

1-Introduction

1.1 -Natural products

A natural product is a chemical compound or substance produced by a living organism—that is, found in nature. In the broadest sense, natural products include any substance produced by life. Natural products can also be prepared by chemical synthesis (both semi-synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients¹.

1.1.1-Tannins

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or tea. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruits. Tannins have molecular weights ranging from 500 to over 3,000 (gallic acid esters) and up to 20,000 (proanthocyanidins)¹.

1.1.2-Saponins

Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative¹.

The aglycone (glycoside-free) portions of the saponins are termed sapogenins. The number of saccharide chains attached to the sapogenin/aglycone core can vary – giving rise to another dimension of nomenclature (monodesmosidic, bidesmosidic, etc.) – as can the length of each chain. A somewhat dated compilation has the range of saccharide chain lengths being 1–11, with the numbers 2-5 being the most frequent, and with both linear and branched chain saccharides being represented. Dietary monosaccharides such as D-glucose and D-galactose are among the most common components of the attached chains¹.

1.1.3-Steroids

Steroids comprise a group of cyclic organic compounds whose most common characteristic is an arrangement of seventeen carbon atoms in a four-ring structure, where the rings are three composed of 6-carbons (rings A, B, and C) followed by one with 5-carbons (ring D). Further common features are an 8-

carbon side chain attached to a carbon on ring D, and two or more methyl groups at the points where adjacent rings are "fused". Hundreds of distinct steroids are found in animals, fungi, plants, and elsewhere, and specific steroids underlie proper structure and function in many biological processes. Their core tetracyclic ring structure is synthesized in each organism by biochemical pathways that involve cyclization of a thirty-carbon chain, squalene, into an intermediate, either lanosterol or cycloartenol. From such intermediates, organisms then derive critical steroids such as cholesterol, the sex hormones estradiol and testosterone and bile acids. Based on such structures, synthetic and medicinal chemists synthesize novel steroids for use as drugs such as the anti-inflammatory agent dexamethasone¹.

1.1.4-Glycoside

In chemistry, a glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body².

1.1.5-Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also termed alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and, more rarely, other elements such as chlorine, bromine, and phosphorus¹.

1.1.6-Flavonoids

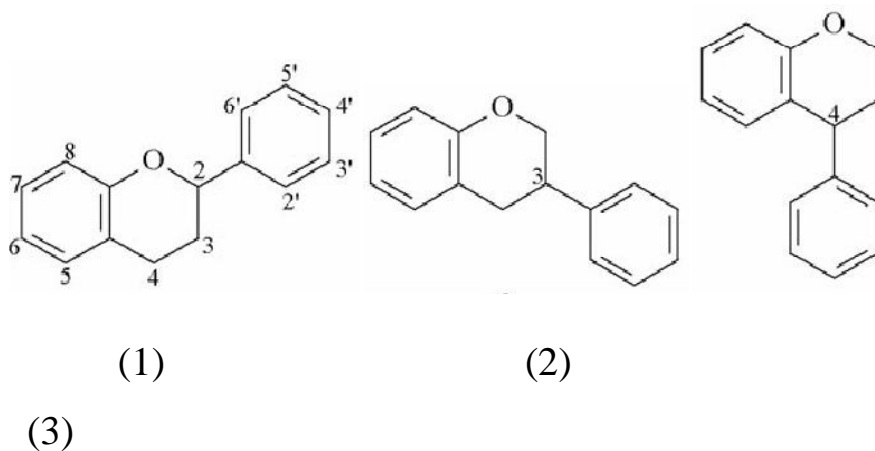
Flavonoids are the low molecular weight polyphenolic secondary metabolic compounds, universally distributed in green plant kingdom, located in cell vacuoles. Flavonoids play a variety of biological activities in plants, animals, and bacteria. In plants, flavonoids have long been known to be synthesized in particular sites and are responsible for color and aroma of flowers, fruit to attract pollinators consequently fruit dispersion; help in seed germination, growth and development of seedling. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, Function as signal molecules, allelopathic compounds, phytoalexins, detoxifying agents, antimicrobial defensive compounds. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimation and freezing tolerance¹.

Flavonoids form a family of well known natural products present in most of the plant families. More than 8000 different flavonoids have been isolated from their natural source to date. The structural variations of these flavonoids are associated with many different biological and pharmacological activities, including anticancer activity, protection against cancer formation (chemo-protection), antioxidant activity, cardiovascular and hepatic protection, antibacterial, antifungal and antiviral activity. Flavonoids have also been reported to play an important role in hormone-related female diseases, such as breast cancer and menopausal syndrome. Natural flavonoids have therefore been subjected to many chemical modifications in order to improve their activity².

1.1.6.1- Nomenclature and classification of flavonoids

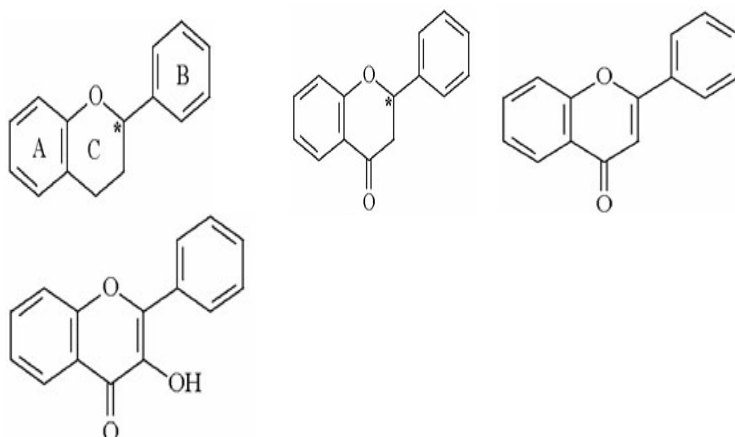
The term “flavonoid” is generally used to describe a broad collection of natural products that include a C₆-C₃-C₆ carbon framework, or more specifically a phenylbenzopyran functionality. Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans) (1), isoflavonoids (3-benzopyrans) (2), and the neoflavonoids (4-benzopyrans)(3). These groups usually share a common chalcone precursor, and therefore are biogenetically and structurally related groups

usually share a common chalcone precursor, and therefore are biogenetically and structurally related ³.



1.1.6.2- Flavonoids (2-phenylbenzopyrans)

Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into the following groups, flavan (4) flavanone(5) flavone (6) flavonol(7) dihydroflavonol (8) flavan-3-ol (9) Flavan-4-ol (10) Flavan-3,4-diol (11) ³.

