H_{12}O_5$), digitoxose ($C_6H_{12}O_4$), etc., are known which do not contain the usual proportions of hydrogen to oxygen.
- Certain carbohydrates are also known which contain nitrogen or sulphur in addition to carbon, hydrogen and oxygen.

The terminology is now commonly used in a much broader sense to denote polyhydroxy aldehydes and ketones, and their derivatives.

Sugars or saccharides are other terms used in a rather broad sense to cover carbohydrate materials. Though these words link directly to compounds with sweetening properties, application of the terms extends considerably beyond this.

A monosaccharide is a carbohydrate usually in the range C_3–C_9, whilst oligosaccharide covers small polymers comprised of 2–10 monosaccharide units. The term polysaccharide is used for larger polymers.

1.1. Monosaccharide:

Six-carbon sugars (hexoses) and five-carbon sugars (pentoses) are the most frequently encountered monosaccharide carbohydrate units in nature.

The majority of natural monosaccharides have the D configuration, except: L- rhamnose, L-arabinose and L- fucose.

Monosaccharide structures may be depicted in open-chain forms showing their carbonyl character, or in cyclic hemiacetal or hemiketal forms obtained after a nucleophilic attack of an appropriate hydroxyl onto the carbonyl. two epimeric structures (anomers) are possible α or β. In practice the β-D-sugars and α-L-sugars have the anomeric hydroxyl directed 'up'.

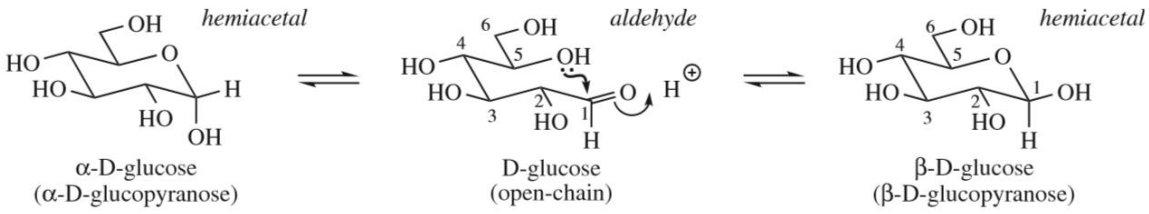


Figure (9): D- glucose conformation

1.1.1. Physiochemical properties of Monosaccharides :

- Reducing power: Sugars having free or potentially free aldehyde or ketone group have an ability to reduce other compounds, for example reduction of Fehling's Solution (reducing sugar + $2Cu^{++} \rightarrow$ oxidized sugar + $2Cu^{+}$).
- Steriochemistry Optical isomers differ from each other in the disposition of the various atoms or groups of atoms in space around the asymmetric carbon atom *C.
- Mutarotation: when a monosaccharide is dissolved in water, the optical rotatory power ([α]D) of the solution gradually changes until it reaches a constant value, indicating an equilibrium between the two anomeric forms α and β.

For ex : when D glucose is dissolved in water, a specific rotation of ([α]D=+112.2) is obtained, but this slowly changes , so that at 24h the value has become ([α]D=+52.7). This gradual change in specific rotation is known as mutarotation. This phenomenon is shown by number of pentoses, hexoses and reducing disaccharides.

- Solubility: they are quite soluble in water, each molecule having several OH groups that readily engage in hydrogen bonding.

1.1.2. Principal pharmaceutical monosaccharides and derivatives

- D-Glucose (dextrose) occurs naturally in grapes and other fruits. It is usually obtained by enzymic hydrolysis of starch, and is used as a nutrient, particularly in the form of an intravenous infusion. Chemical oxidation of glucose converts the aldehyde function to a carboxylic acid and produces gluconic acid. The soluble calcium salt calcium gluconate is used as an intravenous calcium supplement.

- D-Fructose is usually obtained from invert sugar separating it from glucose, and is of benefit as a food and sweetener for patients who cannot tolerate glucose, e.g. diabetics. Fructose has the sweetness of sucrose and about twice that of glucose.
- The sugar alcohol D-sorbitol is found naturally in the ripe berries of the mountain ash (*Sorbus aucuparia*; Rosaceae), but now it is prepared semi-synthetically from glucose. It is half as sweet as sucrose, is not absorbed orally, and is not readily metabolized in the body. It finds particular use as an osmotic laxative and a sweetener for diabetic products.
- D-Mannitol is also produced from glucose, but occurs naturally in manna, the exudate of the manna ash (*Fraxinus ornus* Oleaceae). This material has similar characteristics to sorbitol, but is used principally as a diuretic. It is injected intravenously, is eliminated rapidly into the urine, and removes fluids by an osmotic effect.

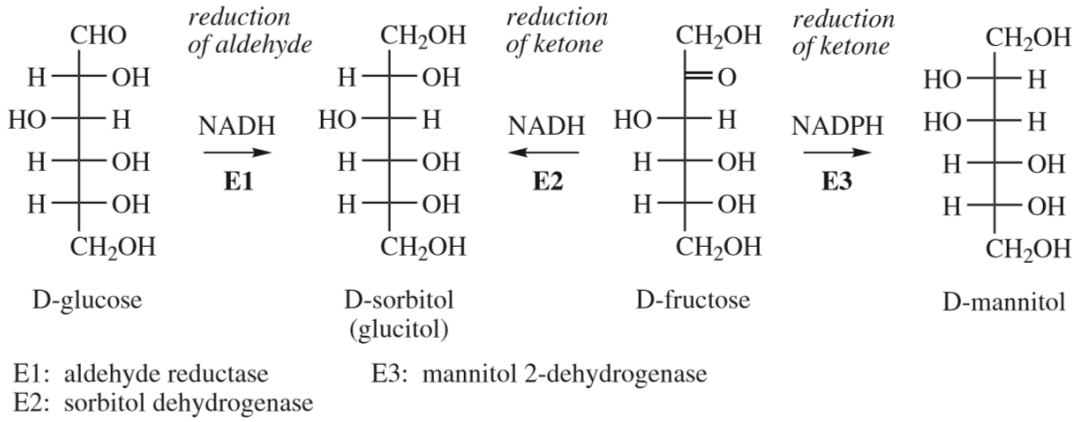


Figure (10): Some Monosaccharides structures

- Vitamin C (ascorbic acid) can be synthesized from glucose by most animals except humans, other primates, guinea pigs, bats, and some birds; for these it is obtained

via the diet. Citrus fruits, peppers, guavas, rose hips, and blackcurrants are especially rich sources, but it is present in most fresh fruit and vegetables.

Vitamin C deficiency leads to scurvy, characterized by muscular pain, skin lesions, fragile blood vessels, bleeding gums, and tooth loss. The vitamin C is essential for the formation of collagen, the principal structural protein in skin, bone, tendons, and ligaments.

Vitamin C does have valuable antioxidant properties, and these are exploited commercially in the food industries.

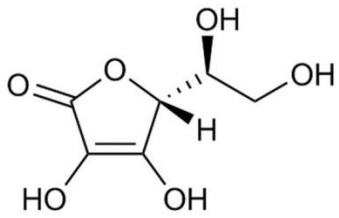


Figure (11): Ascorbic acid (Vit C)

1.2. Principal pharmaceutical polysaccharides and derivatives

1.2.1. Inulin:

Inulin is a linear chains of up to 50 β-1,2- linked fructofuranose units terminated by o single glucose unit; a relatively small polymer of the Compositae / Asteraceae and Campanulaceae.

Inulin BP is obtained from the tubers of *Dahlia variabilis*. *Helianthus tuberosus* and other genera of the Compositae; it derives its name from the dahlia, *Inula helenium*. from which it was first isolated in the 19th century. It is sparingly soluble in cold water but readily dissolves at around 70°C without gelatinizing.

Inulin is not metabolized by the body and is excreted unchanged. As Inulin injection it is used for the measurement of glomerular filtration rate.

Dondelion root The root of the dandelion (*Taraxacum officinale*) is an important drug of herbal medicine. Among other constituents it contains up to 20% of carbohydrates, particularly inulin, in the autumn and about 2 % inulin in the spring.

Chicory root (*Cichorium intybus*) is indigenous to Europe and is now widespread in northern states of the USA. Canada and parts of Asia it is widely cultivated. The dried roots contain a high proportion (up to 58%) of inulin together with sugars. Decoctions of the root are used as a diuretic and to treat liver ailments; the root is also cited as a tonic and laxative

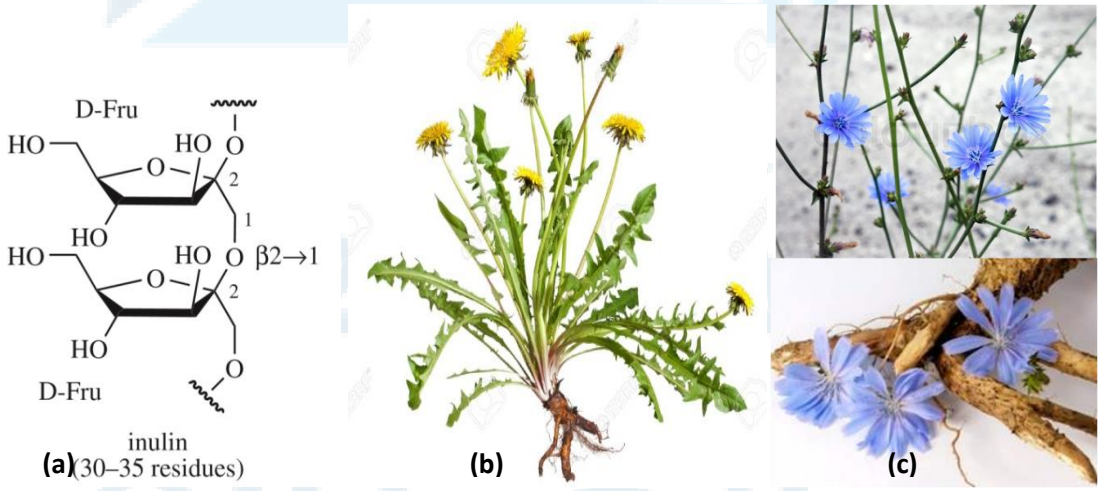


Figure (12): Inulin structure (a), *Taraxacum officinale* (b) & *Cichorium intybus* (c).

1.2.2. Pectin:

Pectins from different sources vary in their complex constitution, the principal components being blocks of D-galacturonic acid residues.

These occur in the middle lamellae of cell walls and are abundant in fruits {e.g. apples, oranges, and roots {gentian}. The parent substance protopectin is insoluble but is easily converted by restricted hydrolysis into pectinic acids (pectins).

Pectin is used as an emulsifier, gelling agent and also as a thickening agent. It is a major component of antidiarrhoeal formulation. Pectin is a protective colloid which assists absorption of toxin in the gastro-intestinal tract. It is used as haemostatic in cases of haemorrhage. As a thickener it largely used in the preparation of sauces, jams and ketchups in food industry.

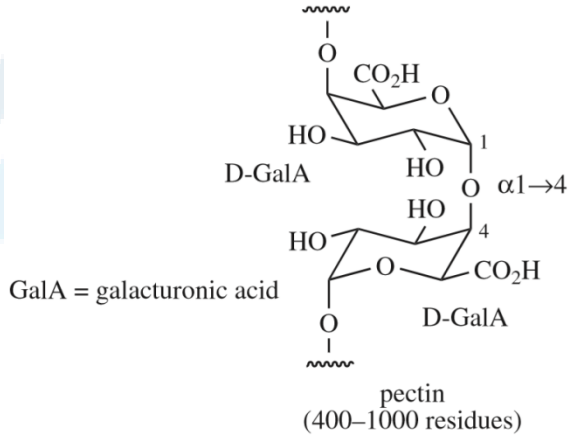


Figure (13): Pectin structure

1.2.3. Algal gelling agents

The two most important pharmaceutical products in this class are the alginates and agar

- Alginic acid is a linear polysaccharide formed principally by β1⟶4 linkage of d-mannuronic acid residues.

  Alginic acid: is obtained by alkaline (Na_2CO_3) extraction of a range of brown seaweeds, chiefly species of Laminaria (Laminariaceae) and Ascophyllum

(Phaeophyceae) in Europe, and species of Macrocystis (Lessoniaceae) on the Pacific coast of the USA.

The carbohydrate material constitutes 20–40% of the dry weight of the algae. The acid is usually converted into its soluble sodium salt or insoluble calcium salt.

Sodium alginate finds many applications as a stabilizing and thickening agent in a variety of industries, particularly food manufacture, and also the pharmaceutical industry, where it is of value in the formulation of creams, ointments, and tablets.

Calcium alginate is the basis of many absorbable haemostatic surgical dressings.

Alginic acid or alginates are incorporated into many aluminium- and magnesium-containing antacid preparations to protect against gastro-oesophageal reflux. Alginic acid released by the action of gastric acid helps to form a barrier over the gastric contents.

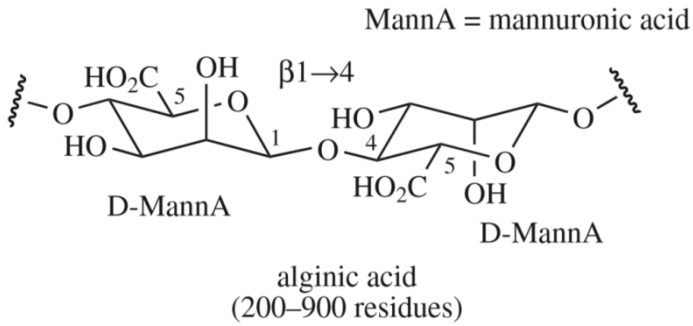


Figure (14): Algenic acid structure

- Agar is a carbohydrate extracted using hot dilute acid from various species of red algae (seaweeds), including *Gelidium* (Gelidiaceae) and *Gracilaria* (Gracilariaceae) from Japan, Spain, Australasia, and the USA. Agar's main application is in bacterial culture media, where its gelling properties are exploited. It is also used to some extent as a suspending agent and a bulk laxative.

- Carrageenan is a carbohydrate polymer extracted from the red alga *Chondrus crispus* (Gigartinaceae) (chondrus, or Irish moss) collected from Irish and other Atlantic coasts in Europe, also North America. Related species of algae, e.g. Gigartina, may also be used. They are widely used in the food and pharmaceutical industries as thickening agents. Laboratory studies suggest that carrageenans can function as topical microbicides, blocking sexually transmitted viruses such as human papillomavirus and herpes, though not HIV.

1.2.4. Gums and mucilages

Gums and mucilages have similar constitutions and on hydrolysis yield a mixture of sugars and uronic acids. Gums are considered to be pathological products formed upon injury of the plant or owing to unfavorable conditions, such as drought, by a breakdown of cell walls (extracellular formation; gummosis). Conversely mucilages are generally normal products of metabolism formed within the cell (intracellular formation) and may represent storage material, a water-storage reservoir or a protection for germinating seeds. They are often found in quantity in the epidermal cells of leaves. e.g. senna, in seed coats (linseed, psyllium etc.), roots (marshmallow) and barks (slippery elm).

- Acacia gum (gum arabic) is a dried gum from the stems and branches of the tree *Acacia senegal* (Leguminosae/Fabaceae), abundant in the Sudan and Central and West Africa. Trees are tapped by removing a portion of the bark. The gum is used as a suspending agent and as an adhesive and binder for tablets.

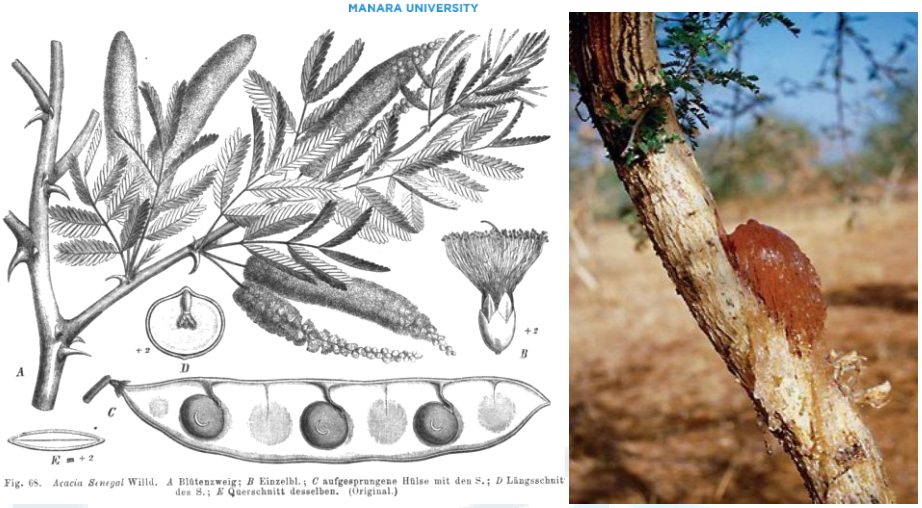


Fig. 68. *Acacia Senegal* Willd. *A* Blütenzweig; *B* Einzelbl.; *C* aufgesprungene Hülse mit den S.; *D* Längsschnitt des S.; *E* Querschnitt desselben. (Original.)

Figure (15): *Acacia Senegal*

- **Psyllium: (Flea Seed)**

The dried. ripe seeds of *Plantago afra*, *P. indica* and *P. ovata* (Plantaginaceae) are used in medicine. The seeds of *P. afra* and *P. indica* are known in commerce as Spanish or French psyllium, while those of *P. ovata* are known as blond psyllium, ispaghula. spogel seeds or Indian plantage seeds.

Uses: Plantago seeds are used as demulcents and in the treatment of chronic constipation. Ispaghula husk is used for similar purposes but has a higher swelling factor.

Figure (16):Psyllium *spp.*

- *Althea officinalis* (Marshmallow root)

Marshmallow root is derived from *Althea officinalis* (Malvaceae), a perennial herb which is found wild in moist situations in southern England and Europe. In general appearance it closely resembles the common hollyhock, *Althea rosea*. The plant has a woody rootstock from which arise numerous roots up to 30 cm in length.

Marshmallow root contains about 10% of mucilage. Marshmallow root and also the leaves, are used as demulcents. particularly for irritable coughs and throat and gastric inflammation.

Figure (17): *Althea officinalis.*

- Cetraria

Cetraria or Iceland moss, is a foliaceous lichen growing amidst moss and grass in central Europe, Siberia and North America. and on the lower mountain slopes of central Europe and Spain. For medicinal purposes it is usually collected in Scandinavia and central Europe.

The dried drug is brittle but becomes cartilaginous on moistening with water. Odour, slight, taste, mucilaginous and bitter. It is used as a demulcent for the treatment of

cough involving throat irritation. Iceland moss has been used as a bitter tonic and for disguising the taste of nauseous medicines.

Figure (18): Iceland moss

1.3. Aminosugars and Aminoglycosides

1.3.1. Chitin and derivatives

The structure of Chitin is rather similar to cellulose, though it is composed of amino sugar residues, N-acetylglucosamine linked β1⟶4.

Chitin is a major constituent in the shells of crustaceans, e.g. crabs and lobsters, and insect skeletons, and, as with cellulose, its strength again depends on hydrogen bonding between adjacent molecules, producing rigid sheets.

Chitosan: is obtained by chemical deacetylation of chitin, it can be used:

- in water purification (chelating properties and adsorption of pollutants)
- pharmaceutical excipient or drug carrier
- obesity treatment (lowering serum cholesterol and controlling obesity)
- and in wound-healing preparations, as It possess antibacterial activity as well as blood-clotting ability.

Glucosamine is usually obtained by hydrolysis of the shells from various shellfish, being liberated from the chitin component. It is used in cases of osteoarthritis, osteoporosis, as it stimulates the formation of collagen and joint function.

These polymers are biocompatible, biodegradable, and non-toxic compounds.

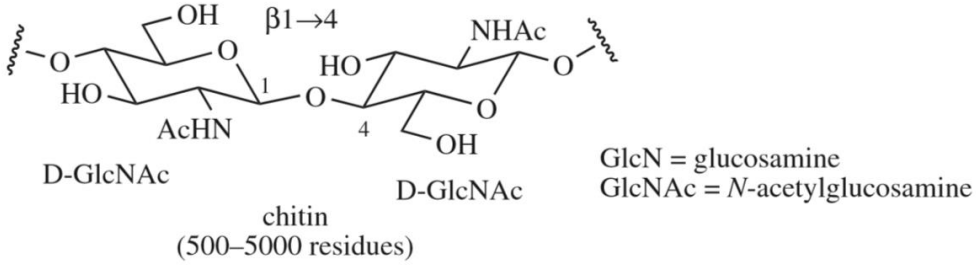


Figure (19): Chitin structure.

1.3.2. Chondroitin sulfate

Chondroitin is usually derived from pig or cow cartilage, though shark and fish cartilage is also used. It may possibly play a role as a protective agent in joints, and is thus used as a dietary supplement to minimize arthritis and cartilage problems. It is often combined with glucosamine, considered a potential precursor of beneficial cartilage glycosaminoglycans.

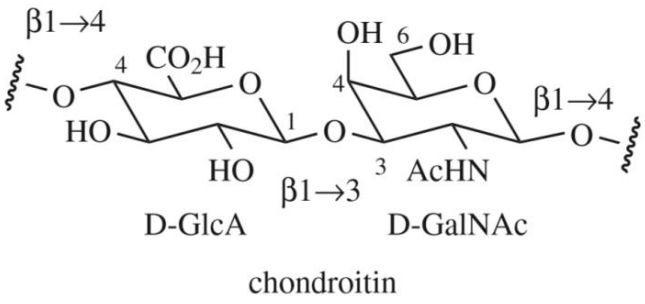


Figure (20): Chondroitin structure.

1.3.3. Heparin

The mammalian blood anticoagulant is also a carbohydrate polymer containing glucosamine derivatives.

Heparin is usually extracted from the intestinal mucosa of pigs or cattle, where it is present in the mast cells. It is a blood anticoagulant and is used clinically to prevent or treat deep-vein thrombosis. It is administered by injection or intravenous infusion and provides rapid action. It is also active *in-vitro*, and is used to prevent the clotting of blood in research preparations. Heparin acts by complexing with enzymes in the blood which are involved in the clotting process.

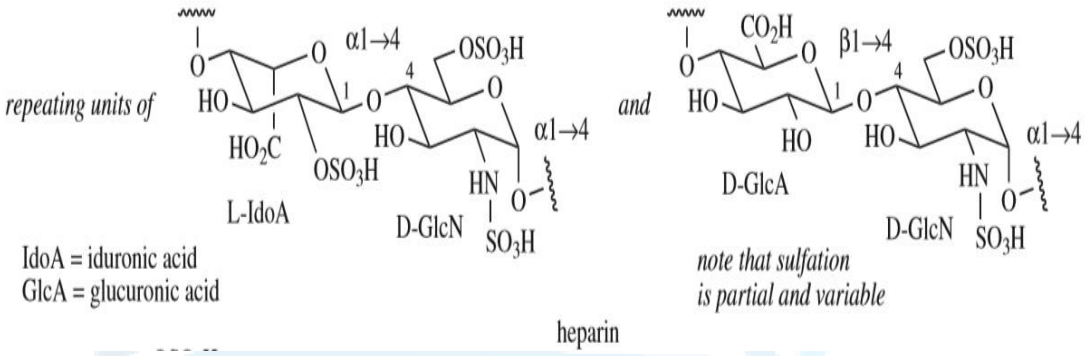


Figure (21): Heparin structure.