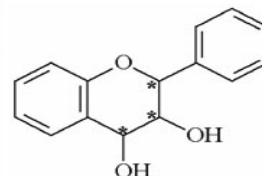
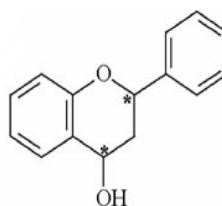
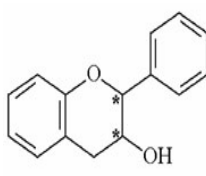
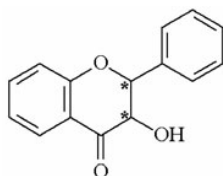


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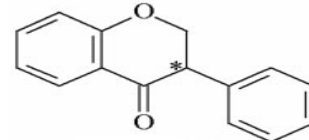
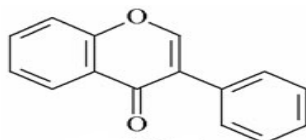
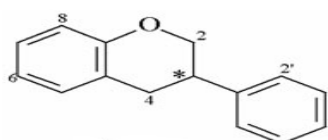
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1.1.6.3- Isoflavonoids

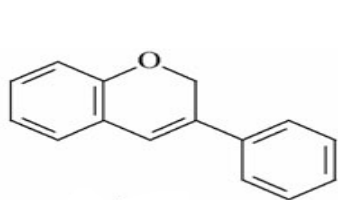
The isoflavonoids are a distinctive subclass of the flavonoids. These compounds possess a 3-phenylchroman skeleton that is biogenetically derived by 1, 2-aryl migration in a 2-phenylchroman precursor. Despite their limited distribution in the plant kingdom, isoflavonoids are remarkably diverse as far as structural variations are concerned. This arises not only from the number and complexity of substituents on the basic 3 phenylchroman system, but also from the different oxidation levels and presence of additional heterocyclic rings. Isoflavonoids are subdivided into the following groups(3) Isoflavan (12) isoflavone (13), isoflavanone(14), Isoflav-3-ene (15), Isoflavanol (16), Rotenoid (17), coumestane (18), 3-arylcoumarin (19), coumaronochromene (20), coumaronochromone [(21), pterocarpans(22)³.



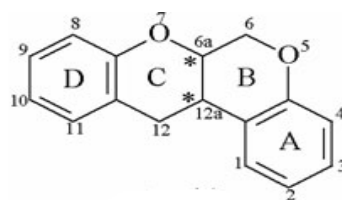
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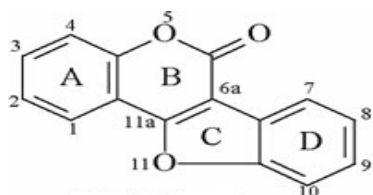


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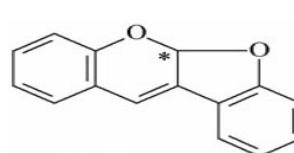


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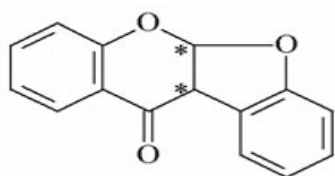


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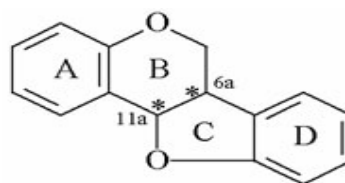


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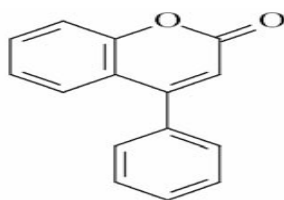
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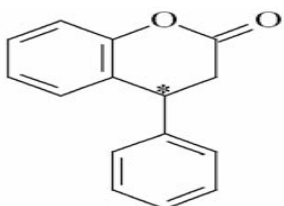
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1.1.6.4- Neoflavonoids

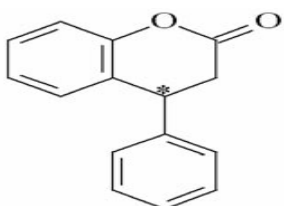
The neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins (4-aryl-2H-1-benzopyran-2-ones) (24), 3, 4-dihydro-4-arylcoumarins (25), and neoflavenes(26)³.



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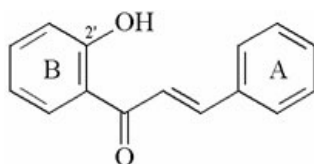
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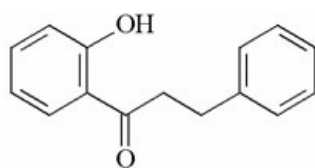
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1.1.6.5- Minor flavonoids

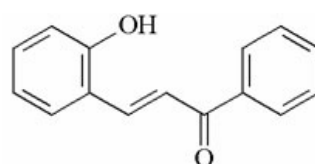
Natural products such as chalcones and aurones also contain a C6-C3-C6 backbone and are considered to be minor flavonoids. These groups of compounds include the 2'-hydroxychalcones(27), 2'-OH-dihydrochalcones(28) , 2'-OH-retro-chalcone(29), aurones (2-benzylidenecoumaranone)(30), and auronols(31) ³.



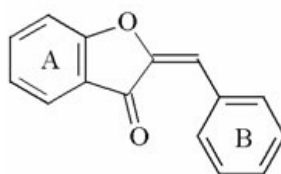
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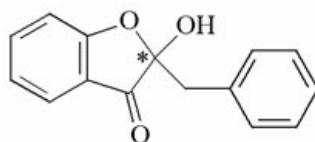
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1.1.6.6- Biosynthesis of flavonoids

The B-ring and part of the heterocyclic ring of the flavonoid skeleton are provided by a suitable hydroxy-cinnamic acid-CoA ester, usually 4-coumaroyl-CoA, whereas the A-ring originates from three acetate units via malonyl-CoA (Fig 2). Both precursors are derived from carbohydrates. Malonyl-CoA is formed from acetyl-CoA and CO₂ catalysed by acetyl CoA

carboxylase. 4-Coumaroyl-CoA and related hydroxycinnamic acid esters are supplied by the first steps of the general phenylpropanoid pathway. This pathway starts from the aromatic amino acid phenylalanine, which is synthesized via the shikimate arogenate pathway. The key reaction is the deamination of phenylalanine catalysed by phenylalanine ammonia-lyase (PAL). This enzyme links the primary metabolism with the phenylpropanoid pathway. The product of the reaction, trans-cinnamate, is hydroxylated to 4-coumarate by cinnamate 4-hydroxylase, a cytochrome P450 mixed-function monooxygenase. Activation of 4-coumarate by formation of the CoA ester is catalysed by 4-coumarate-CoA ligase. 4-Coumaroyl-CoA can be hydroxylated in position 3 to caffeoyl-CoA, which may serve as a substrate for chalcone formation besides 4-coumaroyl-CoA in some plant species. Three different enzyme activities have been demonstrated for caffeoyl-CoA formation from 4-coumaroyl-CoA. The key enzyme for the formation of the flavonoid skeleton is chalcone synthase (CHS), which catalyses the stepwise condensation of three acetate units from malonyl-CoA with 4-coumaroyl-CoA to the fifteen carbon intermediate 2',4',6',4-tetrahydroxychalcone (Fig 1) ⁴. The respective 6'-deoxychalcone, isoliquiritigenin, is likewise synthesized from malonyl-CoA and 4-coumaroyl-CoA by chalcone synthase but in coaction with a (reduced nicotinamide adenine dinucleotide phosphate) (NADPH)-dependent

reductase. Both chalcone types may be the direct precursors for aurones and other diphenylpropanoids. The enzymes involved in these reactions are still unknown. But, in particular, the 6'-hydroxy- and 6'-deoxychalcones are the immediate precursors for all flavonoid compounds. The stereospecific cyclization of the chalcone, catalysed by chalcone isomerase, provides 25-flavanones with the typical flavonoid skeleton (Fig 2). Two types of chalcone isomerases are known: one catalysing cyclization of 6'-hydroxy-chalcone to 5-hydroxyflavanone and another isomerizing both 6'-hydroxy- and 6'-deoxychalcone to 5-hydroxy- and 5-deoxyflavanone, respectively. Flavanones are the direct precursors for other natural products, such as the large class of flavones, isoflavones that are involved in phytoalexin synthesis, and the two flavonoid intermediates, the flavan-4-ols and the dihydroflavonols⁴.

Flavones are synthesized from flavanones by introduction of a double bond between C-2 and C-3. Two types of enzymes, flavone synthase I, (a 2-oxoglutarate-dependent dioxygenase), and flavone synthase II, (a cytochrome P450 mixed-function monooxygenase), were found to catalyse this reaction^[5]. Formation of isoflavones from flavanones is catalysed by 2-hydroxy-isoflavanone synthase, another cytochrome P450 mixed-function monooxygenase, coacting with a dehydratase protein. The enzyme accepts both 5-hydroxy- and 5-deoxyflavanones as substrates. The reaction involves an

oxidative rearrangement of the flavanone, including a shift of the aryl ring from position 2 to 3. The reduction of the carbonyl group of flavanones gives rise to flavan-4-ols. The reaction is catalysed by flavanone-4-reductase and provides the immediate precursors for the formation of 3-deoxy-anthocyanins (Fig 1)⁵.

Finally, flavanones can be hydroxylated in position 3 to dihydroflavonols, which are biosynthetic intermediates in the formation of flavonols, catechins, proanthocyanidins and anthocyanidins. This reaction is catalysed by flavanone 3-hydroxylase, a 2-oxoglutarate-dependent dioxygenase.

Dihydroflavonols are the direct substrates for most of the flavonols and flavan-3,4-diols, which are also known as leucoanthocyanidins. Flavonols are formed from dihydroflavonols(5) by introduction of a double bond between C-2 and C-3. The reaction is catalysed by flavonol synthase, another 2-oxoglutarate-dependent dioxygenase. Reduction of dihydroflavonols in position 4, catalysed by dihydroflavonol-4-reductase, leads to flavan-2,3-trans-3,4-cis-diols, which are intermediates in catechin, proanthocyanidin and anthocyanidin formation. Catechins are synthesized from leucoanthocyanidins by further reduction in position 4. This reaction is catalysed by flavan-3,4-cis-diol-reductase. Proanthocyanidins probably originate from leucoanthocyanidins and catechins by a condensation reaction. The enzyme catalyzing this reaction is not yet known (Fig 1).

The formation of the glycosidic linkage(s) is catalyzed by transferases which are also highly specific, as far as the substrate and the glycosylation positions are concerned. These enzymes require the presence of uridine diphospho-saccharides (UDP-saccharides). Acyltransferases are also specific enzymes for the acylation of some glycosides such as anthocyanins⁵.

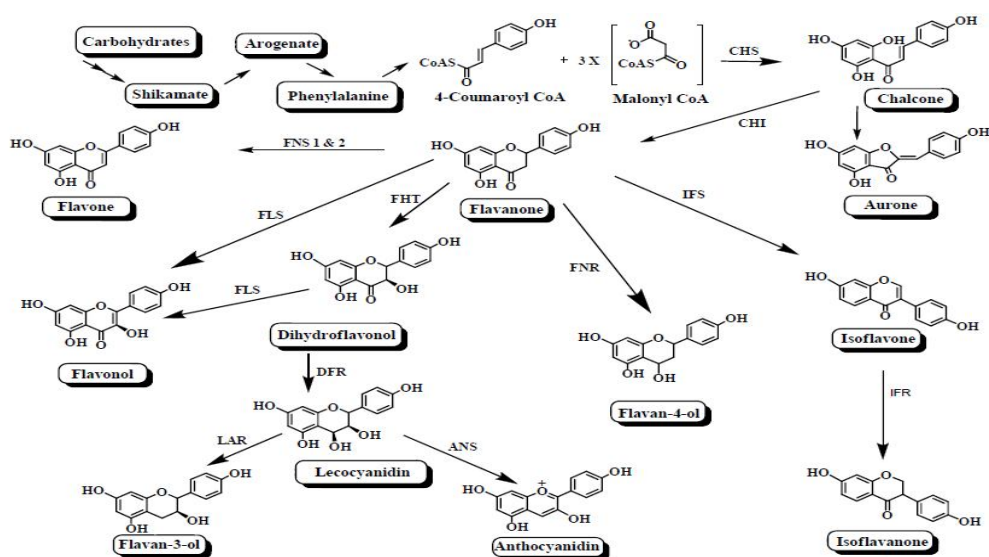


Fig1: Schematic overview of the major branch pathway of Flavonoid biosynthesis, starting from carbohydrates and leading to twelve Flavonoid groups .

1.1.6.7- Metabolism of flavonoids

During metabolism of flavonoids, hydroxyl groups are added (Phase I biotransformation), and then methylated, sulfated or glucuronidated (Phase II biotransformation). In food, flavonoids exist primarily as 3-O-glycosides and polymers⁶.

Several types of higher structures exist, and polymers comprise a substantial fraction of dietary flavonoid intake ⁷. Enzymatic oxidation of green tea leaves during fermentation to black tea results in polymerization of flavanols to tannins and other complex compounds ⁸.