1.3.4. Aminoglycosides

The aminoglycosides form an important group of antibiotic agents and are immediately recognizable as modified carbohydrate molecules. Typically, they have two or three uncommon sugars, mainly aminosugars.

The first of these agents to be discovered was streptomycin from *Streptomyces griseus*.

The aminoglycoside antibiotics (gentamicin, tobramycin, kanamycin, neomycin, amikacin, and netilmicin), have a wide spectrum of activity, including activity against some Gram-positive and many Gram-negative bacteria.

They are not absorbed from the gut, so for systemic infections they must be administered by injection. However, they can be administered orally to control intestinal flora. The widespread use of aminoglycoside antibiotics is limited by their nephrotoxicity, which results in impaired kidney function, and by their ototoxicity, which is a serious side-effect and can lead to irreversible loss of hearing.

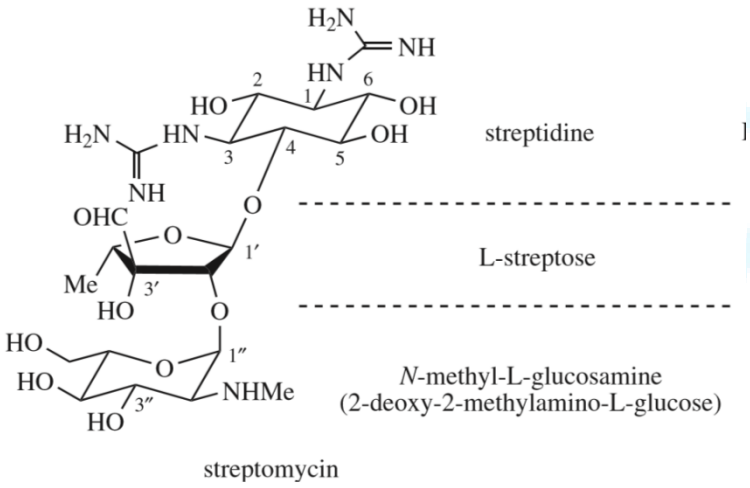


Figure (22): Streptomycin structure

PHENOLIC COMPOUNDS

Phenolic compounds represent a large group of molecules with a variety of functions in plant growth, development, and defense. Phenolic compounds include signaling molecules, pigments and flavors that can attract or repel, as well as compounds that can protect the plant against insects, fungi, bacteria, and viruses. Most phenolic compounds are present as esters or glycosides rather than as free compounds. Tannins and lignin are phenolic polymers.

Definition:

The term phenolics covers a very large and diverse group of chemical compounds. They are compounds that have one or more hydroxyl groups attached directly to an aromatic ring; though some non-phenolic compounds may have hydroxyl groups on a benzene ring e.g. morphine. The accurate classification should be based on the biosynthesis pathway of these compound. Yet it is difficult to find a hypothesis which would fit all cases of phenolic.

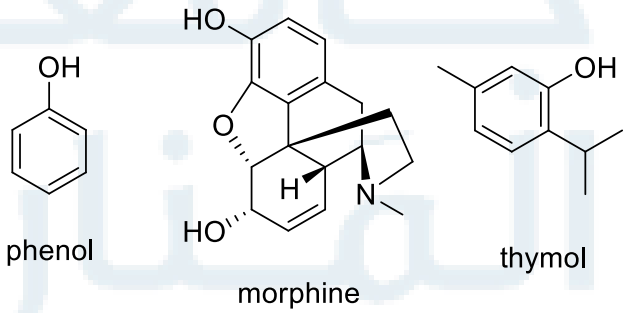


Figure (3.1): Phenolic and non-phenolic compounds

The term phenolics covers a very large and diverse group of chemical compounds. These compounds can be classified in a number of ways. Harborne and Simmonds (1964) classified these compounds into groups based on the number of carbons in the molecule.

Table (3.1): Classification of phenolic compounds

Structure	Class
C_6	simple phenolics
C_6-C_1	phenolic acids and related compounds
C_6-C_2	acetophenones and phenylacetic acids
C_6-C_3	cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols
C_6-C_3	coumarins, isocoumarins, and chromones
C_{15}: C_6-C_3-C_6	chalcones, aurones, dihydrochalcones
C_{15}: C_6-C_3-C_6	flavanoides
C_{30}	biflavonyls
C_6-C_1-C_6 C_6-C_2-C_6	benzophenones, xanthones, stilbenes
C_6, C_{10}, C_{14}	quinones
C_{18}	betacyanins
Lignans	dimers or oligomers
Tannins	oligomers or polymers

1. BIOSYNTHESIS OF PHENOLIC COMPOUNDS

1.1. The shikimate pathway

The shikimate pathway provides a route to aromatic compounds, particularly the aromatic amino acids L-phenylalanine, L-tyrosine, and L-tryptophan. This pathway is employed by microorganisms and plants, but not by animals; accordingly, the aromatic amino acids feature among the essential amino acids for man and animals have to be obtained in the diet.

Phenylalanine and tyrosine form the basis of C_6C_3 phenylpropane units found in many natural products, e.g. cinnamic acids, coumarins, lignans, and flavonoids, and along with tryptophan are precursors of a wide range of alkaloid structures.

The shikimate pathway begins with a coupling of phosphoenolpyruvate (PEP) and d-erythrose 4-phosphate to give the central intermediate in the pathway (shikimic acid).

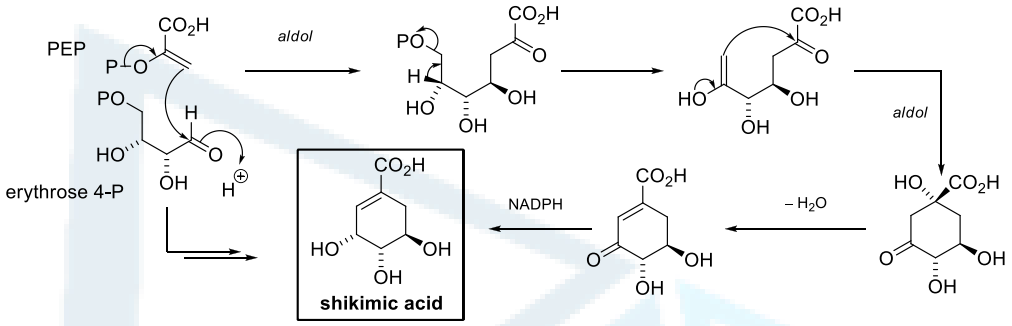


Figure (3.1): Biosynthesis of shikimic acid.

A very important branchpoint compound in the shikimate pathway is chorismic acid, a precursor of the important class of phenolic compounds

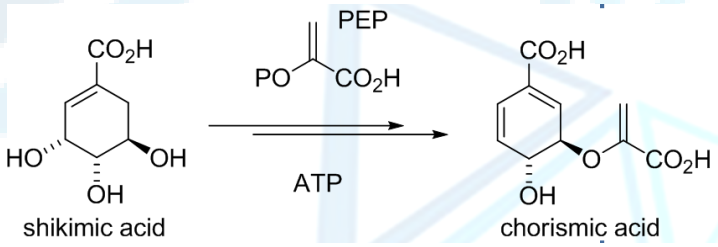


Figure (3.2): Biosynthesis of chorismic acid

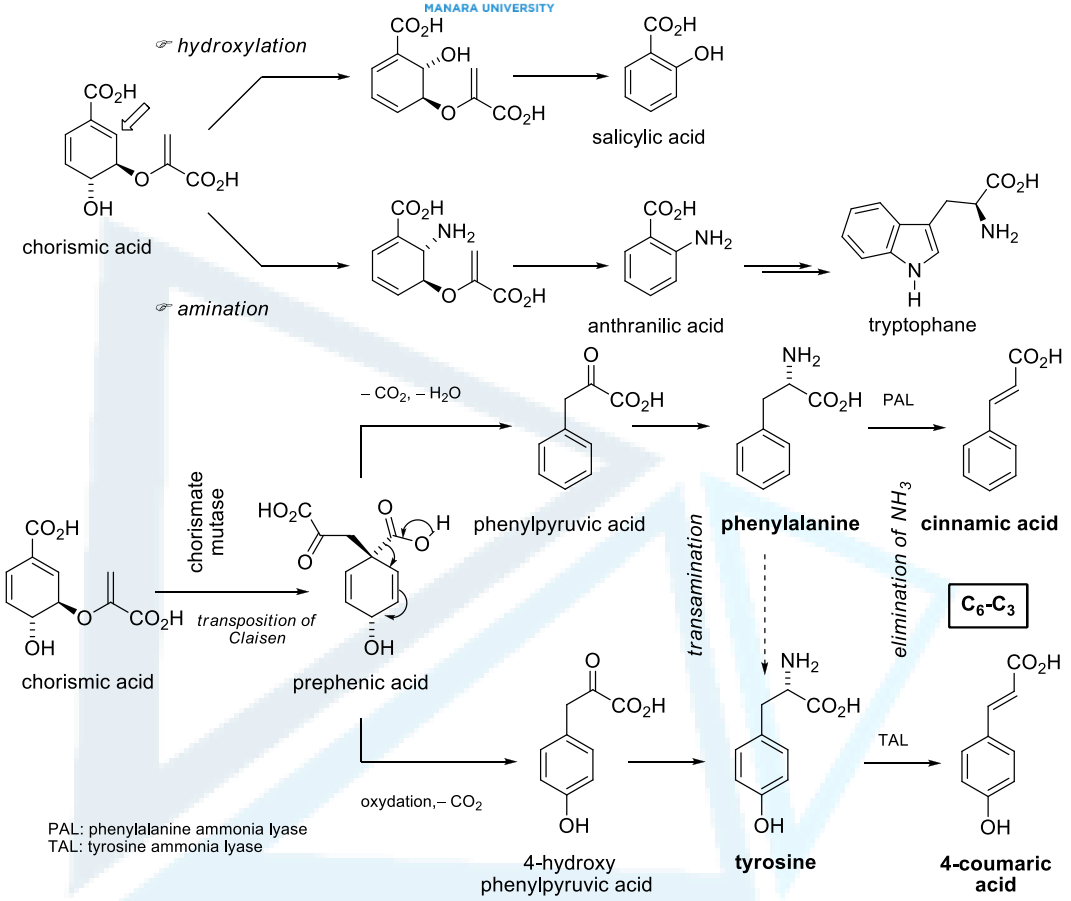


Figure (3.3): Biosynthesis pathway of phenolic compounds.

1.2. The acetate pathway

Two molecules of acetyl-CoA could participate in a Claisen condensation giving acetoacetyl-CoA, and this reaction could be repeated to generate a poly-β-keto ester of appropriate chain length

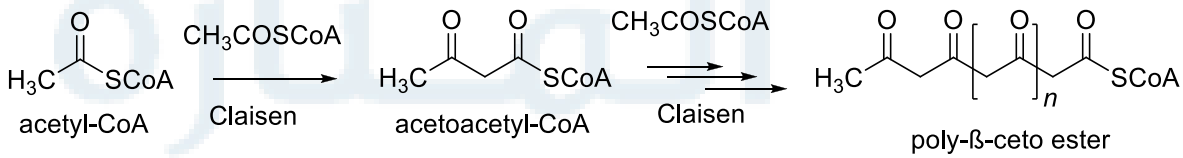


Figure (3.4): Claisen condensation of acetyl-CoA.

A poly-β-keto ester is very reactive, and there are various possibilities for undergoing intramolecular Claisen or aldol reactions leading to the formation of the aromatic cycles.

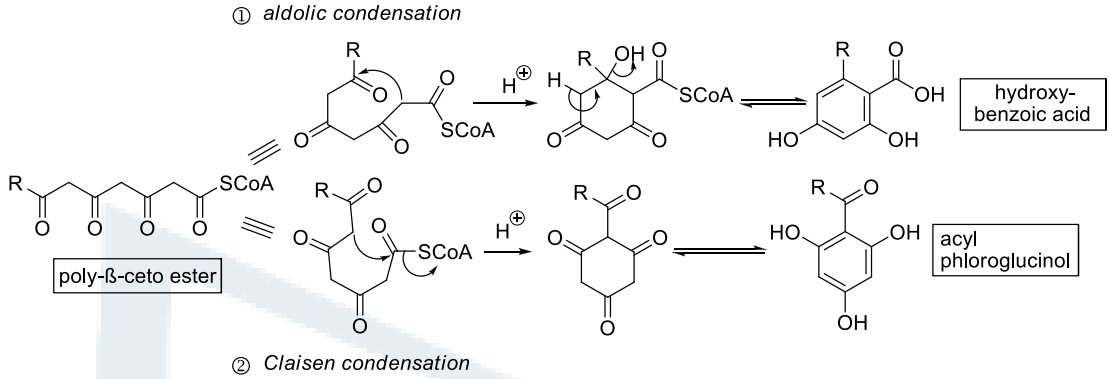


Figure (3.5): Aromatic compounds synthesized by the acetate pathway

2. PHYSICO-CHEMICAL PROPERTIES OF PHENOLIC COMPOUNDS

Since phenol is benzene with a hydroxyl group, the reactivity of phenol and phenolic compounds is in many ways dictated by the chemical properties of the benzene ring.

2.1. The acidic nature of the phenolic hydroxyl group

The first property to consider is acidity. A compound is considered an acid when it can release a proton (H^+) while in solution.

Phenolic compounds are, in general, weak acids. Compared to the hydroxyl group of unsubstituted aliphatic alcohols, however, the phenolic OH-group is more acidic. The reason for this is that the anion formed after abstracting the proton from the hydroxyl group is relatively stable because of the existence of several mesomeric structures. The anion is referred to as the phenolate anion. Hence, phenol is a weak acid, with a pKa value of 10. This places phenol in between carboxylic acids (pKa = 4-5) and aliphatic alcohols (pKa = 16-19).

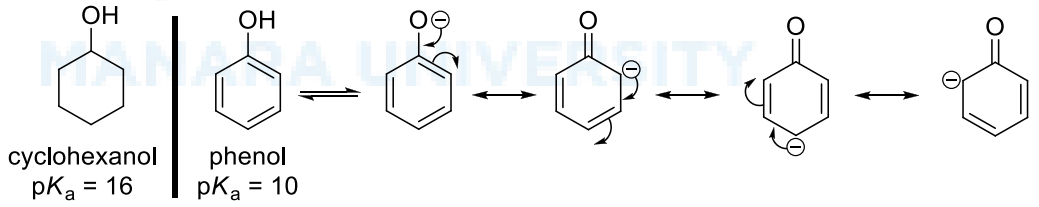


Figure (3.6): The acidic nature of the phenolic function

2.2. The antioxidant activity of phenolic compounds

Phenolic compounds are considered as a good reducing agent that prevents oxidation of other molecules, compounds that can scavenge radicals are also referred to as antioxidants.

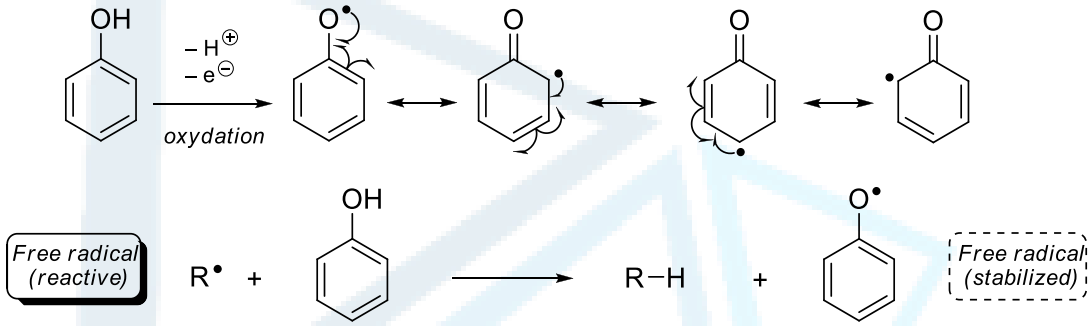


Figure (3.7): The acidic nature of the phenolic function

2.3. Hydrogen bonding and the phenolic hydroxyl group

The hydrogen bond is an electrostatic interaction between a hydrogen atom bound to an electronegative atom such as oxygen, fluorine or nitrogen, and the free electrons of other atoms. Phenolic compounds may form both inter- and intra-molecular hydrogen bonds.

The presence of hydrogen bonds raises the melting and boiling points of compounds, because more energy is required to break intermolecular bonds. The presence of hydrogen bonds can alter the UV and IR spectra of a given compound.

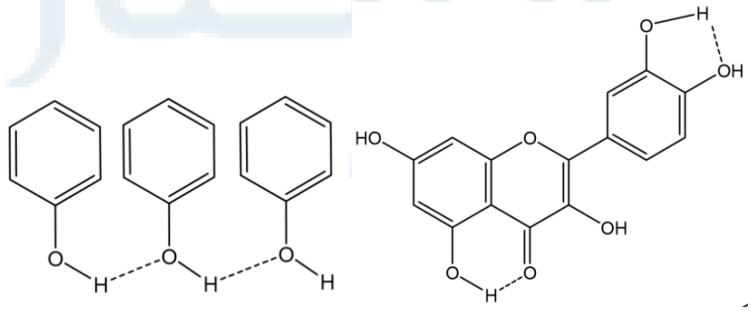


Figure (3.8): Inter- and intra-molecular hydrogen bonds of the phenolic hydroxyl group

2.4. Esterification:

Esters (RCOOR) are formed by reaction of a carboxylic acid with the hydroxyl group of an alcohol. The hydroxyl group of phenolic compounds can participate in ester formation.

2.5. Ethers and glycosides

Ethers (R-O-R) are frequently found as natural products in nature. The most common ether is that of methanol and the phenolic hydroxyl group.

Glycosides formed between a sugar molecule and an alcohol, are in some sense similar to ethers.

2.6. Metal complexes

Several structures are capable of forming metal complexes like iron, aluminum and magnesium ions.

Metal complexes are used for compound identification. They can shift or change absorption spectra.

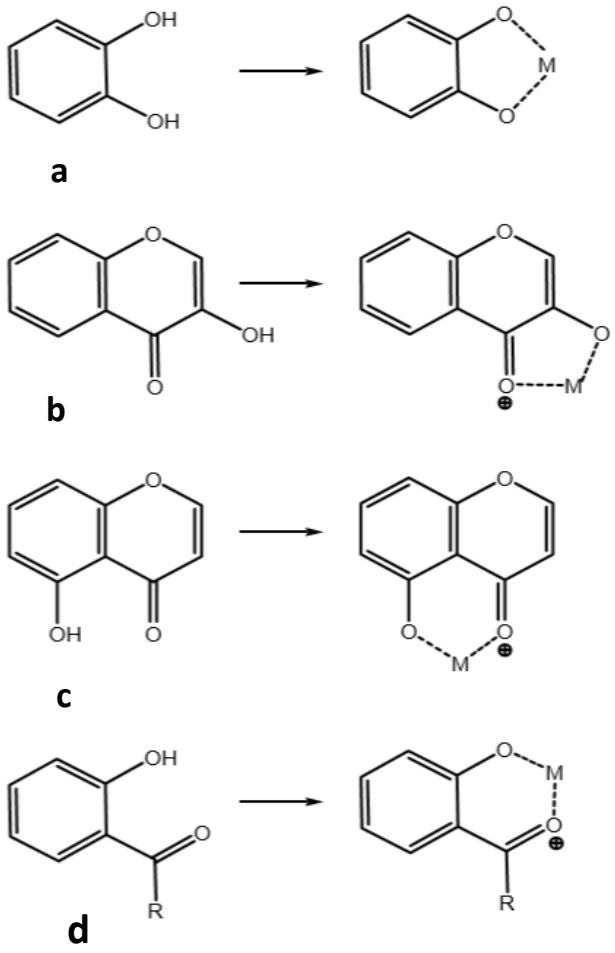


Figure (3.9): Metal complexes of phenolic compounds.

3. CHEMICAL TESTS FOR PHENOLIC COMPOUNDS

- Ammonia test Filter paper dipped in alcoholic solution of drug was exposed to ammonia vapor. Formation of yellow spot on filter paper.
- Shinoda test To the alcoholic extract of drug magnesium turning and HCl was added, formation of red colour indicates the presence of flavonoids.
- test: to the alcoholic extract of drug zinc turning and HCl was added, formation of deep red to magenta colour indicates the presence of dihydro flavonoids.

- Vanillin HCl test: Vanillin HCl was added to the alcoholic solution of drug, formation of pink colour due to presence of flavonoids.
- $FeCl_3$ test: To the concentrated alcoholic extract of drug few drops of alcoholic $FeCl_3$ solution was added to give different coloration according the phenolic derivatives presented in the extract.

4. CLASSES OF PHENOLIC COMPOUNDS

They range from simple structures with one aromatic ring to highly complex polymeric substances such as tannins and lignins. Phenols are important constituents of some medicinal plants and in the food industry they are utilized as colouring agents, flavourings, aromatizers and antioxidants.

This chapter mainly deals with those phenolic classes of pharmaceutical interest, namely: (1) simple phenolic compounds, (2) coumarins and their glycosides, (3) flavone and related flavonoid glycosides, (4) tannins, (5) anthocyanidins and anthocyanins (6) quinones and their glycosides (naphthoquinones + anthraquinones) and (7) lignans and lignin.

4.1. Simple phenolics and phenolic acids

The phenolic compounds in this group often also possess alcoholic, aldehydic and carboxylic acid groups; Glycoside formation is common in nature.

4.1.1. Simple phenolics

Simple phenolics are substituted phenols. The *ortho, meta* and *para* nomenclature refers to a 1,2-, 1,3- and 1,4-substitution pattern of the benzene ring, respectively, where in this case one of the functional groups is the hydroxyl group.

Examples include quinol (hydroquinone, *p*-dihydroxybenzene), resorcinol (1,3-dihydroxybenzene), a *meta*-dihydroxylated simple phenolic, and phloroglucinol (1,3,5-trihydroxybenzene), a *meta*-trihydroxylated simple phenolic.

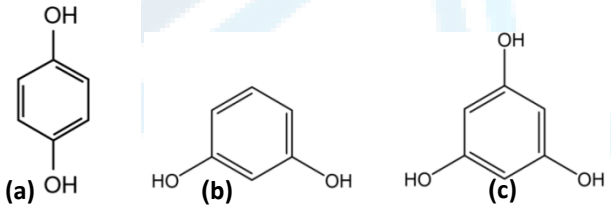


Figure (3.10): Structure of hydroquinone (a), resorcinol (b) and phloroglucinol (c).

- Resorcinol: is commonly used in hair dyes and acne medication. In higher doses it is toxic and can disrupt the function of the central nervous system and lead to respiratory problems. It has also been shown to disrupt the endocrine system, specifically thyroid function.
- Phloroglucinol: The taenicidal constituents of male fern, the bitter principles of hops and the lipophilic components of hypericum are phloroglucinol derivatives. Both terpenoids and phenols bathways are involved in the synthesis of the phloroglucinol derivatives.

4.1.1.1. BEARBERRY LEAVES (UVA URSI)

Bearberry leaf EP/BP/BHP consists of the dried leaves of *Arctostaphylos uva-ursi*, Ericaceae. *A. uva-ursi* is a small evergreen shrub found in central and northern Europe and in North America. The obovate leaves are dark green to brownish-green, 2–3 cm long, The drug is odourless but has an astringent and somewhat bitter taste.

Constituents: Bearberry contains the glycosides arbutin and methyl-arbutin (hydroquinone derivatives), about 6–7% of tannin and the flavone derivative quercetin. The pharmacopoeial drug is required to contain at least 7.0% of hydroquinone derivatives calculated as arbutin.

Uses: Bearberry is diuretic and astringent and during excretion it exerts an antiseptic action on the urinary tract.

Figure (3.11): *Arctostaphylos uva-ursi*

4.1.2. Phenolics acid and aldehydes C_6-C_1

Hydroxy-benzoic acids are characterized by the presence of a carboxyl group substituted on a phenol. Examples include *p*-hydroxybenzoic acid, gallic acid, protocathechuic acid, salicylic acid and vanillic acid. Related are

hydroxybenzoic aldehydes, such as vanillin, which have an aldehyde group instead of a carboxyl group.

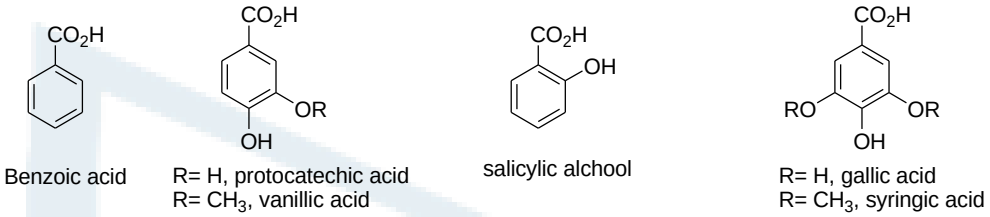


Figure (3.12): Structures of some phenolic acids derivatives C_6-C_1

4.1.2.1. Meadowsweet (*Filipendula ulmaria*)

Meadowsweet BP/EP, Filipendula BHP 1983 consists of the dried flowering tops of *Filipendula ulmaria* (L.) Maxim. [*Spirea ulmaria* L.], family Rosaceae.

This well-known perennial plant is found in wet meadows, marshes, by rivers, etc. throughout most of Europe, temperate Asia and as an escape in the eastern US and Canada. It is up to 120 cm in height with numerous radical longish petioled leaves. Each leaf is composed of up to five pairs of ovate serrated leaflets. Numerous aromatic cream-coloured flowers form irregular cymose panicles, which are particularly dense on the terminal branches of the leafy stems.

Figure (3.13): *Filipendula ulmaria*

Constituents: The BP/EP requires a minimum concentration of 0.1% for the steam volatile fraction of Meadowsweet. The major component of the oil (up to ca 70%) is salicylaldehyde together with methyl salicylate, benzaldehyde, benzyl alcohol, and smaller amounts of other components such as vanillin

Action and uses: The BP/EP cites meadowsweet as a diuretic; traditionally it has also been used for its anti-inflammatory, astringent and stomachic properties.

4.1.2.2. Willow bark (*Salix purpurea* L.)

The bark of various species of *Salix* which include *S. purpurea* L., (purple willow) and *S. fragilis* L. (crack willow, salicaceae) is a source of phenolic compounds (BP/EP, BHP and ESCOP).

Figure (3.14): *Salix* sp. botanical illustration.

Constituents: The composition of the glycoside mixture is variable in the bark depending on species, age of bark and time of collection. The later is usually made in spring when the bark is easily removed from the branches. Willow bark is a source of salicin (fig. 3.14), a phenolic glycoside now seldom used but generally

regarded as the natural forerunner of aspirin. Other phenolic glycosides are salicortin (an ester of salicin), acetylated salicin (fragilin) and salicortin.

During drying, salicortine is hydrolyzed by the temperature and liberates the (salicoside).

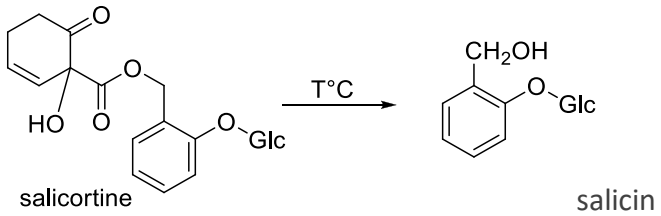


Figure (3.15): Hydrolysis of Salicortine

Action and uses: Willow is employed as an anti-inflammatory in the treatment of rheumatism, arthritis and muscular pains. The salicylic glycosides are hydrolyzed in the intestines to release the salicylic alcohol, which is directly oxidized into salicylic acid, responsible of the anti-inflammatory activity.

4.1.3. Phenolics acid C_6-C_3 cinnamic acids derivatives:

There are five common cinnamic acids, which have a C_6-C_3 skeleton. All plants probably contain at least three of them. Shown below are cinnamic acid, *p*-coumaric acid, caffeic acid, ferulic acid and cinapic acid.

Cinnamic acids are commonly found in plants as esters e.g chlorogenic acid (fig. 3.17) which is an ester of caffeic acid and quinic acid.

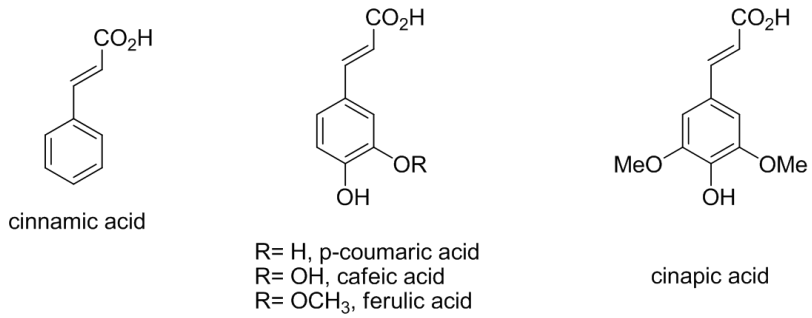


Figure (3.16): Common cinnamic acids.

4.1.3.1. Artichoke leaf (*Cynara scolymus L.*)

The leaves of the globe artichoke, *C. scolymus* L., family Asteraceae/Compositae, have been long-used in traditional medicine and are now included in the BP/EP, the BHP and the Complete German Commission E Monographs. The plant is native to the Mediterranean region and northern Africa.

Leaves, up to ca 70 cm long and 30 cm wide, are collected and dried just before the flowering stage.

Figure (3.17): *Cynara scolymus L.*

<u>Constituents:</u> Phenolic acids are important constituents and include chlorogenic acid, caffeic acid and cynarin (1, 5-di-*O*-caffeoylquinic acid) (see Fig. 3.17). The BP specifies a minimum requirement for chlorogenic acid of 0.8%. Other constituents include flavonoides, volatile oil, sesquiperpene lactones, e.g. cynaropicrin, inulin, tannins and phytosterols.

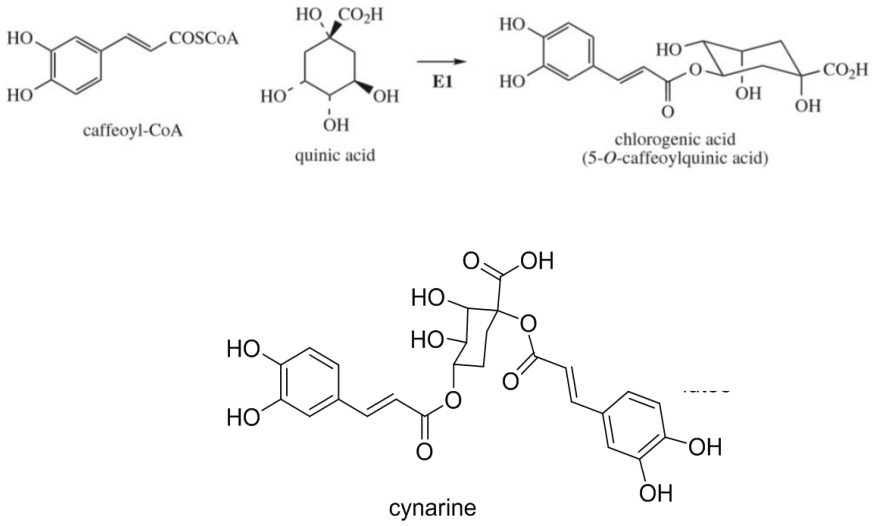


Figure (3.18): chlorogenic acid and cynarine.

Action and uses: Artichoke leaf is used for the treatment of indigestion and dyspepsia; for its use as a hepatic protector.

The drug being used principally as a cholagogue (promotion of emptying of the gall bladder and bile ducts). There has been more recent interest in the hepatoprotective properties of the plant and significant antioxidative activity has been demonstrated involving chlorogenic acid and cynarin.

4.1.3.2. Rosemary leaf (*Rosmarinus officinalis L.*)

Rosemary leaf BP/EP, BHP is the whole dried leaf of *Rosmarinus officinalis* L., family Labiatae. The plant is native to Mediterranean regions and is widely cultivated elsewhere in herb gardens and as an aromatic ornamental.

***R. officinalis* is an aromatic evergreen shrub, variable in its form, but mostly with stems reaching a height of over 1 m. The bilobed corollas of the flowers are pale to dark blue and occur clustered in spikes at the ends of the branches.**

Figure (3.19): *Rosmarinus officinalis*

Constituents: The composition of the essential oil is considered under 'Rosemary Oil', below. Hydroxycinnamic acids include caffeic acid and a dimer rosmarinic acid. For the dried leaf, the BP sets a minimum requirement of 3.0% for total hydroxycinnamic acids expressed as rosmarinic acid.

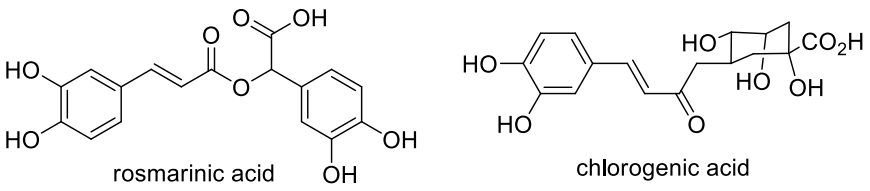


Figure (3.20): rosmarinic acid and chlorogenic acid.

Uses: Rosemary leaves have many traditional uses based on their antibacterial, carminative and spasmolytic actions. in addition to antioxidative, hepatoprotector and cholagogue activity.

4.1.3.3. Tolu balsam *Myroxylon balsamum* L.

Tolu Balsam is obtained by incision from the trunk of *Myroxylon balsamum* (L.) Harms. var. *balsamum* (Leguminosae).

Figure (3.21): *Myroxylon balsamum*

Collection: The drug is collected by making V-shaped incisions in the bark, the secretion being received in a calabash placed in the angle of the V.

Characters: When freshly imported, tolu is a soft, yellow semisolid. On keeping it turns to a brown, brittle solid. It softens on warming, and if a little is then pressed between two glass slides. Odour is aromatic and fragrant; taste, aromatic; the drug forms a plastic mass when chewed.

Constituents: Tolu contains about 80% of resin derived from resin alcohols combined with cinnamic and benzoic acids. The drug is rich in free aromatic acids and contains about 12–15% of free cinnamic and about 8% of free benzoic acid. Other constituents are esters such as benzyl benzoate and benzyl cinnamate and a little amount of vanillin. Total balsamic acids are about (BP/EP, 25–50%).

Uses: Balsam of Tolu has antiseptic and flavouring properties and is commonly added to cough mixtures in the form of a syrup of tincture.

4.1.3.4. Peru balsam

Balsam of Peru is obtained from the trunk of *Myroxylon balsamum* var. *pereirae* (Fabaceae/Leguminosae), after it has been beaten and scorched, a large tree that differs but little from that yielding balsam of tolu.

The drug is produced in Central America (San Salvador, Honduras and Guatemala) and is now included in the European Pharmacopoeia and the BP (2000).

Characters: Balsam of Peru is a viscid liquid of a somewhat oily nature, but free from stickiness and stringiness. When seen in bulk, it is dark brown or nearly black in colour, but in thin layers it is reddish-brown and transparent. The balsam has a pleasant, somewhat vanilla-like odour and an acrid, slightly bitter taste.

Constituents: The official drug is required to contain not less than 45.0% w/w and not more than 70% w/w of esters, The chief balsamic esters present are benzyl cinnamate (cinnamein), benzyl benzoate and cinamyl cinnamate (styracin). The drug also contains about 28% of resin.

Uses: Balsam of Peru is used as an antiseptic dressing for wounds and as a parasiticide. Now it is of less current interest in Western medicine. Taken internally it is used to treat catarrh and diarrhoea. Allergic responses are possible.

4.2. Coumarins and their glycosides

Coumarins also have a C_6-C_3 skeleton, but they possess an oxygen heterocycle as part of the C_3-unit. They are benzo-α-pyrone derivatives of such as coumarin (the lactone of O-hydroxycinnamic acid).

Coumarins are wildly distributed in plants both in the free state and as glycosides; and are commonly found in families such as the Umbelliferae/Apiaceae and Rutaceae. Many of these compounds play a role in disease and pest resistance, as well as UV-tolerance.

4.2.1. Biosynthesis of Coumarins

ortho-Hydroxycinnamic acids undergo intramolecular esterification to yield lactones that are called coumarins. Cinnamic acid and 4-coumaric acid give rise to the coumarin and umbelliferone (7-hydroxycoumarin) respectively.

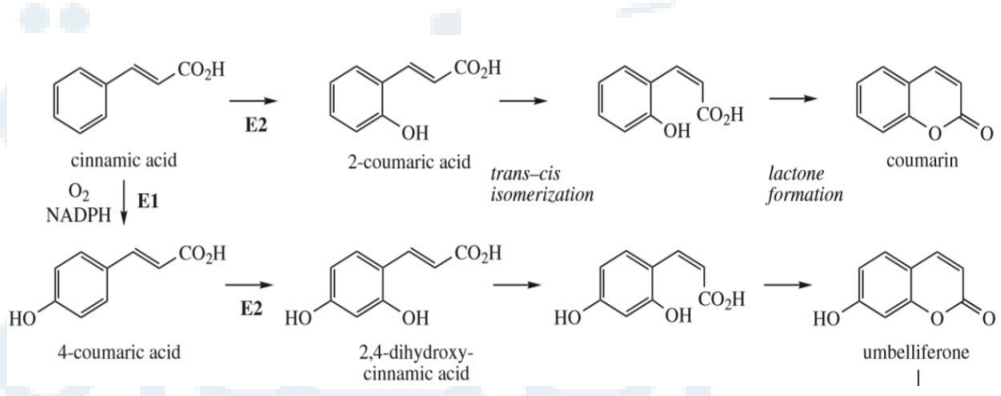


Figure (3.22): Biosynthesis of coumarins.

4.2.2. Physic-chemical properties of coumarins derivatives

- Coumarins, in the free state, are soluble in alcohol and low polar organic solvent, but can be extract by steam distillation.

- These compounds in ammoniacal solution have a blue, blue–green or violet fluorescence.

4.2.3. Biological properties of coumarins derivatives

- Considered as Vitamin P, coumarins increase capillary resistance and decrease capillary permeability.
- More complex coumarins such as the calanolides have received recent attention as potent HIV-1-RT inhibitors (HIV-1- reverse transcriptase).
- Furanocoumarins increase oral bioavailability of various drugs used to treat cancer, hypertension, heart disease and allergies, as coumarins inactivate the cytochrome P450 enzymes (specifically CYP3A4 and CYP3A5); for example modifying drug availability resulting from the consumption of grapefruit juice (dihydroxybergamotin).
- Furanocoumarins are strong photosensitizers, used in the photochemotherapy (PUVA treatment, psoralen and long ultraviolet irradiations) for the treatment of vitiligo and psoriasis. The psoralen absorbs in the near UV and allows this radiation to stimulate formation of melanin pigments where severe blemishes exist (vitiligo).

  These applications were limited due to their risk of skin cancer and hepatotoxicity.
- Reaction with psoralens inhibits DNA replication and reduces the rate of cell division. Because of their planar nature, psoralens intercalate into DNA, and this enables a UV-initiated cycloaddition reaction between pyrimidine bases (primarily thymine) in DNA and the furan ring of psoralens; A second cycloaddition can then

occur, this time involving the pyrone ring, leading to interstrand cross-linking of the nucleic acid.

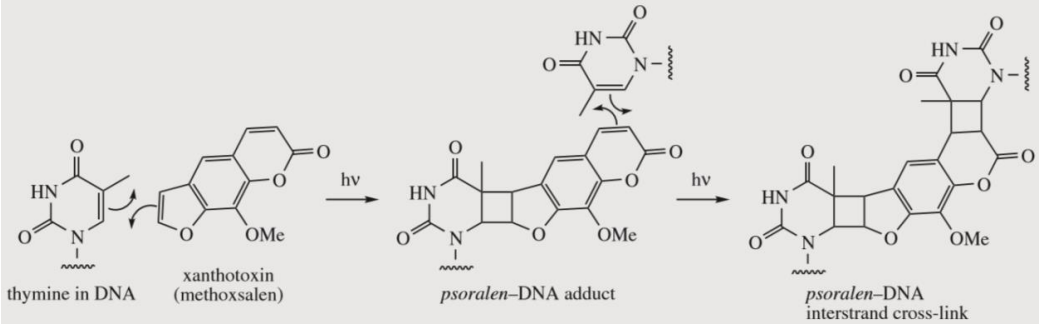


Figure (3.23): cross-linking of the nucleic acid by the furocoumarin.

- Bicoumarins and anticoagulants:

Bicoumarins are formed from two coumarin moieties and the linkage may occur in a number of ways.

Dicoumarol is formed at C3–C3′ through a methylene group; It is a constituent of fermenting hay and is formed by microbial action of coumarin (infected plant). It is a powerful anticoagulant and haemorrhagic and can cause the death of animals (rodenticides) consuming the spoiled fodder.

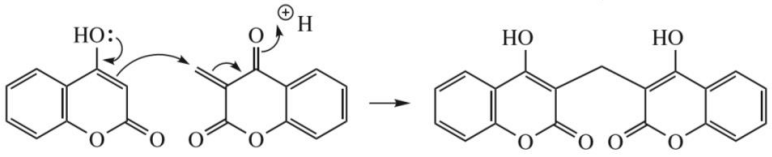


Figure (3.24): Occurrence of dicoumarol

This agent interferes with the effects of vitamin K in blood coagulation; Synthetic dicoumarol, like Warfarin, has been used as an oral blood anticoagulant in the treatment of thrombosis. Warfarin was initially developed as a rodenticide and has been widely employed for many years as the first-choice agent.

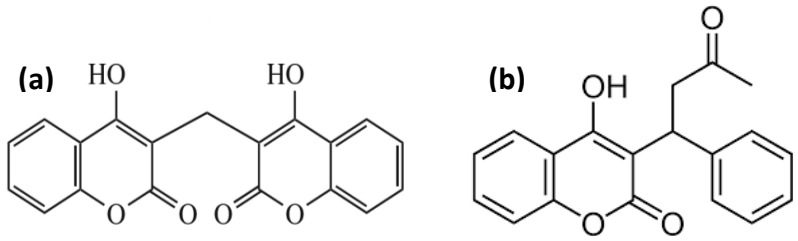


Figure (3.25): dicoumarol (a) & Warfarin (b)

4.2.4. Classification of coumarins derivatives

4.2.4.1. Simple coumarins derivatives

The simple coumarins are derived of umbelliferone by a simple modification, with additional oxygen substituents on the aromatic ring, e.g. aesculetin (esculetin) and scopoletin.

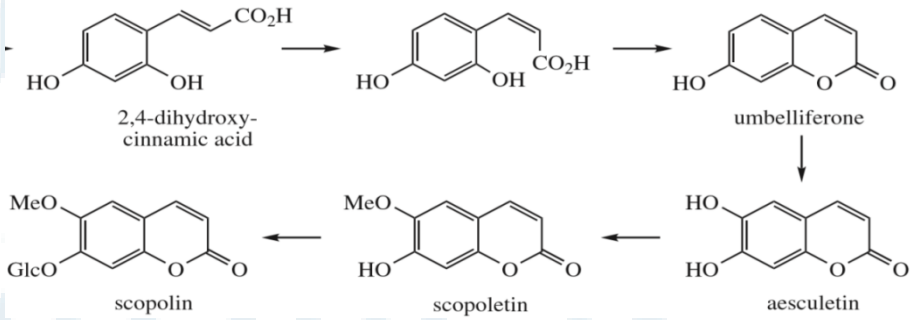


Figure (3.26): Simple coumarins derivatives

4.2.4.2. Furanocoumarins derivatives

The biosynthesis of such compound is initiated by the prenylation (alkylation) of coumarins in position *ortho* of the hydroxyl function C-7; then an oxidative cyclization occurs to form the condensed furano-cycle.

According to the alkylation position we can distinguish the following furocoumarin:

- Linear furocoumarin (Psoralens): the prenylation occurs in C-6 leading to the formation of psoralen, the precursor of linear furocoumarins Figure (3.30).

- Angular furocoumarin: the synthesis of these compounds start with the prenylation on the alternative *ortho* position C-8 of the hydroxyl function, leading to the formation of the angelicin the precursor of angular furocoumarins Figure (3.29).

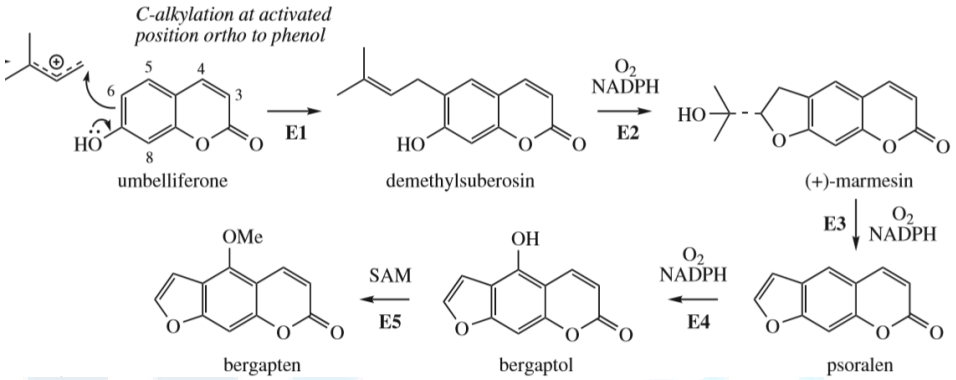


Figure (3.27): Biosynthesis of furocoumarins.

Psoralens are linear furocoumarins which are widely distributed in plants, but are particularly abundant in the Umbelliferae/Apiaceae and Rutaceae. The most common examples are psoralen, bergapten are xanthotoxin Figure (3.27).

Bergamot oil obtained from the peel of *Citrus aurantium* ssp. *bergamia* (Rutaceae) can contain up to 5% bergapten Figure (3.31) and is frequently used in external suntan preparations.