1.1.6.8- Toxicity of flavonoids

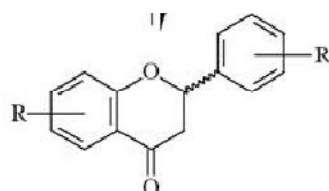
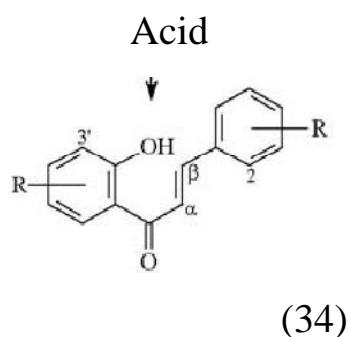
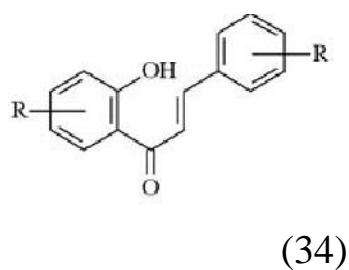
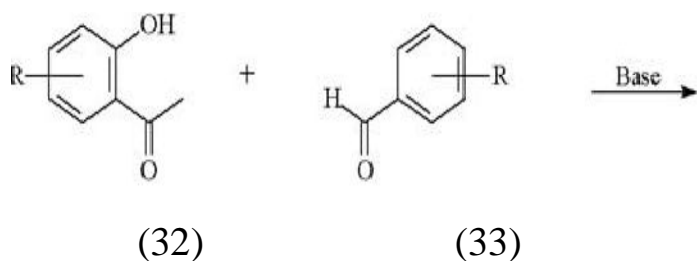
It has been suggested that because flavonoids are widely distributed in edible plants and beverages and have previously been used in traditional medicine, they are likely to have minimal toxicity. However, this family of compounds has a diverse range of activities in mammalian cells⁹⁻¹⁰ and in vivo confirmation of their side effects would be necessary for a full evaluation of their practical usefulness in the field of modern medicine ¹¹. Given that the selectivity of flavonoids for eukaryotic enzymes appears to vary from compound to compound ^{12, 10}, such a study would need to assess the toxicity of these phytochemicals on an individual basis.

1.1.6.9- Synthesis of flavonoids

a) Acid- and base-catalyzed synthesis of chalcones, racemic flavanones

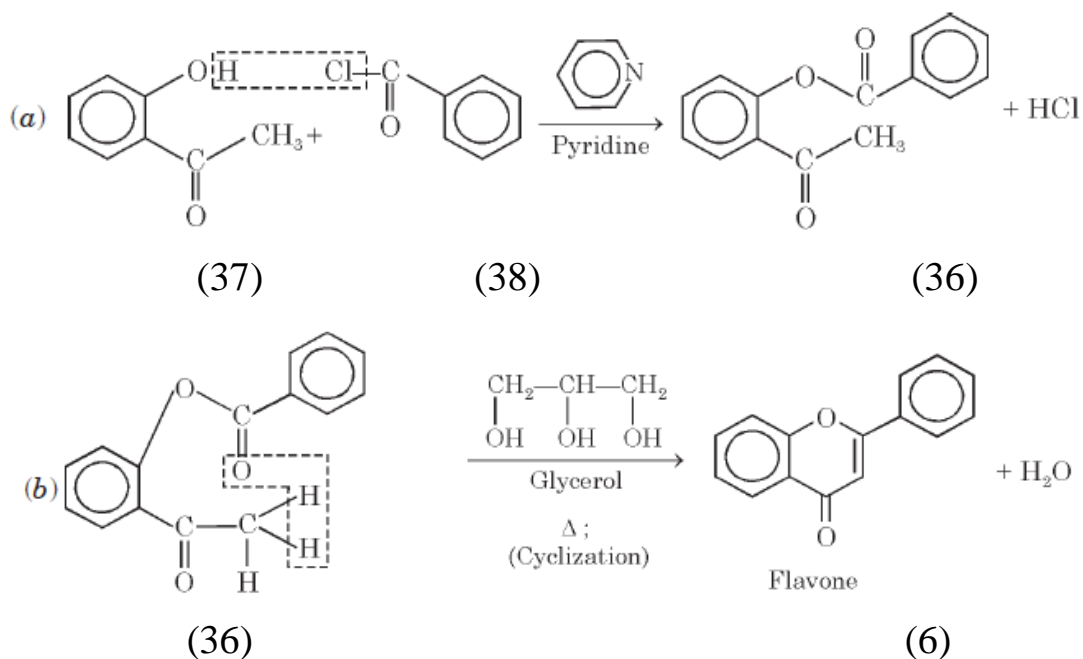
Chalcones and dihydrochalcones are considered to be the primary C₆-C₃-C₆ precursors and constitute important intermediates in the synthesis of flavonoids³. Chalcones are readily accessible via two well-established routes comprising a base-catalyzed aldol condensation or acid-mediated aldolization

of 2-hydroxyacetophenones (32), and benzaldehydes (33). The base-catalyzed aldol condensation is usually the preferred route toward chalcone (34), formation, since under acidic conditions cyclization of the ensuing chalcone leads to formation of corresponding racemic flavanones (35)³.



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There are two methods for the preparation of 'flavone', namely :
(i) From ortho-benzoyloxyacetophenone(36) and conversion of it into flavone by heating with pure redistilled glycerol (2-Step Synthesis), (ii) from ortho-benzoyloxyacetophenone(36), conversion to ortho hydroxybenzoylmethane, and finally to flavones by treatment with sulphuric acid (3-Step Synthesis)¹³.



Equation (a) o-hydroxyacetophenone(37), on benzoylation with benzoyl chloride(38), in the presence of basic medium due to the presence of pyridine gives rise to the formation of obenzoyloxy-Acetophenone(36), and a mole of hydrochloric acid is liberated. The liberated HCl instantly combines with the pyridine (basic) present in the medium to yield the corresponding salt pyridinium chloride¹³.

Equation (b) The o-benzoyloxyacetophenone on heating and treatment with freshly distilled anhydrous glycerol, in an absolute inert atmosphere, abstracts a mole of water ; and ultimately undergoes cyclization to yield flavones(6)¹³.

1.1.6.10-- Isolation of flavonoids

The analysis of flavonoids and their conjugates is one of the most important areas in the field of instrumental analytical methods, helping to solve problems in biological and medical sciences. Different methods of isolation of the natural products may be applied, and the utilization of various strategies is dependent on the origin of the biological material from which the target natural products are to be extracted (plant or animal tissue or body fluids). In the case of polyphenolic compounds, it is often important to initially determine whether the researchers are interested in the identification of individual components present in a mixture of target compounds or whether they would like to estimate the total amount of phenolic compounds in the biological material investigated. This second approach most often takes place during the nutritional studies on different foods or fodders, mainly of plant origin³.

1.1.6.11- Preparation of plant or samples for flavonoid analysis

Free flavonoid aglycones exuded by plant tissues (leaf or root) may be washed from the surface with nonpolar solvents, such as methylene chloride, ethyl ether, or ethyl acetate. However, more

polar glycosidic conjugates dissolve in polar solvents (methanol and ethanol), and these organic solvents are applied for extraction procedures in Soxhlet apparatus. Mixtures of alcohol and water in different ratios are applied for the extraction of flavonoids and their conjugates from solid biological material (plant or animal tissues and different food products)³.

The extraction efficiency may be enhanced by the application of ultrasonication¹⁴ or pressurized liquid extraction (PLE), a procedure performed at elevated temperature ranging from 60°C to 200°C¹⁵. Supercritical fluid extraction with carbon dioxide also may be used¹⁶. However, the temperature conditions during the extraction procedures have to be carefully adjusted because of the possibility of thermal degradation of the flavonoid derivatives. In many cases, further purification and/or preconcentration of the target compound fraction is necessary. In these cases, liquid–liquid extraction (LLE) or SPE are most commonly used. For estimation of the extraction yield it is necessary to spike biological materials with proper internal standards. Most suitable are compounds structurally similar to the studied analytes but not present in the sample¹⁶. Compounds labeled with stable isotopes (²H or ¹³C) are useful when mass spectrometric detection is applied. In the case of the extraction of flavonoids from biological materials, different classes of phenolic compounds are often added. On the other hand,

quantitative analysis of consecutive components of the analyzed flavonoid mixture needs reference standard compounds necessary for preparation of calibration curves essential for a precise quantification. The choice of the extraction procedure for obtaining flavonoid conjugates from biological material is very important and depends on the goals of the conducted research. The evaluation of the spatial distribution of target compounds on the organ, tissue, cellular, or even subcellular level is of special interest in some projects. In these situations, the amount of biological material for the isolation of natural products may be extremely small, and the application of microextraction¹⁶ techniques are necessary¹⁷.

In many cases, it is necessary to avoid the chemical and/or enzymatic degradation of the metabolites. This is of special importance in the profiling of flavonoid glycosides in research directed toward plant functional genomics or during physiological and biochemical studies that need information about all classes of flavonoid conjugates present, even the thermally labile acylated derivatives¹⁷. On the other hand, in the phytochemical analysis of plant species or phytopharmaceutical studies of plant material, the repeatable isolation of all biologically active flavonoid aglycones with a good yield is more important. In these cases, more drastic extraction conditions are acceptable¹⁷.

1.1.6.12-Flavonoids as signal molecules

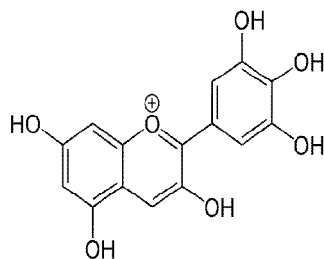
Nearly every class of flavonoid has been shown to have biological activity, with a majority related to antioxidant properties. In plants, flavonoids appear to contribute to a general reduction of reactive oxygen species and therefore impact cellular processes sensitive to REDOX effects. However, flavonoids also have been implicated in more direct interactions with transport and signal transduction pathways. One well-documented example is the role of flavonoids in fertility: while a few flavonoid-deficient plants are able to germinate, grow, and set fertile seed¹⁸ most plants require flavonoids for fertility and normal pollen development. Another is flavonoid modulation of auxin transport as well as localized auxin accumulations observed during nodulation. Perhaps the best-studied example of flavonoid signaling is that of flavonoid mediation of interactions between the plants and other organisms in the environment at both competitive (allelopathy/defense) and cooperative (mycorrhizal association) levels¹⁸.

Flavonoids are bioactive molecules with specific and nonspecific effects on intra- and extraorganismal plant signaling mechanisms. However intraorganismal flavonoid signaling is probably a by-product of the evolution of plant signaling and trafficking mechanisms in an environment where flavonoids are present for purposes of extraorganismal signaling and defense and a role in initial protection from oxidative stress. Recently

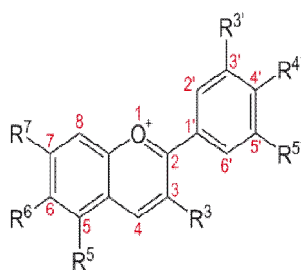
developed molecular biological tools and high throughput metabolic profiling technologies provide new opportunities to identify specific and nonspecific sites of flavonoid regulation¹⁹⁻²⁰

1.1.6.13. Plant–animal interactions

In addition to pollen germination, fertilization, and seed set, flavonoids function in the attraction of animal pollinators. In flower petals, visible flavonoids such as anthocyanins(39), and delphinidin(40) serve as attractants for pollinators like birds, small mammals, and some insects. Natural pollinators can prefer or discriminate against petal color, and therefore play an important role in the evolution of petal color; often a petal color is preferred and flowers of that color are visited more often, which enhances seed yield²¹. UVfluorescent flavonols serve as nectar guides for bees and other insects and enhance the frequency of pollinator visits, indirectly contributing to increased seed yields²².



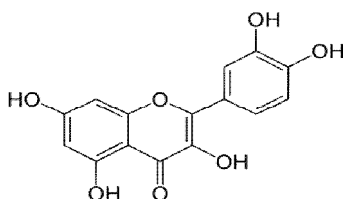
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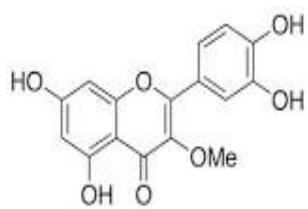
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1.1.6.14- Plant–fungal interactions

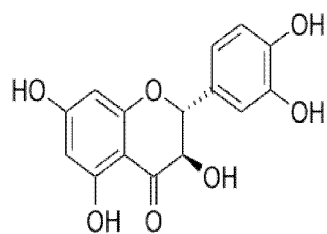
Flavonoids appear to provide defense against fungal infection. A flavone found in rice is allelopathic, to rice fungal pathogens; quercetin(41), quercetin 3-methyl ether(42), and its glycosides inhibited conidia germination in *Neurospora*, and taxifolin(43), appeared to be an antifungal agent in pine²³. In addition to increased amounts of heptamethoxyflavone(44), nobiletin(45), sinensetin(46), and tangeretin(47), the amounts of naringenin(48) decreased while the amounts of the aglycone forms increased after fungal infection in citrus²⁴. C-Glycosylflavonoids accumulated at the plasma membrane immediately at the powdery mildew infection site and inhibition of chalcone synthase expression reduced resistance to fungal infection²⁵. These studies suggest that different classes of flavonoids and their derivatives have differential functions²⁵.