These alkylation reactions between can cause acute dermatitis, with persistent hyperpigmentation, in the case where contact with one or other of these plants is followed by exposure to solar UV; This may be the case Cologne (containing Bergamot from *Citrus bergamia*, Rutaceae), or, mainly, Apiaceae or Rutaceae, in addition to the only Moraceae member.

4.2.5. Natural Drugs containing Coumarins

4.2.5.1. Horse chestnut bark

Horse chestnut, *Aesculus hippocastanum* (Hippocastanaceae) are native tree to western Asia. It is now widely distributed over the world as an ornamental.

Constituents: The bark contain coumarins (esculoside), flavones and tannins; and all of these compounds contribute to the pharmacological effect of this drug.

Figure (3.28): *Aesculus hippocastanum*

Uses: The bark of horse chestnut was used in folk medicine, but now it is no longer in common use and is replaced by the seeds. The esculoside and his semi-synthetic derivative methesculetol have been used in the treatment of peripheral vascular disorders including haemorrhoids, varicose veins. Tannins tone the blood vessel walls and flavonoids are anti-inflammatory.

Horse chestnut bark is contraindicated with anticoagulants such as warfarin, due to the coumarins content.

4.2.5.2. Melilot (dried flowering tops)

Melilot BP/EP, BHP 1996 consists of the dried flowering tops of *Melilotus officinalis* L., (common melilot, ribbed melilot, king's clover, yellow sweet clover), family Leguminosae/Fabaceae.

It is found throughout Europe and eastwards to western China, N. America, except the far north, and elsewhere often as a weed of cultivation. Habitats include fields, hedgerows and waste places.

Melilot is an erect or decumbent branched biennial up to 100 cm tall with alternate stalked trifoliate leaves. The yellow papillionaceous flowers occur in racemes up to 5 cm in length and give rise to almost straight glabrous pods.

Figure (3.29): *Melilotus officinalis*

Constituents: Coumarin derivatives occur in melilot although coumarin itself is not present to any extent in the living plant. It arises when the plant is crushed, or the dried material treated with water, by the action of enzymes giving first the unstable hydrolytic product coumarinic acid, which then cyclizes to coumarin producing the well-known 'new-mown hay' odour.

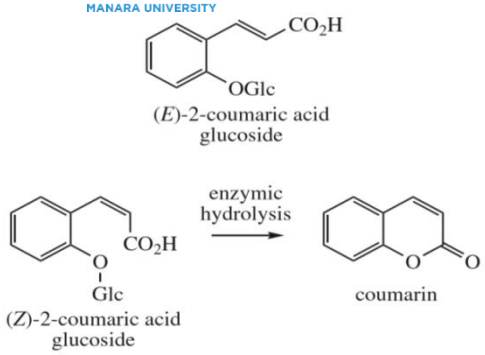


Figure (3.30): Occurrence of coumarins in *Melilotus officinalis*

Other acids isolated from melilot include melilotic acid, caffeic acid and other minor acids. Various oleanene saponins, volatile compounds and flavonoids have also been reported.

Uses: The dried flowering tops of *Melilotus officinalis* are used traditionally in the treatment of peripheral vascular disorders including haemorrhoids, varicose veins.

4.2.5.3. ANGELICA ROOT

The root of the official drug (BP, EP, BHP) consists of the rhizome and root of *Angelica archangelica* L. (Umbelliferae/ Apiaceae), whole or cut and carefully dried.

Perennial plant growing with many, broad leaves comprise numerous small leaflets divided into three principal groups. The edges of the leaflets are finely toothed or serrated. The flowers, which blossom in July, are small and numerous, yellowish or greenish, are grouped into large globular umbels.

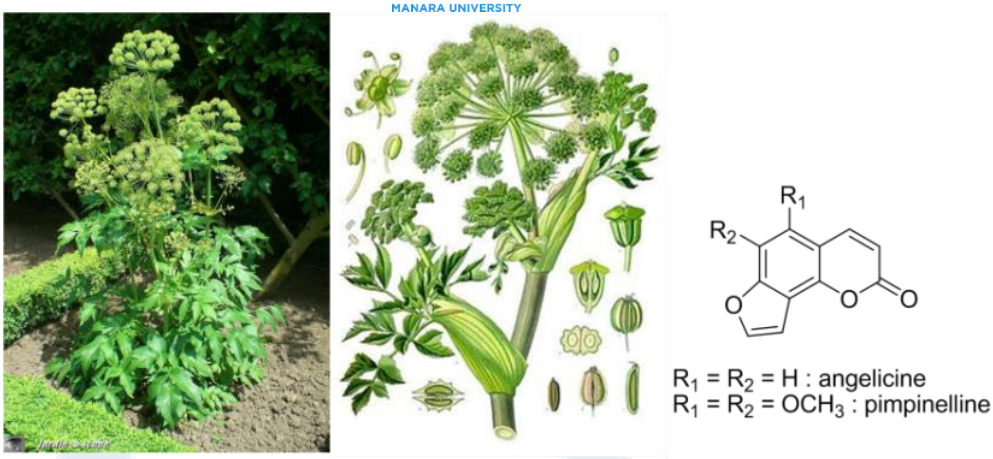


Figure (3.31): *Angelica archangelica* L.

Constituents: volatile oil (BP not less than 0.2%), simple coumarins, furocoumarins and their glycosides (e.g. angelicin).

Uses: In herbal medicine the root is indicated in the treatment of bronchitis associated with vascular deficiency, and dyspeptic conditions (bloating and flatulence).

These uses are limited because of the photosensitivity induced by furano-coumarin, patients should avoid sunlight exposure during treatment.

4.3. Flavonoids and related flavonoid glycosides

Flavonoids are C_{15} compounds all of which have the structure C_6-C_3-C_6. The flavonoids which occur both in the free state and as glycosides are the largest group of naturally occurring phenols. More than 2000 of these compounds are now known.

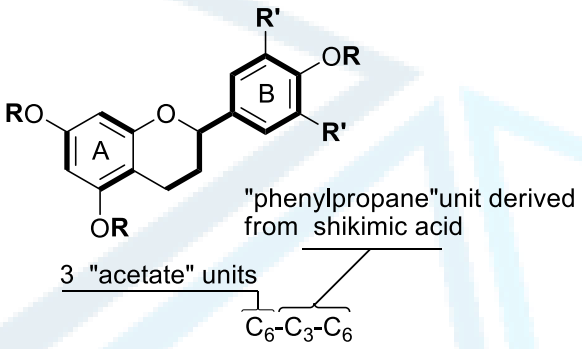


Figure (3.32): Basic structure of flavonoides

The flavones and their close relations are often yellow (Latin flavus, yellow). They are widely distributed in nature but are more common in the higher plants and in young tissues.

The flavonoids are a remarkable group of plant metabolites. No other class of secondary product has been credited with so many (or such diverse) key functions in plant growth and development. Many of these tasks are critical for survival, such as attraction of animal vectors for pollination and seed dispersal, others are employed as agents of defense against herbivores and pathogens. Some flavonoids play an important role in protecting plants from harmful UV-B levels.

4.3.1. Flavonoids biosynthesis:

Flavonoids are formed from three acetate units and a phenyl-propane unit as has already been outlined. They are products from a cinnamoyl-CoA starter unit (shikimate pathway), with chain extension using three molecules of malonyl-CoA (acetate units: acetate pathway).

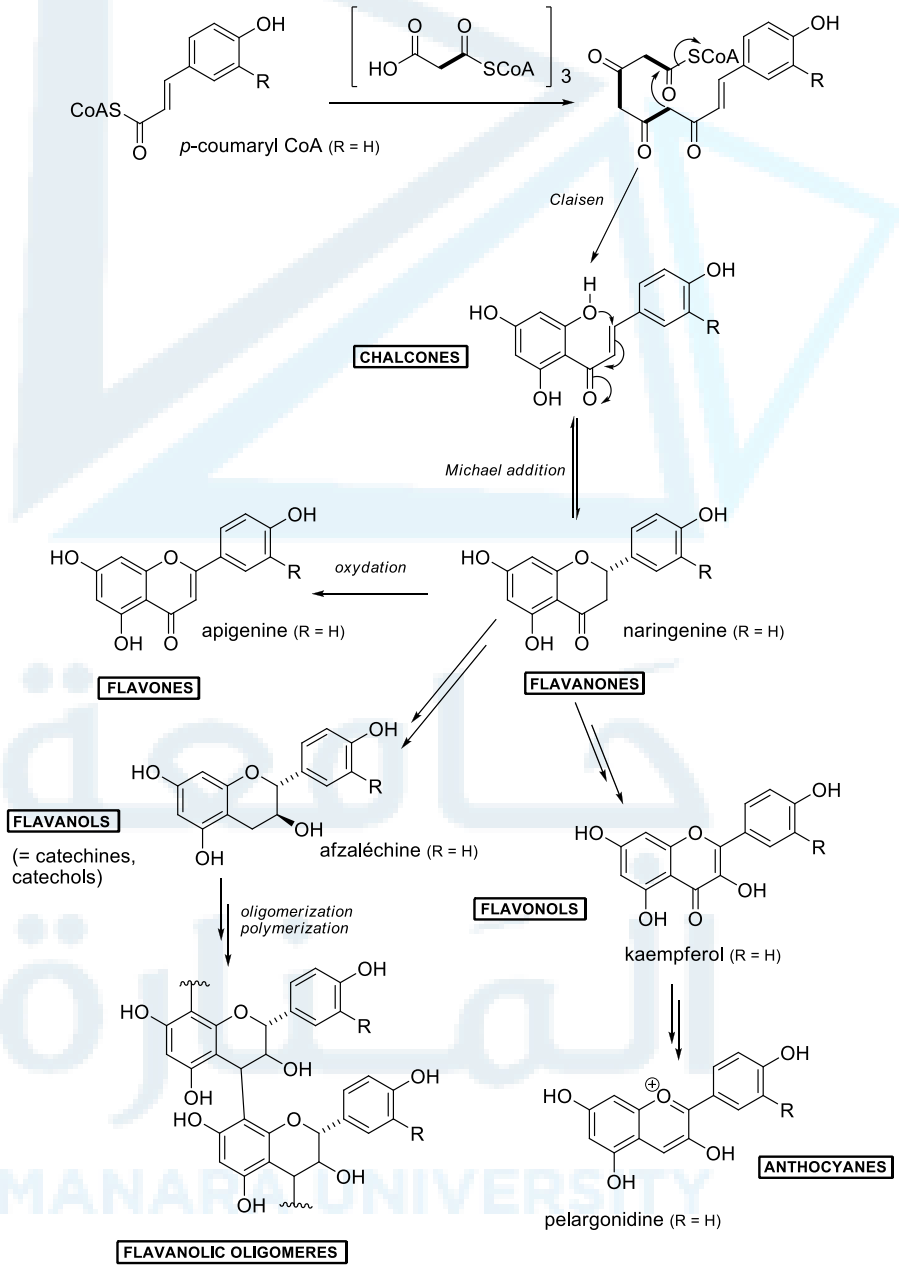


Figure (3.33): Flavonoids biosynthesis

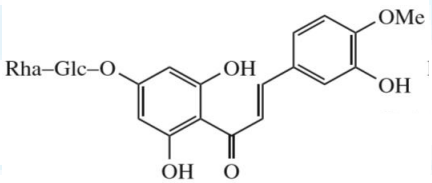
4.3.2. Flavonoids chemical classification:

It is the arrangement of the C_3 group that determines how the compounds are classified.

4.3.2.1. Chalcones and dihydrochalocones

Chalcones have a linear C_3-chain connecting the two rings. The C3-chain of chalcones contains a double bond, whereas the C3-chain of dihydrochalcones is saturated.

Chalcones are yellow pigments in flowers. An example of a dihydrochalcone is phloridzin, a compound found in apple leaves, and which has been reported to have anti-tumor activity.



neohesperidin-chalcone

Figure (3.34): Basic structure of chalcones

4.3.2.2. Aurones

Aurones are formed by cyclization of chalcones, whereby the meta-hydroxyl group reacts with the α-carbon to form a five-member heterocycle. Aurones are also yellow pigments present in flowers.

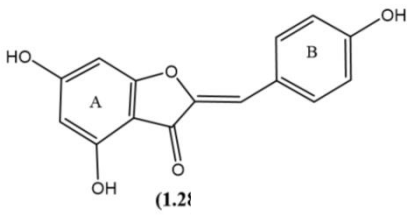


Figure (3.35): Basic structure of aurones

4.3.2.3. Flavonoids

Typical flavonoids, such as flavanone, have a six-member heterocycle. Flavonoids have an A-, B- and C-ring, having the basic structure of 2-phenyl-γ-chromone. They are typed according to the state of oxygenation of the C3 unit.

- Flavanones: The heterocycle of flavanones also contains a ketone group, but there is no unsaturated carbon-carbon bond. The A- and B-ring can be substituted analogous to the flavones, as in naringenin.
- Flavanonols: Flavanonols are also known as dihydroflavonols The heterocycle of flavanones also contains a ketone group and a hydroxyl group.
- Flavones: The heterocycle of flavones contains a ketone group, and has an unsaturated carbon-carbon bond. Flavonols when they have an hydroxyl en C-3. Flavones are common in angiosperms. The most widely distributed flavones in nature are kaemferol and quercetin.
- Anthocyanidins and Anthocyanins: The heterocycle of anthocyanidins is a pyrilium kation. Anthocyanins are water-soluble glycosides of anthocyanidins. The most common glycoside is the 3-glycoside.
- Biflavonyls: Biflavonyls have a C_{30} skeleton. They are dimers of flavones such as apigenin or methylated derivatives and are found in gymnosperms. Few compounds are known. The most familiar is ginkgetin from *Ginkgo biloba*.
- Isoflavonoids: having the basic structure of 3-phenyl-γ-chromone, they are almost restricted to the Leguminosae/Fabaceae plant family e.g. soya beans (Glycine max).

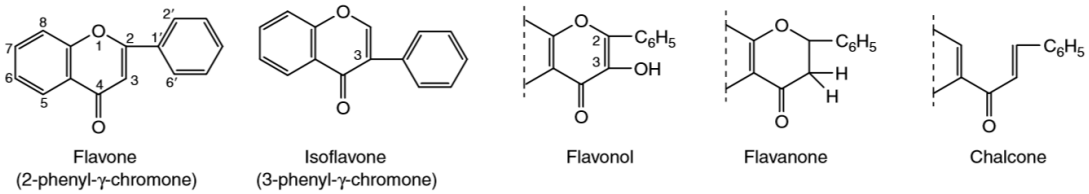


Figure (3.36): Flavonoids derivatives

4.3.2.4. Flavonoids glycosides

Flavonoids occur both in the free state and as glycosides; most are O-glycosides but a considerable number of flavonoid C-glycosides are known.

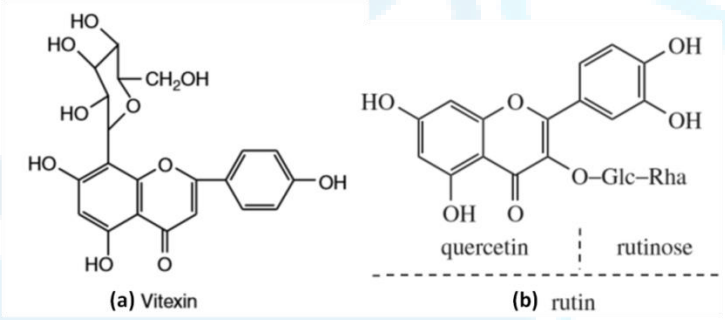


Figure (3.37): Flavonoid C-glycosides (a) and flavonoid O-glycosides.

4.3.3. Flavonoids physico-chemical properties:

The glycosides are generally soluble in water and alcohol, but insoluble in organic solvents; the genins (aglycones) are only sparingly soluble in water but are soluble in ether. Flavonoids dissolve in alkalis, giving yellow solutions which on the addition of acid become colourless.

4.3.4. Flavonoids detection

- Ammonia test: Filter paper dipped in alcoholic solution of drug was exposed to ammonia vapor. Formation of yellow spot on filter paper.

- Shinoda test To the alcoholic extract of drug magnesium turning and HCl was added, A pink or red colour indicates the presence of flavonoid, colours varying from orange to red indicated flavones, crimson to magenta indicated flavonones..
- test: to the alcoholic extract of drug zinc turning and HCl was added, formation of deep red to magenta colour indicates the presence of dihydro flavonoids.
- Vanillin HCl test: Vanillin HCl was added to the alcoholic solution of drug, formation of pink colour due to presence of flavonoids.
- $FeCl_3$ test: To the concentrated alcoholic extract of drug few drops of alcoholic $FeCl_3$ solution was added to give different coloration according the phenolic derivatives presented in the extract.

4.3.5. Flavonoids assay

Total flavonoid content determination is performed spectrophotometrically by using the aluminium chloride colorimetric assay and the absorbance is measured at 415nm.

4.3.6. Flavonoids biological properties

Flavonoids are known for many therapeutic usefulness:

✓ Vitamin P activity increase capillary resistance and decrease capillary permeability.

✓ Protective agents against reactive oxygen species production (ROS):

- Inhibition of oxidative enzyme: xanthine oxydase, proteine kinase C, cyclo-oxygenases, lipoxygenase, succinate oxydase, NADH oxydase.
- Antioxidant agent and free radical scavenger
- Metals chelating agents: like magnesium involved in oxidative stress.

- ✓ Flavonoids are considered as non-specific enzyme inhibitors, e.g. elastase and hyaluronidase
- ✓ Flavonoids are known for its anti-inflammatory and antiallergic effects, for antithrombitic and vasoprotective properties, for inhibition of tumour promotion and as a protective for the gastric mucosa.
- ✓ Many flavonoid-containing plants are diuretic or antispasmodic (e.g. liquorice and parsley), antibacterial or antifungal properties.
- ✓ Non-steroidal phyto-oestrogens like isoflavonoids, some lignans and prenylated chalcones.

4.3.7. Natural Drugs containing flavonoids

4.3.7.1. Citrus glycosides

In botanical characteristics the bitter orange tree *C. aurantium var. amara*-L is not unlike the sweet orange *C. aurantium var. sinensis* L. and both are regarded as subspecies or varieties of *Citrus aurantium* L. (Rutaceae).

The bitter orange tree appears to have been introduced from northern India into eastern Africa, Arabia and Syria, whence it was brought to Europe by either the Arabs or Crusaders about AD 1200. The sweet orange was not known in Europe until the fifteenth century and appears to be of Chinese origin.

- Chemical constituents:

Citrus fruits contain a large number of flavanone glycosides (citroflavonoids). The best-known of these, hesperidin, first isolated in 1828, is present in oranges, both bitter and sweet, and in lemons. An isomer of hesperidin, neo-hesperidin, is present in certain samples of Seville oranges.

Naringin, present in some Seville oranges, is the chief flavonoid constituent of the grapefruit *Citrus paradisi*.

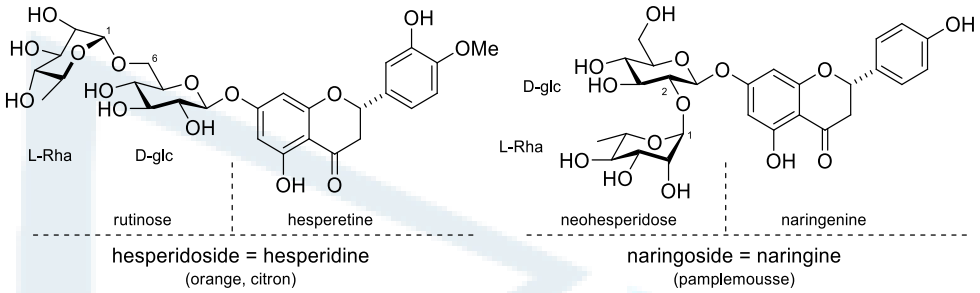


Figure (3.38): Citroflavonoids structures.

- **Uses:**

Citroflavonoids (Daflon®: Diosmin + hesperidin) are widely used in treating venous insufficiency and conditions associated with alteration of blood rheology and capillary fragility.

- natural flavonoids (hesperidin and naringin)
- semi-synthetic flavonoids: Diosmin is synthesized from hesperidin.
- The water-soluble flavonone hesperidin methyl chalcone is prepared from hesperidin.

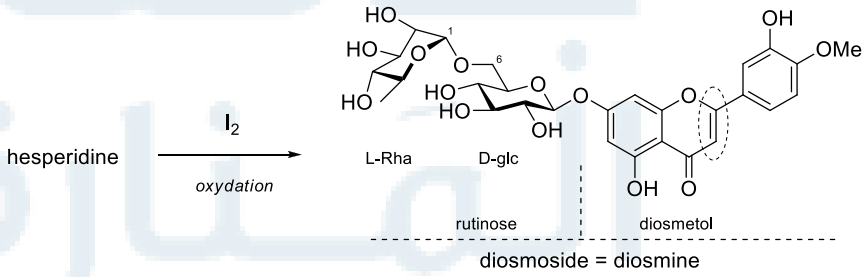


Figure (3.39): Diosmin semi-synthesis.

4.3.7.2. Rutin

Rutin, the rhamnoglucoside of quercetin, is found in many plants, and commercial supplies are made from tobacco residues, *Sophora* and *Eucalyptus* spp. or buckwheat (*Fagopyrum esculentum*).

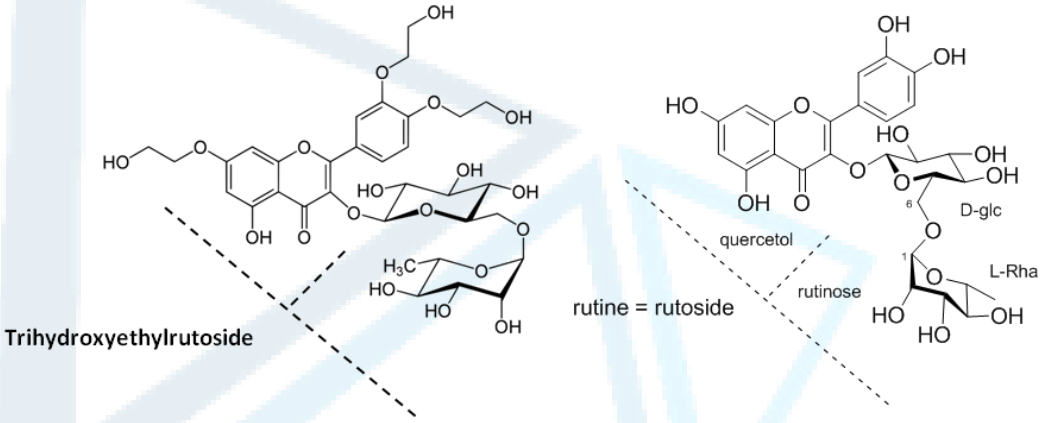


Figure (3.40): Rutin and derivative.

Buckwheat BP/EP (*Fagopyrum esculentum*) Polygonaceae, containing the rutin 3–4%, as the most important therapeutic constituent, is used in the treatment of various circulatory disorders, including varicose veins, chilblains and retinal bleeding.