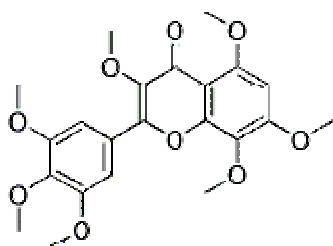
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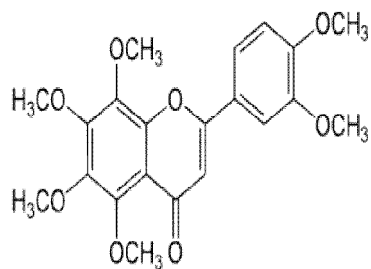
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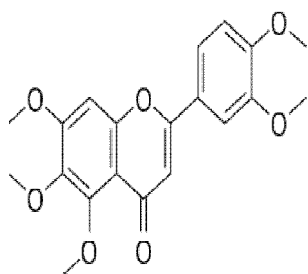
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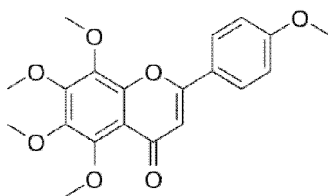
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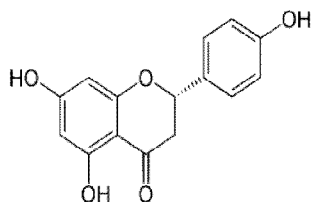
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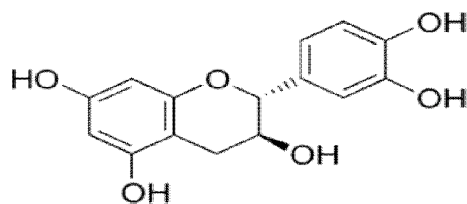
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1.1.6.15- Plant–microbe interactions

It was proposed that plant–microbe interactions occur on a continuum from commensalisms to parasitism²⁶. The multiple roles of flavonoids observed in plant-microbe interactions support this view, as flavonoid signals can attract both beneficial and parasitic bacteria. Similar to antiherbivory or antiparasitic strategies discussed above, flavonoids also are inducible and constitutive components of the defense mechanism against infection²⁷. Several flavones also have been shown to have antimicrobial activity²⁸. Subtle changes in flavonoid speciation can determine the nature of plant microbe interactions. (+)-Catechin and its derivatives appear to be antimicrobial²⁹.



(49)

Nodulation is a special case of plant–microbe signaling. Nodulation is the formation of nitrogen-fixing nodules in roots of legumes (beans, peas, alfalfa, clover, for example) and *Parasponia*, a non-legume, by bacteria in the Rhizobiaceae, and occurs when the host plant and the rhizobium form a symbiotic relationship. Although some rhizobia are generalists, many plant–rhizobium pairs are specialized, with species-specific flavonoids in the roots exudates that are stimulatory to compatible species of rhizobia and inhibitory to noncompatible rhizobia and other soil flora²⁹.

1.1.6.16- Biological Activitiy of flavonoids

Flavonoids are composed of two aromatic rings linked through three carbon atoms that form an oxygenated heterocycle. Variations on the basic structure of flavonoids yield different classes of flavonoids³⁰. These structural variations may explain the observed differences in the bioactivity of these related compounds. . Natural flavonoids have therefore been subjected to many chemical modifications in order to improve their activity³¹.

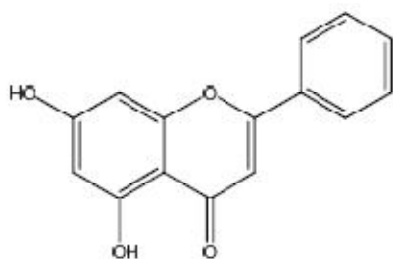
Flavonoids may have existed in nature for over 500 million years and thus have interacted with evolving organisms over the eons. Clearly, flavonoids serve important purposes in nature, having survived in vascular plants throughout evolution³². Flavonoids not only equip the plants themselves with unique properties (such as colors), but also exert an influence on animals living with plants. The long association of plant flavonoids with various animal species and other organisms throughout evolution may account for the extraordinary range of biochemical and pharmacological activities of these chemicals in mammalian and other biological systems.

First of all, we will consider the effects on the plant itself. Flavonoids have important roles in plant biochemistry and physiology, they act as antioxidants, enzyme inhibitors, and precursors of toxic substances, while they also take part in nitrogen fixation, and act as pigments and light screens³³. In addition, these compounds are involved in photosensitization and energy transfer. Certain flavonoids also function as plant growth hormones and growth regulators. They are involved in the control of respiration, photosynthesis, morphogenesis, and sex determination, as well as defence against infections³⁴.

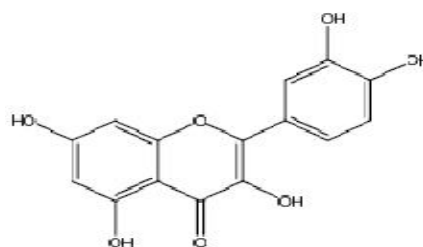
On the other hand, in humans and higher animals, flavonoids have long been recognized to possess anti-inflammatory, antioxidant, ant-allergic and hepato-protective properties. They are also believed to be antithrombotic, antibacterial, antifungal,

antiviral, and cancer protective, and also to protect against cardiovascular disease³⁵⁻³⁷.

Small alternations in the chemical structure of flavonoids may lead to significant changes in biological activities, e.g. chrysin(49) is a poor antioxidant compared to quercetin (50) the latter has increased antioxidant activity due to the presence of three additional hydroxyl groups, yet chrysin is 20 times more effective in the inhibition of the human aromatase enzyme³⁸.



(49)



(50)

1.1.6.17- Antioxidant activity associated with flavonoids

Three important points must be illustrated in order to better understand antioxidant activity of flavonoids and the important role of flavonoids compounds in human biology:

- a. The role of oxidative stress in human diseases.
- b. The antioxidant structure-activity relationship of flavonoids.
- c. The structure characteristics of an effective flavonoid antioxidant.

a. The role of oxidative stress in human diseases

Oxidation is the transfer of electrons from one atom to another. It represents an essential part of our metabolism and aerobic life in general, since oxygen is the ultimate electron acceptor in the electron flow systems that transport energy in the form of ATP³⁹. Problems may arise however when the electron flow generates free radicals, such as O₂-centred free radicals, known as reactive oxygen species (ROS), and including superoxide (O₂^{•-}), peroxy (ROO[•]), alkoxy (RO[•]), hydroxyl (HO[•]) and nitric oxide (NO[•]) radicals.