Figure (3.41): *Fagopyrum esculentum* (Buckwheat).

Extraction: the rutin is extracted with boiling water as it is sparingly soluble in cold water, it is then obtained by crystallization and purified by recrystallization in water or ethanol.

Uses: essentially as a precursor for semi-synthesis or giving associated with citroflavonoids in the treatment of peripheral vascular deficiency.

Troxerutine: are a mixture of hydroxyethylrutosides synthesized from rutin, containing at least 80% of tri-hydroxyethylrutoside.

4.3.8. Natural Drugs containing flavonoids derivatives:

4.3.8.1. *Ginkgo biloba* L. (Ginkgoaceae)

Ginkgo (Maidenhair-tree) is a primitive member of the gymnosperms and the only survivor of the Ginkgoaceae, also called the living fossil. Native to China and Japan but cultivated ornamentally in many temperate regions.

The leaves of ginkgo are official in the BHP 1996 and the BP/EP.

Figure (3.42): *Gikkgo biloba* (Maidenhair-tree).

- **Chemical composition:**

From among the many groups of compounds isolated from ginkgo it is the diterpene lactones and flavonoids which have been shown to possess therapeutic activity.

Five diterpene lactones (ginkgolides A, B, C, J, M) have been characterized.

Some 33 flavonoids have now been isolated from the leaves and involve mono-, di- and tri-glycosides of kaempferol, quercetin, myricetin and isorhamnetin derivatives. The tree also synthesizes a number of biflavonoids (ginkgetin); there has been recent interest in these compounds arising from their antilipoperoxidant, antinecrotic and radical-scavenging properties.

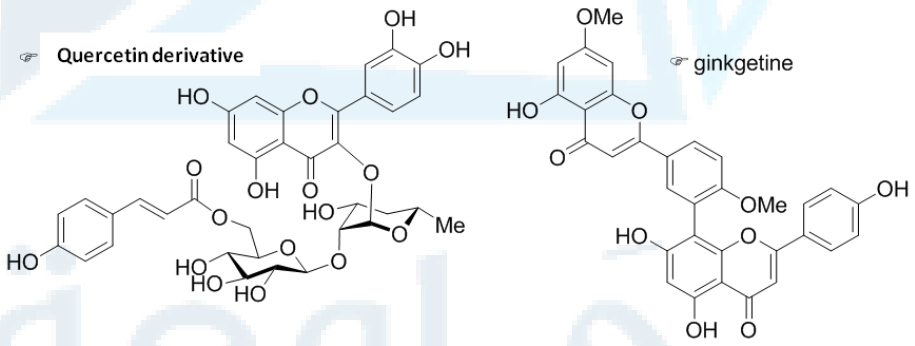


Figure (3.43): Flavonoids derivatives from *Gikkgo biloba*.

- **Uses:**

Ginkgolides, the diterpene lactones, are platelet-activating factor (PAF) antagonists. The later (PAF) is an important mediator involved in both allergic and nonallergic inflammatory diseases as well as thrombotic disorders.

Flavonoids derivatives have vitamin P and radical-scavenging properties.

Ginkgo has a traditional use as an antiasthmatic, bronchodilator, and for the treatment of chilblains. Extracts of the leaf containing selected constituents are

used especially for improving peripheral and cerebral circulation in those elderly with symptoms of loss of short-term memory, hearing and concentration; it is also claimed that vertigo, headaches, anxiety and apathy are alleviated and positive results have been obtained in trials involving the treatment of dementia and Alzheimer's disease.

4.3.8.2. *Silybum marianum* & flavonolignans

Silybum marianum (Asteraceae) is one of the milk-thistles. Indigenous to the Mediterranean region, it has been introduced to most areas of Europe, North and South America and Southern Australia. The glabrous leaves are dark green, oblong sinuate-lobed or pinnatifid, with spiny margins forming a flat rosette. White veins give the leaves a diffusely mottled appearance. The terminal heads which appear from July to September are also spiny with deep violet and slightly fragrant flowers.

Milk-thistle fruit BP/EP, BHP 1996 consists of the mature fruit, devoid of pappus, of *Silybum marianum* L.

Figure (3.44): *Silybum marianum* L.

- **Chemical composition:**

The seeds yield 1.5–3% of flavonolignans collectively termed silymarin. This mixture contains mainly silybin together with silychristin and isosilybin. Commercial extracts typically contain about 80% flavonolignans, in addition to 20% of polyphenolic compounds.

These flavonolignans are formed by various couplings of the flavonoid taxifolin and the lignan precursor coniferyl alcohol.

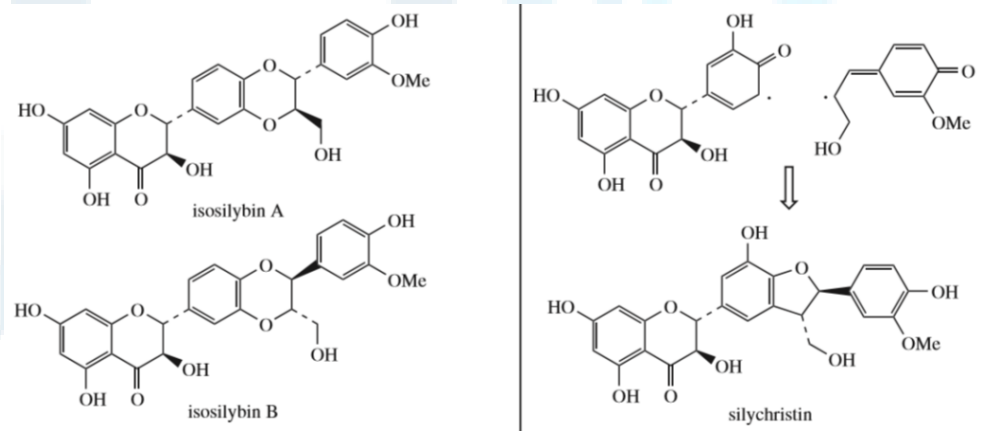


Figure (3.45): Flavonolignans from *Silybum marianum* L.

- **Uses:**

Silymarin may be used in many cases of liver disease and injury, including hepatitis, cirrhosis, and jaundice. These flavonolignans appear to have two main modes of action. They act on the cellular membrane of hepatocytes, inhibiting absorption of toxins; second, because of their phenolic nature, they can act as antioxidants and scavengers for radicals. Such radicals originate from liver detoxification of foreign chemicals and can cause liver damage.

4.3.8.3. *Glycine max* and isoflavones

Glycine max (Fabaceae) contains significant levels of the isoflavones daidzein and genistein. isoflavonoids are almost entirely restricted to the Fabaceae plant family.

Foods rich in isoflavonoids are valuable in countering some of the side-effects of the menopause; So isoflavones are regarded as phyto-oestrogens.

Figure (3.46): *Glycine max* and isoflavones.

Phyto-oestrogen (phytoestrogen) is a term applied to non-steroidal plant materials displaying oestrogenic properties. These planar molecules mimic the shape and polarity of the steroid hormone estradiol. and are able to bind to an oestrogen receptor, though their activity is much less than that of estradiol. In some tissues, they stimulate an oestrogenic response, whilst in others they can antagonize the effect of oestrogens.

There is mounting evidence that phyto-oestrogens also provide a range of other beneficial effects, helping to prevent heart attacks and other cardiovascular diseases, protecting against osteoporosis, lessening the risk of breast and uterine cancer, and in addition displaying significant antioxidant activity which may reduce the risk of Alzheimer's disease. Whilst some of these benefits may be

obtained by the use of steroidal oestrogens, particularly via HRT (hormone replacement therapy), phyto-oestrogens offer a dietary alternative.

4.3.9. Anthocyanidins and glycosides

Anthocyanidins are flavonoids structurally related to the flavones. Their glycosides are known as anthocyanins. These names are derived from the Greek antho-, flower, and kyanos, blue. They 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 cornflower and the red of roses is due to the same glycosides.

Anthocyanins are derivatives of 2-phenylbenzopyrylium (flavylium cation), and consist of an aglycone (anthocyanidin) and sugar(s)

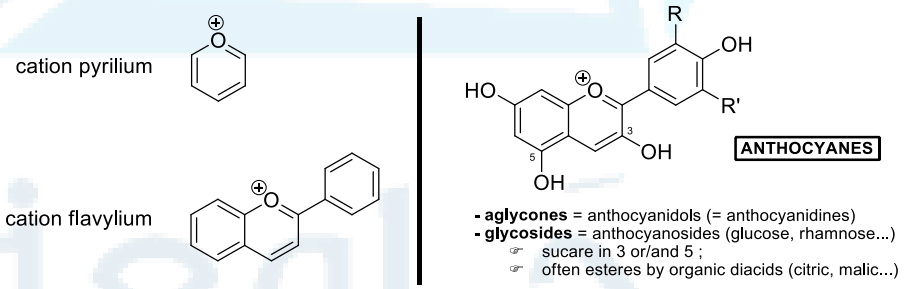


Figure (3.46): Anthocyanidine structure.

Up to now (2014) the literature has reported more than 500 different Anthocyanidines. Among them only six are commonly found in fruits and vegetables: pelargonidin, cyanidin, delphinidin, petunidin, peonidin, and malvidin.

4.3.9.1. Anthocyanidines functions in plants:

- They are responsible for most of the red, blue, purple, and even black colors of fruits, vegetables, grains and flowers.

- anthocyanin made plants more outstanding, thus attracting animals to spread pollens and seeds to aid in breeding.
- anthocyanins are produced as a protective mechanism against environmental stress factors including UV light, cold temperatures

4.3.9.2. Anthocyanidins physic-chemical properties:

- Anthocyanidines pH indicators:

The anthocyanins in aqueous solution coexist in equilibrium with four main species: the flavylium cation, the quinoidal base, the hemiacetal base (carbinol pseudo-base) and chalcone.

In an aqueous solution at pH 1–3, the predominant form of the anthocyanin is the flavylium cation, which is responsible for the red color.

Increasing pH is accompanied by loss of a flavylium cation proton, thereby generating a blue quinoidal base, which is a colorless carbinol pseudo-base that accumulates from hydration of the flavylium cation. This species tautomerizes through opening the C-ring to generate a yellow chalcone.

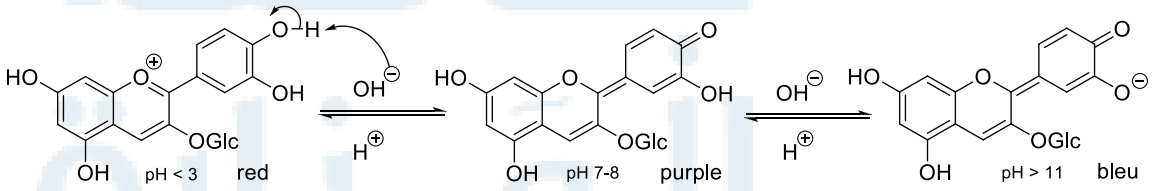


Figure (3.47): Anthocyanidine structure.

- Anthocyanidins stability:

In their purified form, anthocyanidins are highly unstable and susceptible to degradation. Their stability is affected by several factors such as pH,

temperature, chemical structure, concentration, light, oxygen tension, solvents, the presence of specific enzymes, proteins, metallic ions, and of other flavonoids.

4.3.9.3. Anthocyanidins biological properties:

Play an important role in the prevention of diverse diseases such as cancer; cardiovascular diseases involving mechanisms of antioxidant and detoxification activity.

Anthocyanidins are considered as vitamin P, anti-inflammatory agents; digestive enzymes inhibitors (elastase & collagenase), justifying the use of anthocyanines in treating the capillary fragility and vision troubles associated with circulatory disorders.

4.3.9.4. Natural Drugs containing Anthocyanidins:

- Bilberry fruit

Bilberry (*Vaccinium myrtillus* L., /Ericaceae) also known as blaeberry, its fresh and dried fruits are now included in the BP/EP known for containing anthocyanins glucosides.

- *Ribes nigrum,* Grossulariaceae leaves and fruits anti-inflammatory activity
- *Grapes, Vitis vinifera,* Vitaceae. The leaves are used in treating vision troubles associated with circulatory disorders

4.3.10. Tannins

Tannins comprise a group of compounds with a wide diversity in structure that share their ability to bind and precipitate proteins. The name tannins refers to the process of tanning animal skin to form leather. This process has been known since prehistoric times, when animal hides were treated with tannins derived from plants. Chemically this resulted in the cross-linking of the collagen chains in the hide; So that tannins prevent their putrefaction and convert them into leather. During the last century minerals such as aluminum and chromium replaced the use of plant tannins.

As part of Japanese and Chinese natural medicine tannins have been used as anti-inflammatory and antiseptic compounds. They have also been used to treat a wide array of illnesses, including diarrhea and tumors in the stomach or duodenum. Another application of tannins is in wine and beer production, where they are used to precipitate proteins.

The above tannin-protein co-precipitation is important not only in the leather industry but also in relation to the physiological activity of herbal medicines, taste of foodstuffs and beverages, and in the nutritional value of feeds for herbivores.

Tannins are abundant in many different plant species, in particular oak (*Quercus* spp.), chestnut (*Castanea* spp.) and staghorn sumac (*Rhus typhina*). Tannins can be present in the leaves, bark, and fruits, and are thought to protect the plant against infection and herbivory.

Tannins can be classified in three groups: condensed tannins, hydrolysable tannins, and complex tannins. These groups can then be further divided.

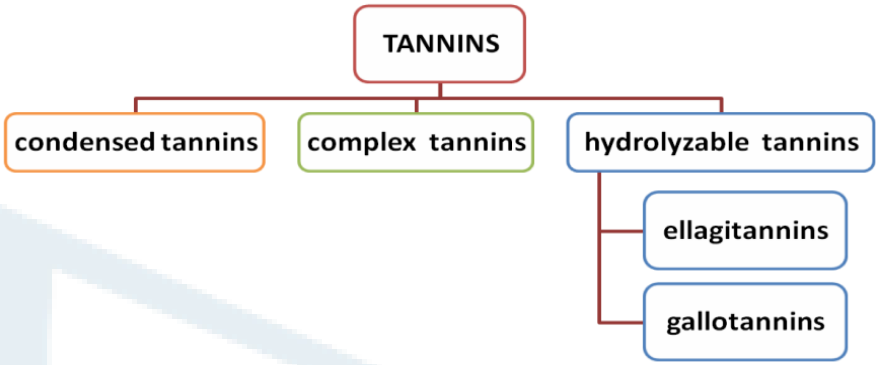


Figure (3.48): Tannins classification.

4.3.10.1. Chemical classification

4.3.10.1.1. Hydrolysable tannins

They are formed from several molecules of phenolic acids such as gallic and hexahydroxydiphenic acids which are united by ester linkages to a central glucose molecule (polyol). These may be hydrolysed by acids or enzymes such as tannase.

Two principal types of hydrolysable tannins are gallitannins and ellagitannins which are, respectively, composed of gallic acid and hexahydroxy-diphenic acid units.

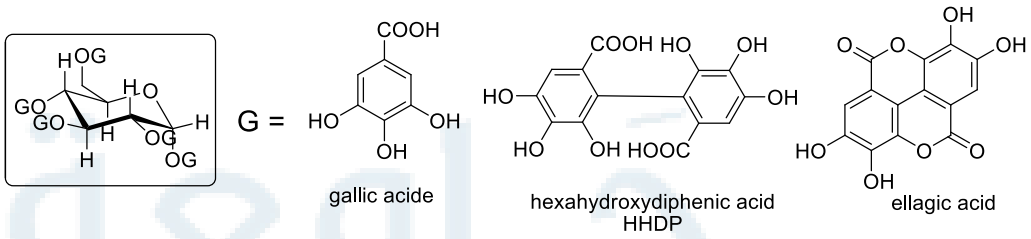


Figure (3.49): Basic sturcture of hydrolysable tannins.

4.3.10.1.1.1. Gallotannins

Gallotannins are hydrolysable tannins with a polyol core, referring to a compound with multiple hydroxyl groups (glucose), substituted with 10-12 gallic acid residues.

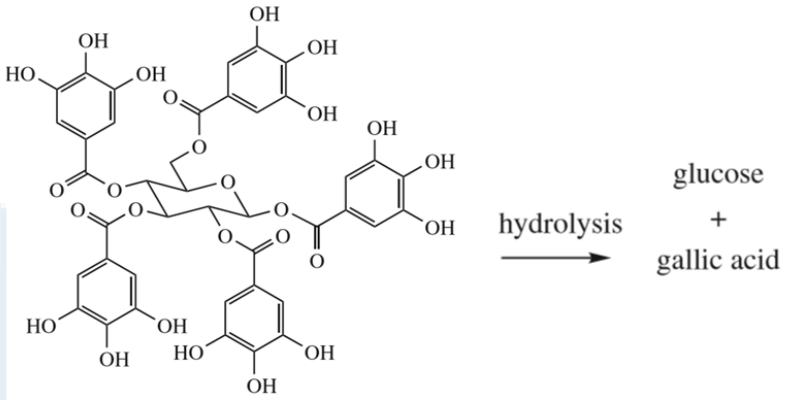


Figure (3.50): Gallotannin structure and hydrolysis products.

4.3.10.1.1.2. Ellagitannins

Ellagitannins are also hydrolysable tannins derived from hexahydroxydiphenic acid esters formed by phenolic oxidative coupling between galloyl functions and polyols.

The name ellagitannins is derived from ellagic acid (the depside of gallic acid) , which is formed spontaneously from hexahydroxydiphenic acid in aqueous solution via an intra-molecular esterification reaction.

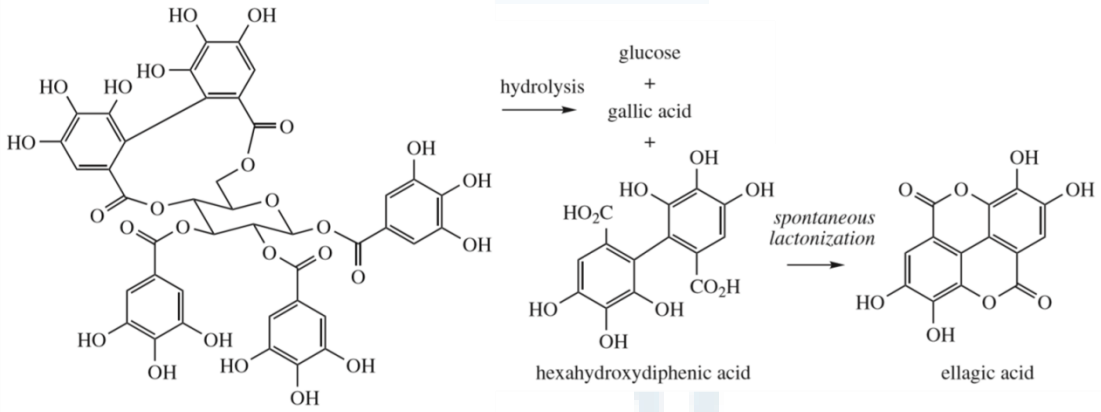


Figure (3.51): Ellagitannins structure and hydrolysis products.

4.3.10.1.2. Condensed tannins

Condensed tannins are also referred to as proanthocyanidins. They are oligomeric or polymeric flavonoids consisting of flavan-3-ol (catechin/ epicatechin) units. The linkage between units is assured by a carbone-carbone bond usually between C4-C8 and C4-C6. Hydrolysis under harsh conditions, such as heating in acid, yields anthocyanidins.

The degree of polymerization affects the ability to precipitate proteins. This is of importance in wine making, where a high level of condensed tannins, especially in red wines, can result in the dry feeling on the inside of the mouth.

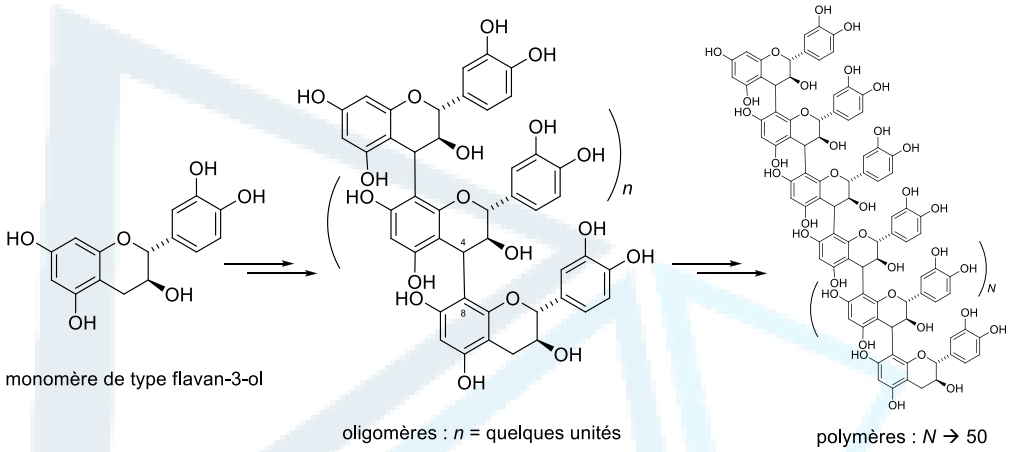


Figure (3.52): Condensed Tannins.

4.3.10.1.3. Complex tannins

Complex tannins are defined as tannins in which a catechin unit is bound to either a gallotannin or an ellagitannin unit.

4.3.10.2. Physico-chemical properties and tests:

Tannins are soluble in water, dilute alkalis, alcohol, glycerol and acetone, but sparingly soluble in other organic solvents. Solutions precipitate heavy metals, alkaloids, glycosides and gelatin. With ferric salts, gallitannins and ellagitannins give blue-black precipitates and condensed tannins brownish-green ones.

4.3.10.3. Biological properties:

- Tannin-containing drugs will precipitate protein and have been used traditionally as haemostatic (styptics), internally for the protection of inflamed surfaces of mouth and throat (gargles).
- They act as antidiarrhoeals and have been employed as antidotes in poisoning by heavy metals, alkaloids and glycosides.

- Proanthocyanidins prepared from cranberries were shown to inhibit the adherence of E. coli to uroepithelial-cell surfaces; other *Vaccinium spp.*, including blueberries had similar bioactivity, suggesting their contribution to the useful effects in urinary tract infections.
- Tannins also contribute to the astringency of foods and beverages, especially tea, coffee, and wines, through their strong interaction with salivary proteins.
- In addition, they have beneficial antioxidant properties.

4.3.10.4. Natural Drugs containing Tannins:

4.3.10.4.1. *Hamamelis virginiana* L. Hamamelis leaf

Hamamelis leaf (witch hazel leaves) consists of the dried leaves of *Hamamelis virginiana* L. (Hamamelidaceae), a shrub or small tree 2–5 m high, which is widely distributed in Canada and the USA. It is official in the BP/EP.

- Chemical constituents: Hamamelis contains gallitannins, ellagitannins, free gallic acid, proanthocyanidins, bitter principles and traces of volatile oil. The pharmacopoeia requires the leaves to contain not less than 3.0% tannins.
- Uses: Hamamelis owes its astringent and haemostatic properties to the tannins. Hamamelitannin and the galloylated proanthocyanidins isolated from *H. virginiana* are reported to be potent inhibitors of 5-lipo-oxygenase, supporting the anti-inflammatory action of the drug.

  Hamamelis Water or Distilled Witch Hazel is widely used as an application to sprains, bruises and superficial wounds and as an ingredient of eye lotions.

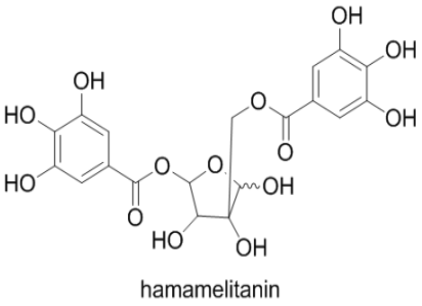


Figure (3.53): *Hamamelis virginiana* L. (Hamamelidaceae).

4.3.10.4.2. *Alchemilla xanthochlora* Rothm., (A. vulgaris L.)

The flowering and aerial parts of the lady's mantle, *Alchemilla xanthochlora* Rothm., (*Alchemilla vulgaris* L.), family Rosaceae, are described in the BP/EP and BHP 1996.

The plant is widespread in Europe, North America and Asia; commercial supplies are obtained principally from Eastern Europe.

- Chemical constituents: The BP/EP drug is required to contain not less than 6.0% of tannins, the dimeric alchemillin characterized as an ellagitannin. Other constituents are flavonoids, quercetin 3-O-β-d-glucoside having been isolated as the major flavonoid in leaves of French origin.
- Uses: Alchemilla acts as an astringent against bleeding and diarrhoea and has a long tradition of use for gynaecological conditions such as menorrhagia.

Figure (3.54): *Alchemilla xanthochlora* Rothm. (Rosaceae).

4.3.10.4.3. Green and black tea

Green and black tea, obtained from dried leaves and leaf buds of the Chinese tea plant C*amellia sinensis* (Theaceae), an evergreen shrub cultivated in India, Sri-Lanka, East Africa, Mauritius, China and Japan.

Green tea is produced by steaming and drying the leaves to prevent oxidation. The oxidase may be destroyed by steaming for 30s, this treatment inactivates oxidases, so that the catechins in the leaves remain stable and thus contribute to the characteristic color and smell of green tea.

Whilst black tea, the leaves are allowed to ferment, allowing enzymic oxidation of the polyphenols; During oxidation, colourless catechins (up to 40% in dried leaf) are converted into intensely coloured theaflavins and thearubigins.

- Chemical constituents: Tea contains 1–5% of caffeine and 10–24% of tannin; also small quantities of theobromine, theophylline and volatile oil. The alkaloid content of the leaves is very much dependent on age and season.

The main polyphenols of green tea are (-)-epicatechin EC, (-)-epicatechin 3-gallate ECG, (-)-epigallocatechin EGC, and (-)-epigallocatechin 3-gallate EGCG, whereas in black tea theaflavin and thearubigin are the most abundant.

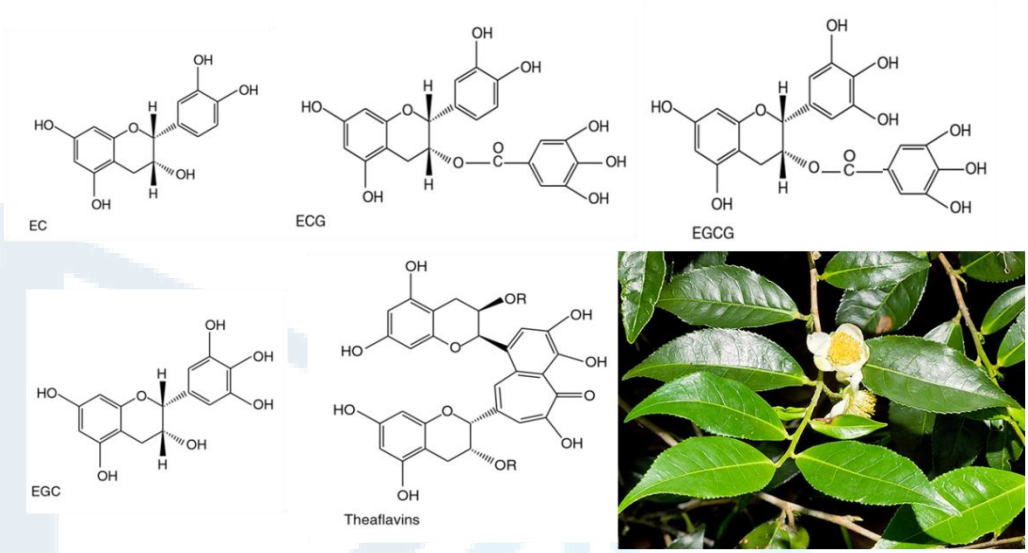


Figure (3.55): *Camellia sinensis* and its phenol derivatives.

- **Uses:**

Tea, an aromatic beverage, is the most widely consumed drink in the world. Astringency and flavour come from tannins and volatile oils.

Theaflavins, dimeric catechin structures, are believed to act as radical scavengers/antioxidants, and to provide beneficial effects against cardiovascular disease, cancers, and the ageing process generally. Green tea, in particular, contains significant amounts of epigallocatechin gallate, a very effective antioxidant regarded as one of the more desirable dietary components. Tea leaf dust and waste is a major source of caffeine.

Caffeine stimulates the central nervous system and has a weak diuretic action. Theophylline is used as bronchodilator in respiratory disease therapy (namely chronic obstructive pulmonary disease and asthma).

4.3.10.4.4. *Lytrhrum salicaria*

***L. salicaria*, commonly known as purple loosestrife, is an herbaceous perennial in the family Lythraceae. It reaches up to two meters tall; has square or angular stems with lance-shaped, stalkless leaves up to ten centimeters long; and ends in dense, towering spikes of pink-purple, 5-7 petaled flowers.**

- Chemical constituents: The phytochemical examination carried on this plant reported that tannins were the main compounds ellagitannins and gallotannins (1,6-di-O-galloylglucose). It also contains a notable amount of flavon C-glycosides (vitexin) and anthocyanins.
- Uses: the flowering branch tops are used internally for diarrhea, chronic intestinal catarrh, in the form of a decoction or a fluid extract. Externally, they are used to treat varicose veins, venous insufficiency, bleeding of the gums and hemorrhoid.

Figure (3.56): *Lythrum salicaria*.

4.3.10.4.5. Rhatany

Rhatany of the BP and EP (Krameria) is the dried root of *Krameria triandra* (Krameriaceae, a small family related to the Leguminosae), a small shrub with decumbent branches about 1 m long. The drug is collected in Bolivia and Peru and is known in commerce as Peruvian rhatany.

- Chemical constituents: The tannins of krameria root are entirely of the condensed (proanthocyanidin).
- Uses: The drug is used as an astringent and the significant antimicrobial activity of the extract gives rational support for its use in mouth and throat infections.

Figure (3.57): *Krameria triandra* (Krameriaceae).

4.3.10.4.6. Galls and tannins

Turkish galls (Turkey Galls; Galla) are vegetable growths formed on the young twigs of the dyer's oak, *Quercus infectoria* (Fagaceae), as a result of the deposition of the eggs of the gall-wasp *Adleria gallaetinctoriae*.

Aleppo galls are globular in shape and from 10 to 25 mm in diameter. They have a short, basal stalk and numerous rounded projections on the surface. Galls are hard and heavy, usually sinking in water. The so-called 'blue' variety are actually of a grey or brownish- grey colour.

Crowned Aleppo galls are sometimes found in samples of ordinary Aleppo galls. They are about the size of a pea, are stalked, and bear a crown of projections near the apex. The insect producing them is *Cynips polycera*.

The dyer's oak is a small tree or shrub about 2 m high which is found in Turkey, Syria, Persia, Cyprus and Greece. Abnormal development of vegetable tissue round the larva is due to an enzyme-containing secretion, produced by the young insect after it has emerged from the egg, which by the rapid conversion of starch into sugar stimulates cell division. As starch disappears from the neighbourhood of the insect, shrinkage occurs and a central cavity is formed in which the insect passes through the larval and pupal stages. Finally, if the galls are not previously collected and dried,

the mature insect or imago bores its way out of the gall and escapes. During these changes the colour of the gall passes from a bluish-grey through olive-green to almost white.

Galls are collected by the peasants of Turkey and Syria. After drying they are graded according to colour into three grades, blue, green and white, which are found on the London market.

- Constituents: Galls contain 50–70% of the tannin known as gal- lotannic acid (Tannic Acid BP/EP); this is a complex mixture of phe- nolic acid glycosides varying greatly in composition
- Uses: Galls are used as a source of tannic acid, for tanning and dyeing, and in the manufacture of inks. Tannic acid is used as an astringent and styptic.

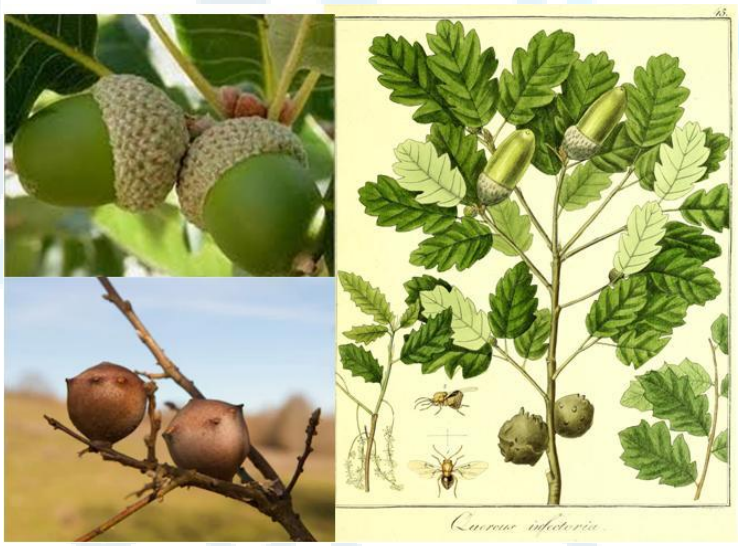


Figure (3.58): *Quercus infectoria* (Fagaceae) and the galls.

4.4. Quinones and their glycosides

The quinones are phenolic compounds that typically form strongly colored pigments covering the entire visible spectrum. Typically, however, they are found in the internal regions of the plant and, thus, do not impart a color to the exterior of the plant. Generally, quinones are derived from benzoquinone, naphthoquinone, or anthraquinone structures. Quinones play an important role in the respiration of plants. They act as electron carriers that function by converting between hydroquinones and quinones, thus acting as redox couples. Benzoquinones, such as ubiquinones, is known as Coenzyme Q and has a role in electron transport in the mitochondria.

Naphthaquinones are rare. Among the naphthaquinones juglone is relatively common. It is found in walnuts.

Anthraquinone is the most widely distributed of the quinones in higher plants and fungi.

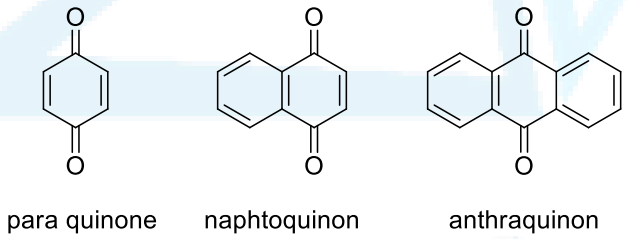


Figure (3.59): Quinones derivatives.

4.4.1. Benzoquinones

Benzoquinones are rather rare in higher plants, where they seem specific to a limited number of families; Myrsinaceae, Primulaceae, and Boraginaceae.

The fundamental properties of benzoquinones are their interconversion to hydraquinones (which makes them mild oxidation reagents), and their tendency to add nucleophiles. Free quinines are practically insoluble in water and can be extracted with common organic solvents.

Natural benzoquinones in the strict sense of the term have no therapeutical application. Note, however, that the reduced form of 1,4 benzoquinone (i.e. hydroquinone) occurs as a glycoside,

named arbutine, and that this molecule has a very strong urinary antiseptic properties (simple phenol-containing drugs).

4.4.2. Naphthaquinone

the distribution of naphtoquinones is limited in fungi and a some families of higher plants such as Droseraceae, juglandaceae, plumbaginaceae, Lythraceae and Boraginaceae. Many naphtoquinones are antibacterial and fungicidal (explaining the resistance of some tropical woods such as teak to fungi and insects). Generally their nucleophilicity explains their cytotoxicity. Currently, no natural naphtoquinone is marketd for therapy, except few drugs containing them remain in use in some galenical preparations (e. g. *Drosera sp.*).

4.4.2.1. *Drosera rotundifolia; Droseraceae*: Drosera

The European sundew, *Drosera rotundifolia*, has long been employed in folk medicine and has been included in some pharmacopoeias (BHP 1983).

It is a small plant 5 cm with a rosette of leaves with long petioles, they are covered with red long trichomes secreting a viscous refractory liquid (hence the name sundew).

- Chemical constituents: the drug consists of the entire plant. It contains naphthoquinone derivatives mainly plumbagone, which has antimicrobial activity, and 7-methyl-juglone.
- Uses: The common form of utilization, as antispasmodic, of sundew is the tincture. In Germany, this tincture and extract are ingredients of syrup used for spasmodic and irritating cough. This drug is not listed in the French Explanatory Note.

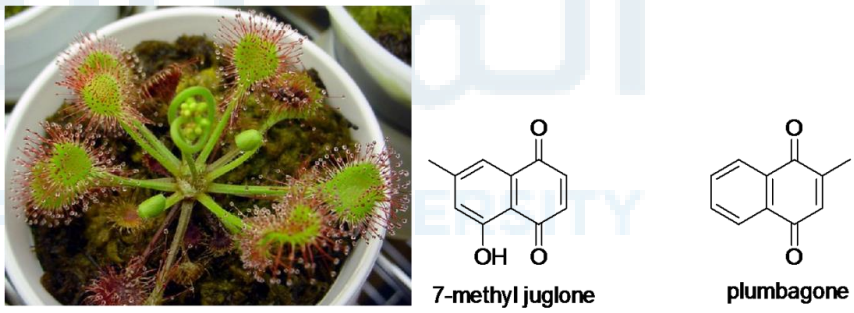
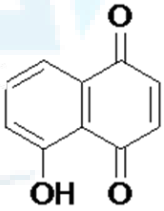


Figure (3.60): *Drosera rotundifolia* & its main naphtoquinons derivatives .

4.4.2.2. *Juglans regia*, Juglandaceae: walnut tree leaves

The part of the walnut tree that is used is the dried leaves. the seeds are used as food.

- Chemical constituents: the leaves contain not less than 2% total flavonoids, naphtoquinon ,juglone as a chief constituent is 0.6%, in addition to hydrolysable tannins. The seeds are good source of unsaturated fatty acids (ω6, ω3), which decrease the incidence of cardiovascular diseases. they contain 50% and more of an oil rich in linoleic (55-65%) and α–linolenic acid (9-15%),.
- Uses: Orally traditionally used in venous insufficiency and piles, in mild diarrhea, localy is used to treat scalp itching, peeling and dandruff.
- Juglone is mutagenic and maybe even carcinogenic, caution should be taken especially in case of the abuse or daily application.



Juglone

Figure (3.61): *Juglans regia* .

4.4.2.3. *Lawsonia inermis* L. Lythraceae, leaves

The henna plant is a tall flowering shrub or tree about 5 m in height, native to tropical and subtropical regions of Africa, Asia and northern Australia (tropical savannah, the tropical arid zone, oases in the Sahara). Henna shrubs need light and warmth and are regarded as rather pest resistant. Henna flowers exhale a distinctive scent, a reason why henna also makes an ornamental bush in oriental house gardens.

- Chemical constituents: Henna leaves contains naphtoquinones (lawsone), flavonoides and coumarins.

Figure (3.62): *Lawsonia inermis* L.

- Uses: Henna has been used as a coloring and cosmetic ingredient; Henna itself is not an allergen, nor could rumours be proved that it might be a carcinogen. Caution should be exercised especially with a kind of so-called "black henna"; This is a mixture of henna with synthetic para-phenylenediamine (PPD) intended to create a black stain. Many cases have been reported in which this PPD additive has caused an immediate severe allergic contact dermatitis reaction. Since ancient times, henna leaves have been used in traditional medicine as an astringent, antiseptic and antipyretic. Henna was used in ancient times also to treat serious diseases (leprosy, smallpox, chickenpox, tumours) by Arabian doctors.

4.4.3. Anthraquinone

They are phenolic compounds derived from anthrecene and have a variable degree of oxidation (anthrone, anthranols and anthraquinones). The active compounds have in common a double hydroxylation at C-1 and C-8.

The botanical distribution of the species containing 1,8 dihydroxyanthraquinone glycosides is very limited: Liliaceae (Aloe), polygonaceae (rhubarb), Rhamnaceae (buckthorn, cascara) and Fabaceae (senna).

4.4.3.1. Chemical classification:

They are phenolic glycosides, hence we may classify these compound according to the nature of the aglycone or the sugar-linking type.

4.4.3.1.1. Aglycones:

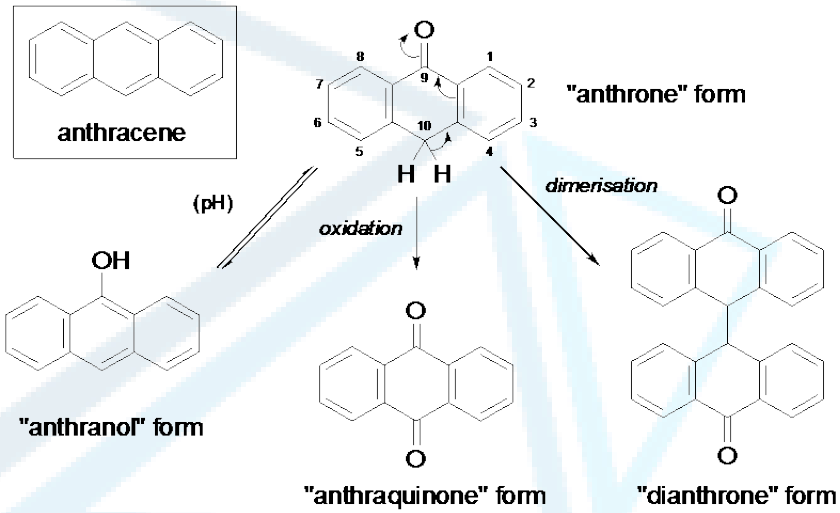


Figure (3.63): Anthracenes derivatives.

- Anthraquinones

The derivatives of anthraquinone present in purgative drugs may be di, tri or tetra hydroxy-substituted.

- Anthranols and anthrones

These reduced anthraquinone derivatives occur either free or combined as glycosides. They are isomeric and one may be partially converted to the other in solution. The parent substance, anthrone, is a pale yellow, non-fluorescent substance which is insoluble in alkali; its isomer, anthranol, is brownish-yellow and forms a strongly fluorescent solution in alkali.

- Dianthrones

These are compounds derived from two anthrone molecules, which may be identical or different; they readily form as a result of mild oxidation of the anthrone or mixed anthrones (e.g. a solution in

acetone and presence of atmospheric oxygen). They are important aglycones in species of Cassia, Rheum and Rhamnus.

4.4.3.1.2. Glycosides type:

- *O*-glycosides: are more frequents in natural drugs.
- *C*-glycosides: in which the sugar is joined to the aglycone with a direct C–C linkage, were also isolated. Although one of the first glycosides to be isolated, there was a problem for investigators for a long time. It is strongly resistant to normal acid hydrolysis but may be oxidized with ferric chloride.

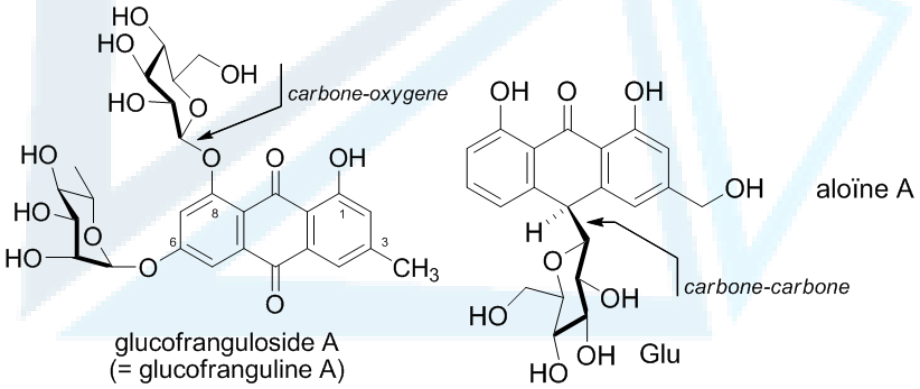


Figure (3.64): Anthranoides glycosides.

4.4.3.2. Physico-chemical properties and characterization :

Anthraquinone derivatives are often orange-red compounds, sparingly soluble in cold water, and soluble in organic solvents and alcohols. The carboxylic aglycones can be extracted with an aqueuous sodium bicarbonate solution. The glycosides are soluble in water and hydroalcoholic solutions.

Treating the O-glycosides in acidic medium causes their hydrolysis, but the cleavage of the carbon-carbon bond of C- glycosides can only be obtained in the presence of ferric chloride under reflux.

the characterization of hydroxyanthraquinone derivatives applies the Bornträger reaction: upon dissolving the quinones in alkaline aqueous medium (KOH), a red color, more or less purplish, develops. This reactions is only positive with the free anthraquinone forms, to characterize glycosides with this reaction, preliminary hydrolysis is required, and if the aglycones are anthrones,

they must first be oxidized to anthraquinones. Another color reaction, specicific to 1,8-dihydroxyanthraquinones, uses magnesium acetate in methanol. The resulting red color is more intense and more stable to light than that formed by the simple reaction with potassium hydroxide, consequently, it is preferable to quantitation.

There are substantial difference in composition between the fresh plants and the dried drugs. in the fresh vegetable, anthracene-type compound occur chiefly as glycosides of monomeric anthrones. During desiccation, two transformation processes take place: oxidation, which leads to anthraquinone glycosides (e.g. buckthorn, franguline), and dimerization which yields glycosides of dianthrones for example: the enzymatic dimerization in senna only observed if drying is accomplished at moderate temperature 40°C.

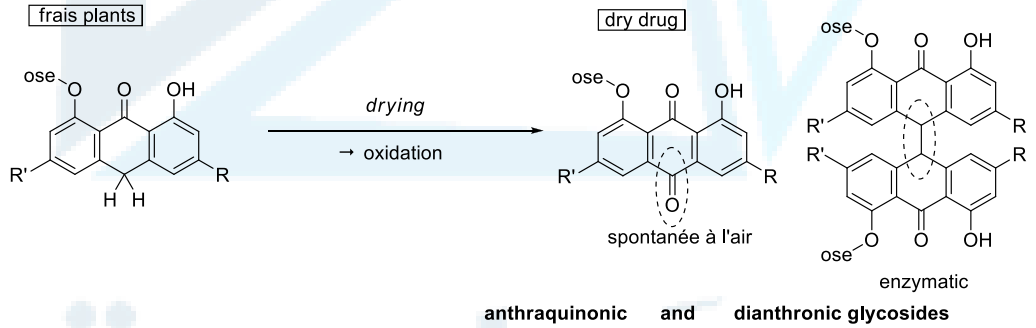


Figure (3.65): Anthranoides transformation.