The contribution of free radical-mediated processes to the pathogenesis of human disease is indicated by biomarkers of oxidative damage to lipids, proteins, and DNA (Fig 2). Such markers have been identified in patients with atherosclerosis, certain cancers, neurodegenerative diseases, and lung disorders, especially those with an inflammatory component to their etiology. A range of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in the mechanisms of damage associated with disease development, including superoxide radical (O₂^{•-}), hydrogen peroxide (HOO[•]), hypochlorite radical (ClO[•]), ferryl heme protein species, lipid alkoxy (RO[•]) and peroxy radicals (ROO[•]), peroxynitrite (ONOO⁻), nitric oxide (NO[•]), and nitrogen dioxide radicals (NOO[•])⁴⁰.

Reactive oxygen species obey different rules in vivo. Some are involved in energy production, phagocytosis, regulation of cell growth and intracellular signaling, as well as recognizing of biologically important compounds⁴⁰.

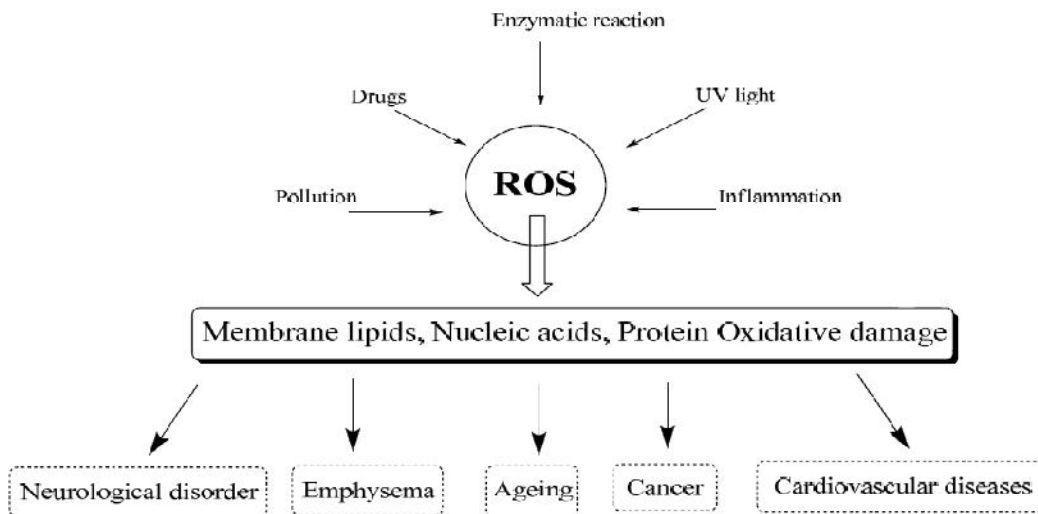


Fig 2: Formation and impact of ROS on the human body.

Reactive oxygen species ROS may be very damaging, since they can attack lipids in cell membranes, proteins in tissues or enzymes, carbohydrates and DNA to induce oxidative modifications, which cause membrane damage, loss of protein function and DNA damage⁴⁰. This oxidative damage is considered to play a causative role in ageing and several degenerative diseases associated with it, such as heart disease, congestive dysfunction and cancer. Humans have evolved antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body

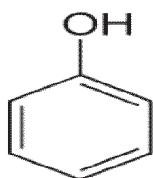
(endogenous antioxidants) and others obtained from the diet (exogenous antioxidants)⁴⁰.

Defense systems against damage induced by reactive oxygen species ROS fall into three categories:

- Preventative antioxidants that suppress free radical formation.
- Radical-scavenging antioxidants that inhibit initiation of chain reactions and intercept chain propagation, including catalytic antioxidants.
- Antioxidants involved in repair processes.

b. The antioxidant structure-activity relationship of flavonoids

In order for a phenol (51) to be classified as an antioxidant it must possess two properties: firstly, it should be oxidized properly when present in low concentrations compared to the substrate, hence delaying or preventing the autoxidation or free radical-mediated oxidation; secondly, the phenol free radical formed after scavenging must be stable (through intramolecular hydrogen bonding) to further oxidation and cannot act as oxidant in its own right³⁹⁻⁴¹. Oxidisable substrates include almost all organic molecules found in food and living tissues including lipids, carbohydrates, proteins and DNA.



(51)

The chemistry of the flavonoids is predictive of their free radical scavenging activity as the reduction potentials of flavonoids and the consequently radical form, are lower than those of alkyl peroxy radicals and the superoxide radical, which therefore means the flavonoids may inactivate these radical species and prevent the deleterious consequences of their reactions⁴²⁻⁴⁴.

The electron/H-donating properties of flavonoids are considered to be the basis of their antioxidant action. Their free radical scavenging properties are best approached through structure-antioxidant activity relationships. The ability of flavonoids to act as antioxidants by electron donation depends directly on the reduction potentials, and inversely on the reactivity of the flavonoid molecules with dioxygen, as the generation of peroxy radicals will propagate oxidative reactions. These concepts have been reviewed⁴⁵.

In essence, three types of structural properties have been recently known to appear to increase the antioxidant activity of the flavonoids;

i- The ortho-dihydroxy structure in the B ring ⁴⁶, which confers higher stability to the radical form and participates in electron delocalization.

ii- The 2,3 double bond in conjugation with a 4-oxo function in the C ring is responsible for electron delocalization from the B ring ⁴¹. The antioxidant potency is related to structure in terms of electron delocalization of the aromatic nucleus. When these compounds react with free radicals, the phenoxyl radicals produced are stabilized by the resonance effect of the aromatic nucleus.

iii- The 3,5-OH and 4-oxo functional groups in the A and C rings are required for maximum radical scavenging potential⁴⁶.

c. The structure characteristics of an effective flavonoid antioxidant

Quercetin fig (3) is perhaps the best example of a flavonoid that displays these three structural properties, and so efficiently captures free radicals⁴⁷.