4.4.3.3. Biological properties:

Depending on the dose administered, 1,8-anthraquinone derivatives exert a more or less violent laxative or purgative activity, at therapeutic doses they are stimulant laxatives.

The activity is linked to the structure of these compounds: the most interesting derivatives are the O-glycosides of dianthrone and anthraquinones, as well as the C-glycosides of anthrones, in other words the groupe of compounds without a CH_2 in position 10. The activity of the glycoside of monomeric anthrones is excessive, which explains why the drugs containing them (buckthorn bark)

are only used after prolonged storage or after the appropriate heat treatment during which they are oxidized to anthraquinones glycosides. the free aglycones (anthraquinones) are practically inactive.

The free aglycones found in the drug or formed by initial gastric hydrolysis, upon reaching the intestine, are absorbed in the small intestine, glucoconjugated in the liver and almost totally excreted in urine (turning the urine a dark yellow or even red if there is a positive alkaline reaction).

The glycosides of anthraquinone and dianthrones are polar molecules, are water-soluble and have high molecular weight, so they are not absorbed nor hydrolyzed in the small intestine. In the colon, they are hydrolyzed by the β-glucosidases of the intestinal flora, and the freed anthraquinones are reduced: thus the active forms are the anthrones formed *in situ*. Which explains the latency observed between compound (or drug) intake and the laxative effect, so the Pharmacological action of the anthraquinone laxatives is restricted to the large bowel; and the effect is delayed for up to 6 h or longer.

For some authors anthraquinone glycosides may be considered *prodrugs*: the sugars would act as transporters by preventing the active moiety from being absorbed prior to being freed in the colon under the influence of bacterial enzymes.

Anthraquinones are thought to affect the absorption of water and electrolytes. By inhibiting the Na-K ATPase activity of enterocytes, they cause an inhibition of water, sodium and chloride resorption and an increase in the secretion of potassium by the intestinal mucosa.

True adverse side effects result almost entirely from long-term abuse leading to severe electrolyte and water losses and eventual hyperaldosteronism. The chronic hypokalemia worsens constipation and may cause damage to the renal tubules. These toxic side effects should not occur when anthranoid laxatives are taken intermittently and at low doses.

Anthranoid preparations are contraindicated in partial or complete bowel obstruction, pregnancy, and lactation. Interactions with cardiac glycosides (digoxin) and other drugs may occur indirectly as a result of electrolyte imbalance (hypokalemia).

Observation in humans, have led to suspect a relationship between the abuse of anthraquinone laxatives and an increased risk of colon cancer, yet the carcinogenicity and the teratogenicity of anthraquinones have to be confirmed.

4.4.3.4. Natural drugs containing anthraquinones:

4.4.3.4.1. Polygonaceae members

4.4.3.4.1.1. *Rheum palmatum L.*

Rhubarb (Chinese Rhubarb) consists of the dried underground parts of *Rheum palmatum* L. (Polygonaceae) or *R. officinale* Baillon.

Figure (3.66): *Rheum palmatum L.*

- Chemical constituent:

The BP/EP drug is required to contain not less than 2.2% of hydroxyanthraquinone derivatives calculated as rhein. In addition to the above purgative compounds, rhubarb contains astringent compounds 5% such as Tannins, free gallic acid, (−)-epicatechin gallate and catechin.

- Uses:

Rhubarb is used as a bitter stomachic and in the treatment of diarrhoea, purgation being followed by an astringent effect. The drug is suitable as an occasional aperients but not for the treatment of chronic constipation.

Besides the anthranoids, which have a cathaltic action, rhubarb also contains tannins and pectins, which produce an antidiarrheal effect. Both actions are superimposed during use. The overall effect is dose-dependent because emodins and tannins appear to have different dose-response characteristics. Rhubarb taken in smaller doses (0.1-0.3 g) has an astringent action in gastritis and dyspepsia and an antidiarrheal action in mild forms of diarrhea. Higher doses (1.0-4.0 g) produce a mild laxative effect.

4.4.3.4.2. Fabaceae members

4.4.3.4.2.1. *Cassia angustifolia*: Senna

Senna leaf and fruit are obtained from *Cassia angustifolia* (India and Pakistan), or less commonly from *Cassia senna* (*syn Cassia acutifolia*), which is described as Alexandrian senna (Sudan). Early harvests provide leaf material, whilst both leaf and fruit (senna pods) are obtained later on.

Figure (3.67): *Cassia spp.* Senna

- Chemical constituent:

The active constituents are dianthrone glycosides, principally sennosides A and B. There are no significant differences in the chemical constituents of the two sennas, or between leaf and fruit drug. However, amounts of the active constituents do vary, and this appears to be a consequence of cultivation conditions and the time of harvesting of the plant material.

- **Uses:**

Senna is a stimulant laxative and acts on the wall of the large intestine to increase peristaltic movement. After oral administration, the sennosides are transformed by intestinal flora into anthrone, which appears to be the ultimate purgative principle.

The use of laxatives is increasing and senna constitutes a useful purgative for either habitual constipation or occasional use. Despite the availability of a number of synthetics, sennoside preparations remain among the most important pharmaceutical laxatives.

4.4.3.4.2.2. Cassia fistula L., golden shower tree

Cassia pods are the dried ripe fruits of *Cassia fistula* (Leguminosae/ Fabaceae), a large tree thought to be indigenous to India but now widely cultivated in the tropics. The drug is chiefly obtained from the West Indies (Dominica and Martinique) and Indonesia. The fruit is a cylindrical pod about 25–30 cm long and 20–25 mm diameter. It is dark chocolate brown to black in colour and contains from 25 to 100 oval, reddish-brown seeds separated by membranous dissepiments.

Figure (3.68): *Cassia fistula*

- **Chemical constituents:**

The most important anthraquinone derivatives of the fruits appear to be rhein and combined sennidin-like compounds. It also contains about 50% of sugars (mucilage).

- **Uses:**

Cassia pulp was formerly used in the form of Confection of Senna. In Ayurvedic medicine the plant is used to treat a variety of ailments. Its antifungal, antibacterial and laxative properties have been established and more recently its antitussive activity has been demonstrated. The low content of anthraquinones and the presence of mucilage make the *C. fistula* act as a mild natural laxative.

4.4.3.4.3. Rhamnaceae members:

4.4.3.4.3.1. *Rhamnus frangula L.* Frangula bark

Frangula bark, alder buckthorn, is obtained from *Rhamnus frangula* L. (*Frangula alnus* Mill) (Rhamnaceae), a shrub 3–5 m high and found in Britain and Europe. Commerical supplies are available from Balkan countries and a little from Russia.

Although much used in England, the demand decreased with the increased popularity of cascara; on the Continent, particularly in France, cascara has not replaced it to the same extent.

- Chemical constituents:

The bark, included in the BP/EP, is required to contain not less than 7.0% glucofrangulins calculated as glucofrangulin *A. Frangula* contains anthraquinone derivatives present mainly in the form of glycosides.

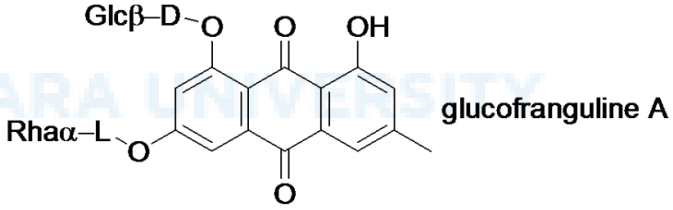


Figure (3.69): *Rhamnus frangula* L. and derivative.

4.4.3.4.3.2. *Rhamnus purshianus* DC (*Frangula purshiana*) cascara bark

The bark is collected from wild trees, which are 6–18 m high, growing on the Pacific coast of North America. The bark is collected from mid-April to the end of August, when it separates readily from the wood.

- Chemical constituents:

It has long been recognized that cascara bark stored for at least 1 year gave galenicals which were better tolerated but as effective as those prepared from more recently collected bark. This is presumably due to hydrolysis or other changes during storage. Cascara contains about 6–9% anthracene derivatives which are present both as normal O-glycosides and as C-glycosides.

Figure (3.70): *Rhamnus purshianus* and derivative.

4.4.3.4.4. Aloeceae / Asphodelaceae (APGIV)

4.4.3.4.4.1. Aloe *vera* or *A. barbadensis* and *Aloe ferox*

Aloe refers to the dried juice or latex obtained from the peri-cyclic tubules of various Aloe species. If should not be confused with aloe gel, a mucilage from the inner parenchymal tissue of the leaf.

It is the solid residue obtained by evaporating the liquid which drains from the transversely cut leaves of various species of Aloe. The juice is usually concentrated by boiling and solidifies on cooling. The drug occurs in dark-brown or greenish-brown, glassy masses.

- Chemical constituents: Aloes contain C-glycosides and resins. The crystalline glycosides known as 'aloin' were first prepared from Barbados aloes in 1851; Aloin (BP, 1988) contains not less than 70% anhydrous barbaloin.

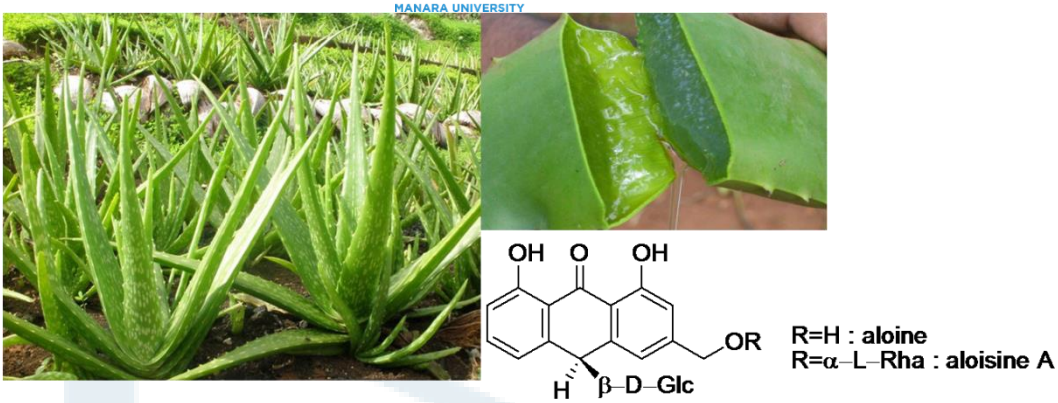


Figure (3.71): *Aloe vera* and derivative.

- Uses: Aloes is employed as purgative. It is seldom prescribed alone; Aloe is the most powerful herbal anthranoid laxative and also the most widely used in Europe. Because of its drastic cathartic action it is not commonly employed in the United States.

4.5. LIGNANS AND LIGNIN

4.5.1. Lignans

Lignans are dimeric compounds formed essentially by the union of two molecules of a phenylpropene derivative. At one time it was thought that these compounds were early intermediates in the formation of lignin. Some 300 lignans have been isolated and categorized into a number of groups according to structural features. One of the most important of the natural lignans having useful biological activity is the aryltetralin lactone podophyllotoxin of *Podophyllum spp.*

Podophyllotoxin and related lignans are found in the roots of *Podophyllum species* (Berberidaceae), and have clinically useful cytotoxic and anticancer activity.

4.5.1.1. *Podophyllum*

Podophyllum consists of the dried rhizome and roots of *Podophyllum hexandrum* (*Podophyllum emodi*) or *Podophyllum peltatum* (Berberidaceae). *Podophyllum hexandrum* is found in India, China, and the Himalayas, and yields Indian podophyllum, whilst *Podophyllum peltatum* (May

apple or American mandrake) comes from North America and is the source of American podophyllum.

Plants are collected from the wild. Both plants are large-leafed perennial herbs with edible fruits, though other parts of the plant are toxic.

Figure (3.72): *Podophyllum.*

- Chemical constituents:

The roots contain cytotoxic lignans and their glucosides, *Podophyllum hexandrum* containing about 5% and *Podophyllum peltatum* about 1%. A concentrated form of the active principles is obtained by pouring an ethanolic extract of the root into water and drying the precipitated podophyllum resin or 'podophyllin'. Indian podophyllum yields about 6–12% of resin containing 50–60% lignans, and American podophyllum 2–8% of resin containing 14–18% lignans.

The lignan constituents of the two roots are the same, but the proportions are markedly different. The Indian root contains chiefly podophyllotoxin (about 4%). The main components in the American root are podophyllotoxin (about 0.25%), β-peltatin (about 0.33%) and α-peltatin (about 0.25%).

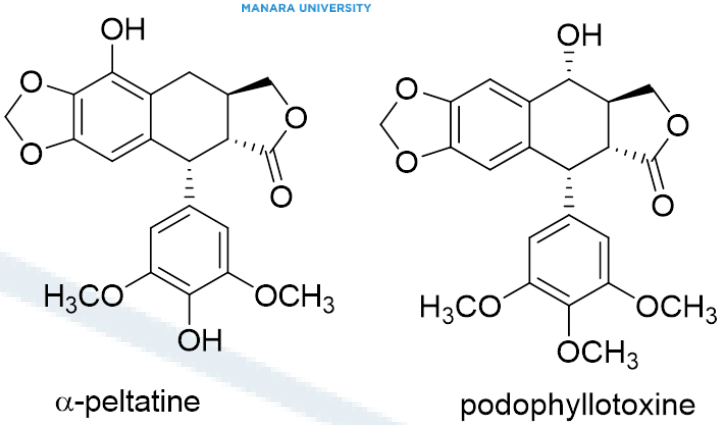


Figure (3.73): *Podophyllum* Lignans' structures.

- Action and uses:

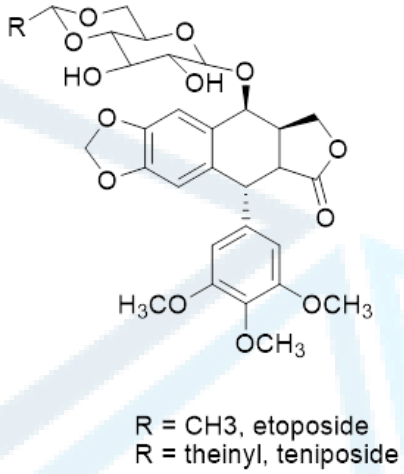
Podophyllum resin has long been used as a purgative, but the discovery of the cytotoxic properties of podophyllotoxin and related compounds has now made podophyllum a commercially important drug plant.

Preparations of podophyllum resin (the Indian resin is preferred) are effective treatments for warts, and pure podophyllotoxin is available as a paint for venereal warts, a condition which can be sexually transmitted. The antimitotic effect of podophyllotoxin and the other lignans is by binding to the protein tubulin in the mitotic spindle, preventing polymerization and assembly into microtubules

Podophyllotoxin and other Podophyllum lignans were found to be unsuitable for clinical use as anticancer agents due to toxic side-effects, but the semi-synthetic derivatives etoposide and teniposide, which are manufactured from natural podophyllotoxin, have proved excellent antitumour agents.

Etoposide is a very effective anticancer agent, and is used in the treatment of small-cell lung cancer, testicular cancer, and lymphomas, usually in combination therapies with other anticancer drugs. It may be given orally or intravenously.

The water-soluble pro-drug etopophos (etoposide 4_-phosphate) is also available; this is efficiently converted into etoposide by phosphatase enzymes and is preferred for routine clinical use.



R = CH3, etoposide
R = theinyl, teniposide

Figure (3.74): Podophyllum lignans semi-synthetized derivatives.

4.5.2. Lignin

Lignin is an important polymeric substance, $(C_6–C_3)n$, laid down in a matrix of cellulose microfibrils to strengthen certain cell walls. It is an essential component of most woody tissues and involves vessels, tracheids, fibres and sclereids.

4.6. **Phloroglucinol:**

The taenicidal constituents of male fern, the bitter principles of hops and the lipophilic components of hypericum are phloroglucinol derivatives.

Both terpenoids and phenols bathways are involved in the synthesis of the phloroglucinol derivatives. for example the synthesis of cannabinoids, where the olivetol, a phenolic coumpound, condense with the geraniol perophosphat a terpenic precursor.

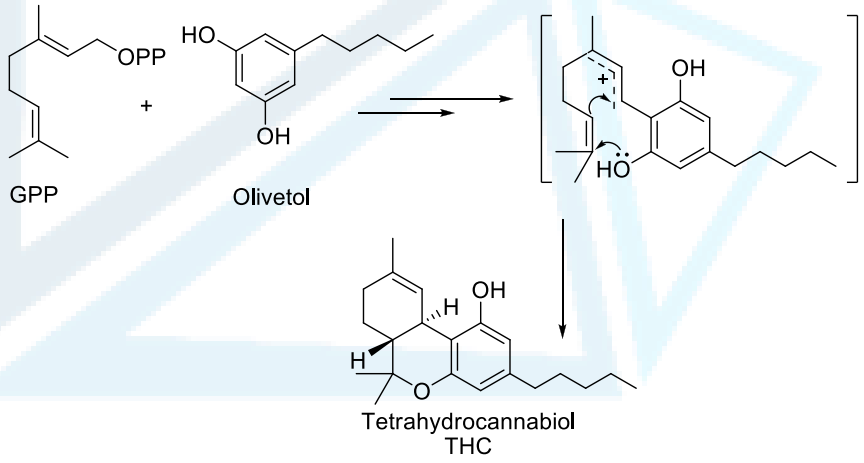


Figure (3.75): synthesis of cannabinoids.

4.6.1. INDIAN HEMP

The Indian hemp plant was originally considered as a distinct species but came to be regarded as a variety or sub-species of *Cannabis sativa* (*Indian sativa*, Cannabinaceae).

The drug consists of the dried flowering and fruiting tops of the pistillate plants from which no resin has been removed. In America and Europe the product used by addicts is known as marihuana, in north Africa as kief, in South Africa as dagga, and in Arabia and Egypt as hashish.

Constituents: The narcotic resin is a brown, amorphous semisolid; soluble in alcohol, Some principal components are cannabinol, tetrahydrocannabinol (THC), cannabidiol (CBD). Δ9-THC is

the principal psychoactive constituent; cannabinol is less potent; although lacking psychotropic properties cannabidiol CBD has anticonvulsant and possible analgesic effects. The cannabis preparations can be evaluated on their Δ9-THC content.

Figure (3.76): *Cannabis sativa*, tops of the pistillate plants and resin.

Uses: Medicinal properties of cannabis were recognized some 5000 years ago. In the mid-nineteenth century it was used in Europe as a hypnotic, anticonvulsant, analgesic, antianxiety and antitussive agent and was still official in the BPC 1949. Over many years it fell into disuse in human and veterinary medicine, and because of its narcotic properties, importation into many countries became illegal. Promising results on the use of Δ9-THC (dronabinol) for the relief of nausea and vomiting caused by cancer chemotherapy led to its use in the USA as an antiemetic. It is also employed to stimulate the appetite of AIDS patients. It can be prescribed in the UK under licence on a named-patient basis.

4.6.2. HYPERICUM - ST JOHN'S WORT

Hypericum consists of the dried aerial parts of *Hypericum perforatum*, family Hypericaceae (Clusiaceae) gathered usually at the time of flowering or shortly before. The generic name derives from the Greek hyper—above, and icon (eikon)—picture, referring to the ancient practice of hanging the plant above religious pictures to ward off evil spirits. It is a herbaceous perennial, usually forming a colony with a spreading root system. The bright yellow flowers are in handsome terminal corymbs.

The drug is now included in the BP/EP, a number of European pharmacopoeias, the British Herbal Pharmacopoeia and the American Herbal Pharmacopoeia.

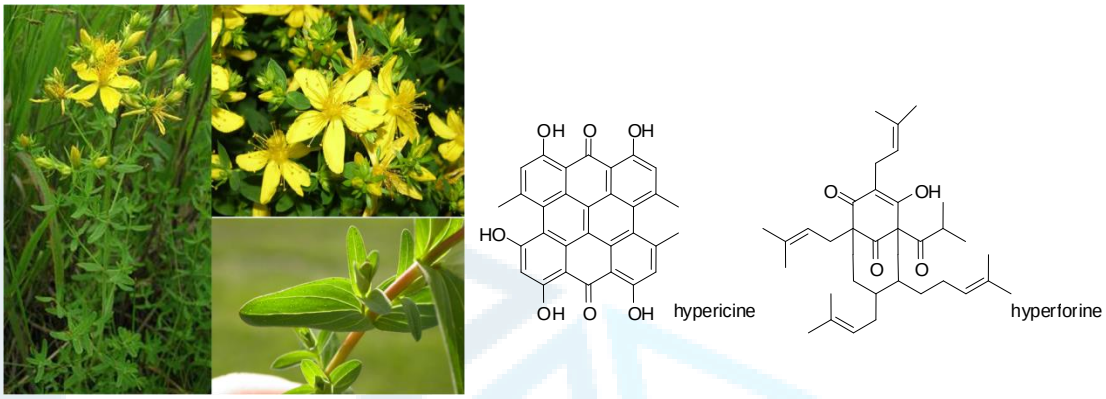


Figure (3.77): *Hypericum perforatum*

Constituents: Hypericum contains a variety of constituents with biological activity. Anthraquinones, principally hypericin and pseudohypericin. Prenylated phloroglucinol derivatives, mainly hyperforin (2.0–4.5%). These phloroglucinols constitute the principal components of the lipophilic extract of the plant and are considered to be the most important active constituents regarding antibiotic and antidepressant properties.

Action and uses: An explosion in the popularity of St John's wort related to its unregulated availability for the treatment of mild to moderate depression. In the USA, for the first eight months of 1999, it ranked second to ginkgo as the best-selling product of the herbal mainstream market, with retail sales valued at over 78 million. In Germany, it represented 25% of all antidepressant prescriptions. It was described as 'nature's Prozac', without the disadvantageous side-effects of the latter.

However, a cautionary warning reported that St John's wort would adversely affect the performance of a number of common drugs (warfarin, digoxin, tricyclic antidepressant agents, simvastatin, cyclosporin and others) by causing their rapid elimination from the body.

Care should be taken during collecting as contact photosensitivity has been reported: due to the presence of dianthrone derivatives hypericin and pseudohypericin.

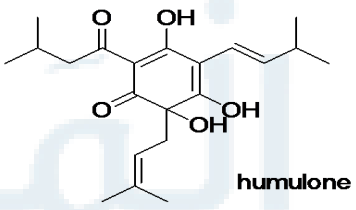
4.6.3. HOPS, *Humulus lupulus* L.

Hops are the dried strobiles of *Humulus lupulus* L. (Cannabinaceae). Only the pistillate plants are cultivated, large quantities being produced in England (particularly Kent), Germany, Belgium, France, Russia and California. The strobiles are collected, dried in kilns and pressed into bales known as 'pockets'.

Hops are included in the EP, BP, BHP and in monographs of the British Herbal Compendium, ESCOP and German Commission E. The hop strobiole consists of external and internal sessile bracts which overlap one another and enclose the ovary. Together they form a petiolate greenish-yellow inflorescence 2–5 cm in length. The odour is characteristically aromatic.

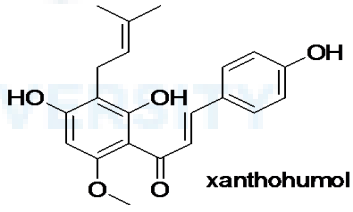
- Chemical constituents:

The distillation of the drug yields volatile oil containing terpenes and sesquiterpenes including humulene and compounds such as 2-methyl-but-3-ene-2-ol and 3-methylbutanoic acid. The bitterness is due to phloroglucinol derivatives known as α-acids (e.g. humulone), β-acids (e.g. lupulone) and also about 10% of resins.



humulone

methylbutenol



xanthohumol

Figure (3.78): *Humulus lupulus* L. and related compounds.

- **Uses:**

The mildly sedative properties of hops are ascribed, in part, to 2-methyl-3-buten-2-ol (methylbutenol); which is too volatile to persist in hop extracts but may form there from bitter acids. Their principal use is as an aromatic bitter in the preparation of beer.

There has been considerable recent interest in the wide-ranging biological activities of the constituents of hops. Thus prenylated chalcones such as xanthohumol for their antioxidant and oestrogenic properties.

LIPIDS

1. Introduction:

The lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone, and benzene) and are generally insoluble in water. There is great structural variety among the lipids and comprise of fixed oils, fats, and waxes. The lipids of physiological importance for humans have the following major functions:

1. They serve as structural components of biological membranes.
2. They provide energy reserves, predominantly in the form of triacylglycerols.
3. Both lipids and lipid derivatives serve as vitamins and hormones.
4. Lipophilic bile acids aid in lipid solubilization.

2. Fixed oils and fats

Fixed oils and fats are obtained from plants or animal. They are rich in calories and in plant source, they are present mostly in the seeds, as reserve substances and in animals they are present in subcutaneous and retroperitoneal tissues.

They differ only according to their melting point and chemically they belong to the same group. If a substance is liquid at 15.5–16.5°C it is called fixed oil and solid or semisolid at the above temperature, it is called fat.

2.1. Chemical characteristics:

They are made from two kinds of molecules: glycerol and three fatty acids joined by Esterification, so called triglycerides: Esters of glycerol (a type of alcohol with a hydroxyl group on each of its three carbons) and three fatty acids.

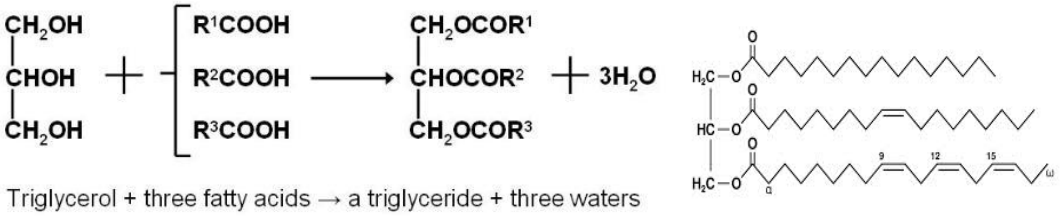


figure (4.1): Triglycerides

- **fatty acids may be saturated, monounsaturated or polyunsaturated.**

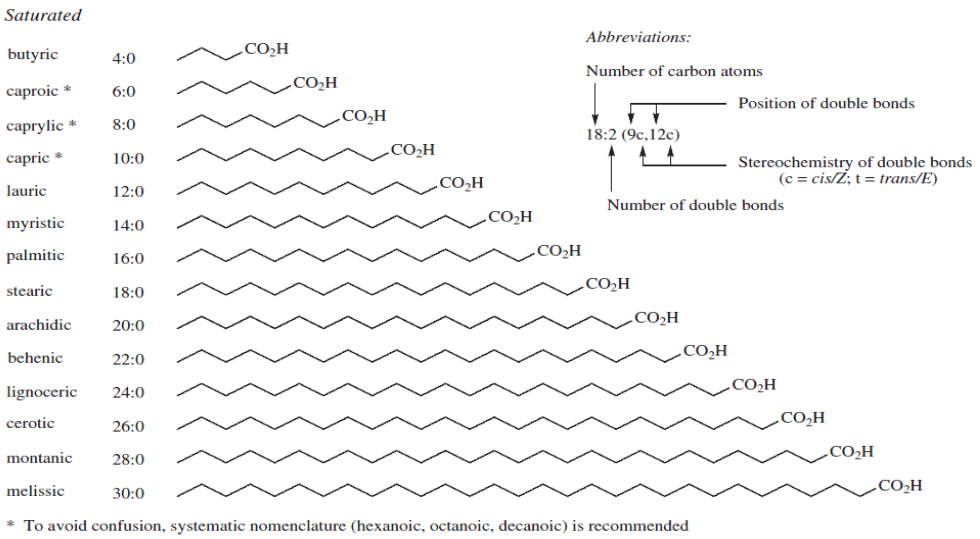


Figure (4.2): common naturally occurring fatty acids

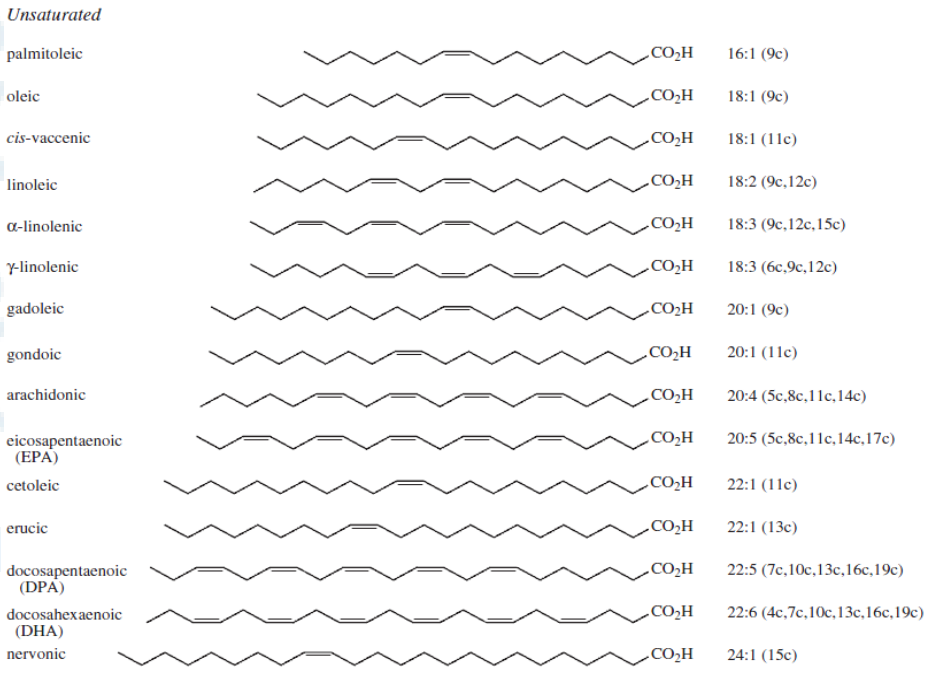


Figure (4.3): common naturally occurring fatty acids

- **Fats:** are mostly from animal sources, are saturated fatty acides. The hydrocarbon chains in these fatty acids are, thus, fairly straight and can pack closely together, making these fats solid at room temperature.
- **Fixed oils:** mostly from plant sources, have some double bonds between some of the carbons in the hydrocarbon tail, causing bends or 'kinks' in the shape of the molecules. Therefore these oils are called unsaturated fats. Because of the kinks in the hydrocarbon tails, unsaturated fats can't pack as closely together, making them liquid at room temperature.

2.2. Physicochemical properties:

Fixed oils and fats are insoluble in water and alcohol and are soluble in lipid solvents like light petroleum, ether, chloroform, and benzene. Only exception in this solubility is castor oil that is soluble in alcohol because of its hydroxy group of ricinoleic acid.

2.3. Analytical Parameters for Fats and Oils

Following are the parameters used to analyze the fats and oils.

1- Iodine value: The iodine value is the mass of iodine in grams that is consumed by 100 g of fats or oil. It is a measure of the extent of unsaturation and higher the iodine value, the more chance for rancidity.

2- Saponification value: The saponification value is the number of milligrams of potassium hydroxide required to saponify 1 g of fat under the conditions specified. It is a measure of the average molecular weight of all the fatty acids present.

3- Acid value: It is the amount of free acid present in fat as measured by the milligrams of potassium hydroxide needed to neutralize it. As the glycerides in fat slowly-decompose the acid value increases.

4- Peroxide value: One of the most widely used tests for oxidative rancidity; peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation.

3. WAXES

Waxes are esters of long-chain fatty acids and alcohols. The fatty acids are same in wax and fats, but the difference being saponification. Waxes are saponified only by alcoholic alkali but the fats may be saponified either by alcoholic alkali or by aqueous alkali. Along with fatty acids it also contains monohydroxy alcohols of high molecular (Some- times cholesterol or phytosterols are also present)

As such they are not suitable as food because hydrolysing enzymes of wax are not present in system. Waxes are widely distributed in nature. The leaves and fruits of many plants have waxy coatings, which may protect them from dehydration and small predators. The feathers of birds and the fur of some animals have similar coatings which serve as a water repellent. Spermaceti, beeswax, carnuba wax, etc. are the examples of waxes.

4. Pharmacopeal drugs containing lipids:

4.1. CASTOR OIL

Synonyms Castor bean oil, castor oil seed, oleum ricini, ricinus oil, oil of palma christi, cold-drawn castor oil.

- Biological Source:

Castor oil is the fixed oil obtained by cold expression of the seeds of *Ricinus communis* Linn., belonging to family Euphorbiaceae.

- Chemical Constituents:

Castor oil consists of glyceride of ricinoleic acid, isorici- noleic, stearic, and dihydroxy stearic acids. Ricinoleic acid is responsible for laxative property. Castor oil also contains vitamin F. 90% of the fatty acid

content is ricinoleic acid. The ricinoleic acid is an 18-carbon acid having a double bond in the 9–10 position and a hydroxyl group on the 12th carbon. This combination of hydroxyl group and unsaturation occurs only in castor oil.

- Uses:

Castor oil is mild purgative, fungistatic, used as an ointment base, as plasticizer, wetting agents, as a lubricating agent. Ricinoleic acid is used in contraceptive creams and jellies; it is also used as an emollient in the preparation of lipsticks, in tooth formulation, as an ingredient in hair oil. The dehydrated oil is used in the manufacture of linoleum and alkyl resin. The main use of castor oil is the industrial production of coatings, also employed to make pharmaceuticals and cosmetics in the textile and leather industries and for manufacturing plastics and fibres.

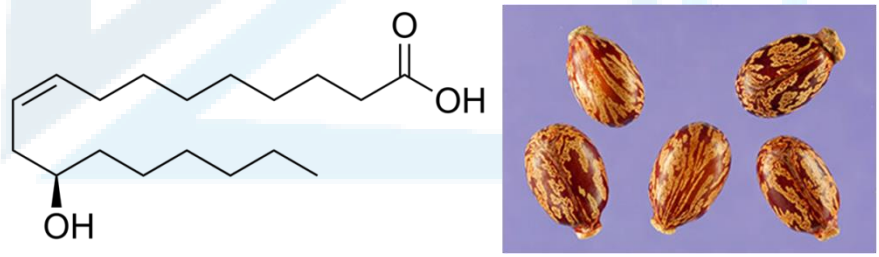


Figure (4.4): Ricinoleic acid and of *Ricinus communis*.

4.2. COD LIVER OIL

- Biological Source:

It is processed from fresh liver of cod fish, *Gadus morrhua* and other species of *Gadus*, belonging to family Gadidae.

Geographical Source It is mainly found in Scotland, Norway, Germany, Iceland, and Denmark.

Characteristics The oil is pale yellow in colour; it has fishy odour and taste. Cod liver oil is slightly soluble in alcohol and fully soluble in chloroform, ether, carbon disulphide and petroleum ether.

- Chemical Constituents

The medicinal properties of cod-liver oil are mainly due to vitamin A and vitamins of the D group. The main antirachitic activity appears to be due to D3 (cholecalciferol). The oil consists of glycerides of unsaturated (about 85%) and saturated (about 15%) acids. In the unsaturated group the acids possess 14, 16, 18, 20 or 22 carbon atoms, and up to 6 ethylenic linkings; in the ω-3 series eicosapentaenoic acid EPA (C20:5) and decosahexaenoic acid DHA (C22:6) are preeminent with smaller amounts of docosapentaenoic acid (C22:5) (see Fatty acids, Chapter 19 for explanation of nomenclature). Evidence is increasing that these polyunsaturated acids are significant for human health. The saturated acids include myristic acid (C14:0), palmitic acid (C16:0) and traces of stearic acid (C18:0).

When both types are present, as in crude cod-liver oil, cooling results in the deposition of saturated acylglycerols such as stearin. In most medicinal cod-liver oils these solid materials are removed by freezing and filtration.

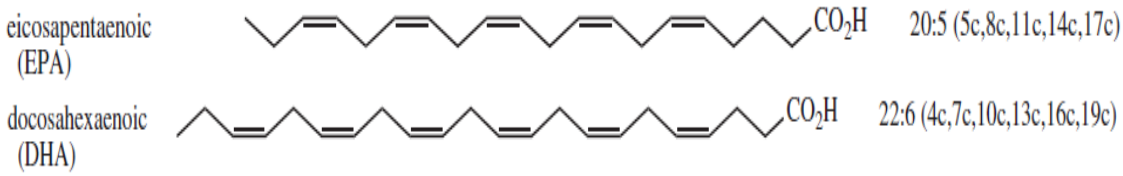


Figure (4.5): ω-3 series eicosapentaenoic acid EPA & decosahexaenoic acid DHA.

- Uses:

Oil is used as source of vitamins, in treatment of rickets, tuberculosis, and also as a nutritive. in addition to its traditional use as a vitamin supplement,it now finds application in the relief of rheumatic pains and joint and muscle stiffness.

Cod-liver oil has the established activity of reducing blood cholesterol levels and affording protection against cardiovascular disease.

4.3. SAFFLOWER OIL

- **Biological Source:**

It is a fixed oil obtained from the ripe and dry seeds of *Carthamus tinctorius* Linn., belonging to family Compositae/Asteraceae.

Safflower oil is obtained by expression or extraction from the seeds (achenes) of Carthamus tinctorius L. (Compositae) or hybrids of this species. Commonly known as safflower, false saffron, saffron thistle, the plant is native to Mediterranean countries and Asia and has been used for colouring and medicinal purposes from Ancient Egyptian and Chinese times. The pigment from the flowers (carthamin) is yellow in water and red in alcohol and was the traditional dye for the robes of Buddhist monks. It is now cultivated largely for the seed oil in its countries of origin, as well as in the US, Australia, Africa and S.E. Asia.

- **Chemical Constituents:**

Safflower oil contains glycerides of palmitic (6.5%), stearic (3.0%), arachidic (0.296%), oleic (13%), linoleic (76–79%), and linolenic acids (90.15%). The polyunsaturated fatty acid content of the oil is highest (75%) and is said to be responsible to control cholesterol level in the blood, and thereby, reduces incidence of heart attacks.

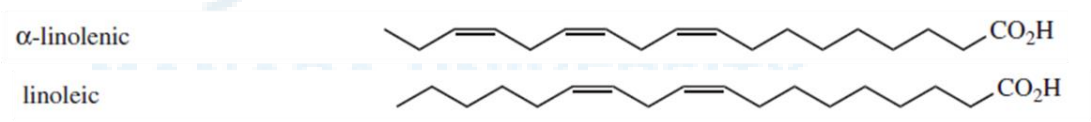


Figure (4.6): Safflower and its major fatty acids.

- **Uses:**

The edible oil is used in the manufacture of oleomargarine, as a dietary supplement in hypercholesteremia and also in treatment of atherosclerosis. Due to its high linoleic acid content, it is consumed for preparation of vegetable ghee. Industrially, it is used for preparation of soft-soap varnishes, linoleum and water-proofing material.

4.4. EVENING PRIMROSE OIL

- **Biological Source:**

Evening primrose oil The fixed oil obtained by extraction and/or expression from the seeds of *Oenothera* spp. (*O. biennis* L., *O. lamarkiana* L.) Onagraceae contains substantial mounts of esterified γ-linolenic acid (GLA), a C18 6,9,12-triene.

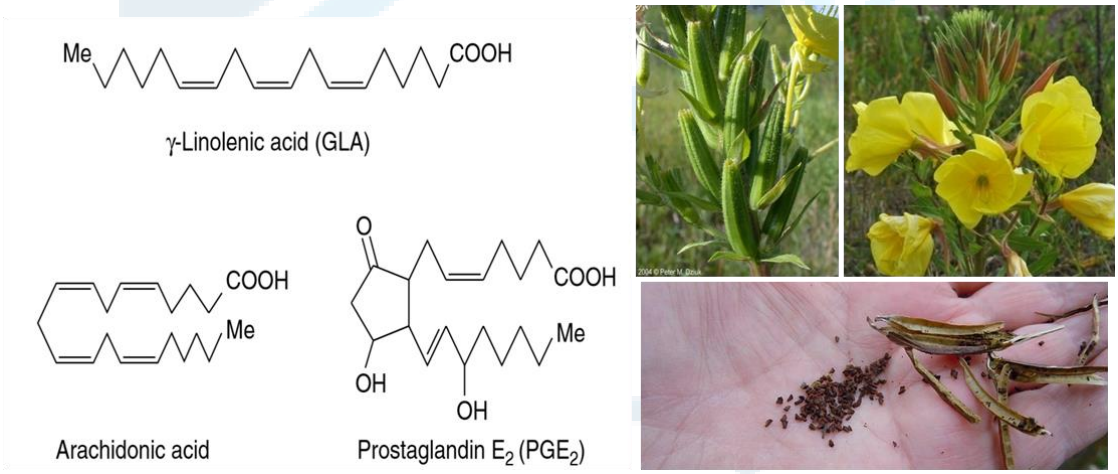


Figure (4.7): Evening primrose its major fatty acid.

In animal tissues it appears that the prostaglandins are formed from dietary linoleic acid by conversion to GLA which undergoes C2 addition and further desaturation to give acids such as arachidonic acid, an immediate precursor of some prostaglandins. The pharmacopoeial tests for the oil are similar to those quoted for borage oil.

- Chemical constituents:

The principal species cultivated in the UK is *O. biennis* which yields an oil containing 7–9% GLA, although more recent work shows higher yields for the oils of some other species, namely *O. acerviphilla nova* (15.68%), *O. paradoxa* (14.41%) and an ecotype of *O. rubricaulis* (13.75%).

- Uses

Beneficial effects of evening primrose oil may well be related to affording a precursor of the prostaglandins for those individuals whose enzymic conversion of linoleic acid to GLA is deficient. The oil is now widely marketed as a dietary supplement, for cosmetic purposes, and more specifically for the treatment of atopic eczema and premenstrual syndrome (prostaglandin E may be depleted in this condition). Further possibilities include its use in diabetic neuropathy and rheumatoid arthritis.

4.5. ECHINACEA spp.

Echinacea species (coneflowers), are perennial herbs of the Compositae/ Asteraceae native to the prairie regions of Ohio where they were used by the Plains tribes to treat a variety of conditions, particularly wounds. Three species are currently important. Roots *of Echinacea angustifolia* DC., the narrow-leaved coneflower, and *E. pallida* Nutt., the pale corn- flower, are included in the BP/EP. The whole plant of *E. purpurpea* (the purple coneflower) is much used for the commercial preparation of herbal medicaments, it being the largest of the three species and easy to cultivate.

- Chemical constituents:

A caffeic acid derivative, echinacoside, is present in the roots of both *E. augustifolia* and *E. pallida*. Cynarin, a quinic acid derivative, occurs only in the former. Esters involving tartaric acid, such as caftaric acid and cichoric acid, occur in small amounts in both species. Other constituents include high molecular weight polysaccharides, alkylamides. The alkamides comprise a complex mixture of unsaturated fatty acids as amides with 2-methylpropanamine (isobutylamine). The BP/EP requires minimum contents of

echinacoside *for E. augustifolia* root (0.5%) and *E. pallida* root (0.2%) determined by liquid chromatography with spectrometric detection at 330 nm.

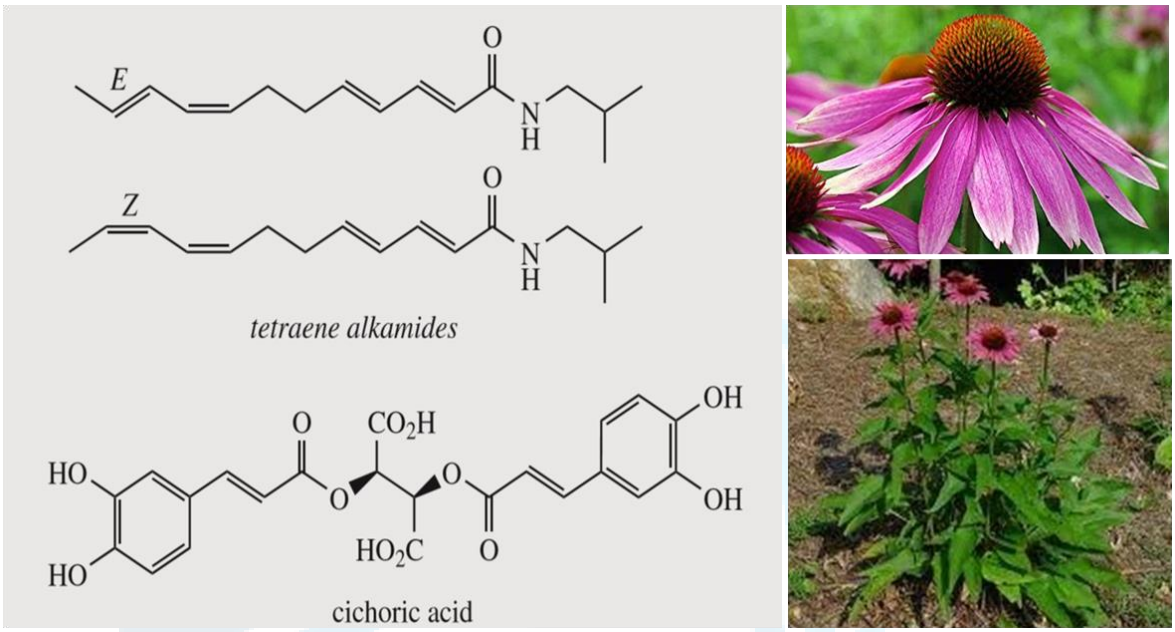


Figure (4.8): *Echinacea purpurpea* and its major constituents.

- Uses:

Echinacea is considered to have immunostimulant properties based on its alkylamide, polysaccharide and cichoric acid components. Preparations of the drug have become popular for the prevention and treatment of the common cold and other respiratory complaints. Activity has variously been assigned to lipophilic alkamides, polar caffeic acid derivatives, high molecular weight polysaccharide material, or to a combination of these. Compounds in each group have been demonstrated to possess some pertinent activity, e.g. immunostimulatory, anti-inflammatory, antibacterial, or antiviral effects.