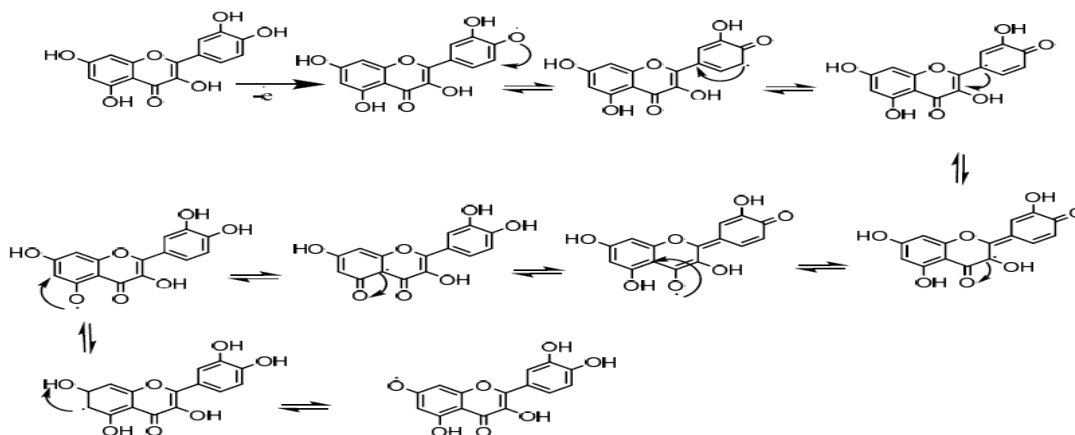


Fig 3: Delocalization and resonance of quercetin radical

Quercetin has five free hydroxyl groups which can donate electrons and a complete resonance system which can stabilize the quercetin radical (Fig 3). Furthermore the hydroxyl groups at position 3, 5, 3' and 4' have a specific configuration that enables the quercetin molecule to bind with up to three metal ions (Fig 4) such as Cu^{2+} or Fe^{3+} . These redox active metal ions may contribute to ROS production ⁴⁷, via a Fenton-type radical generating chemistry. Sequestration of such adventitious metal ions may therefore also be considered as an antioxidant activity ⁴⁸.

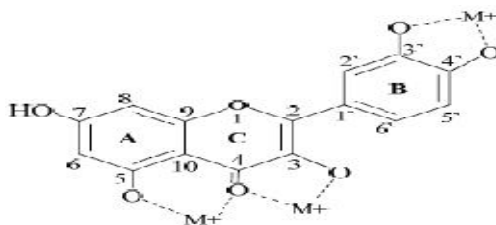
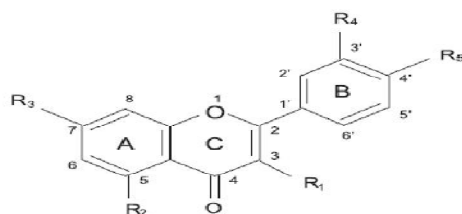


Fig 4: Metal binding site of quercetin.

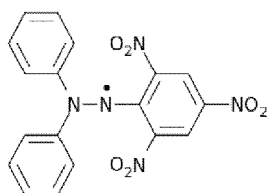
The free radical scavenging activity of five pure flavonoids was evaluated through their ability to quench the synthetic 2,2-diphenyl-1-picrylhydrazyl (DPPH)(52) radical. The assay has been used worldwide as a screen to determine the free radical scavenging capacity of various diet compounds because of its simplicity and requiring relatively short time compared to other methods. Antioxidant activity of flavonoids was observed in the first minute of their incubation with DPPH radical(52), suggesting rapid kinetics of interaction of these compounds. Scavenging activity differs between tested compounds fig 5. It is

shown that chemical structure has an important impact on radical scavenging activity. The more hydroxyl moieties the higher antioxidant activity⁴⁹. Two hydroxyl groups in B ring are required to stronger antioxidant potential, lack of one of them (apigenin) significantly reduces this activity. Methyl groups in rhamnetin and isorhamnetin decrease the radical scavenging properties at lower concentrations compared to the strongest flavonoid quercetin. The location of methyl group also affect an antioxidant potential, rhamnetin, which has this group at C7 in A ring possesses higher antioxidant activity than isorhamnetin (methyl group at C4' in B ring)⁴⁹.



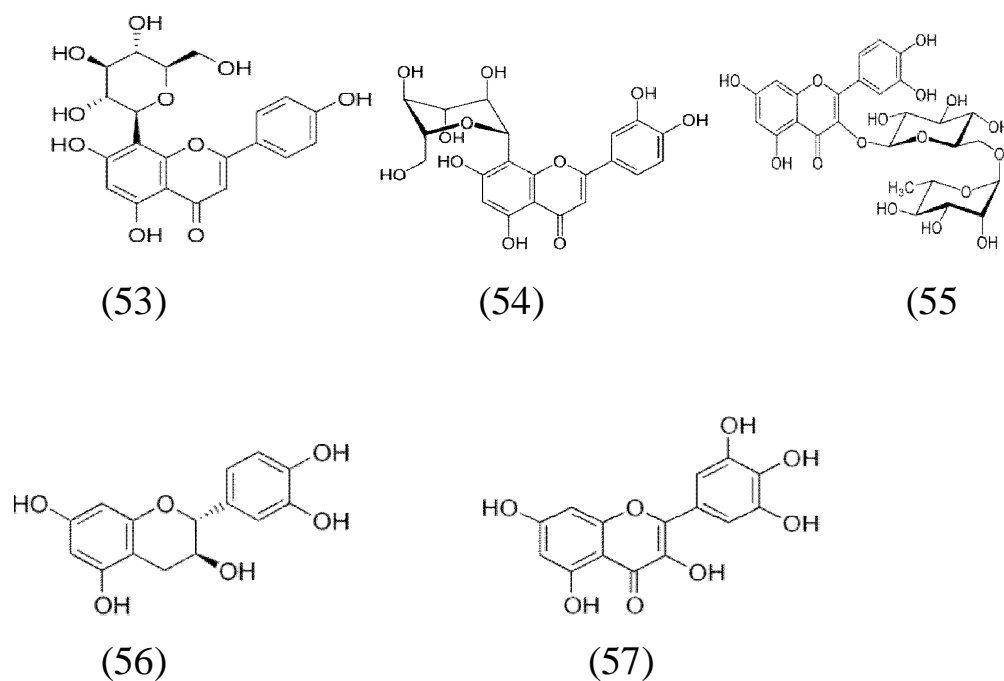
| Flavonoids | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ |
|--------------|----------------|----------------|-----------------|----------------|-----------------|
| quercetin | OH | OH | OH | OH | OH |
| rhamnetin | OH | OH | CH ₃ | OH | OH |
| isorhamnetin | OH | OH | OH | OH | CH ₃ |
| luteolin | - | OH | OH | OH | OH |
| apigenin | - | OH | OH | - | OH |

Figure 5 structure of tested flavonoids (quercetin, rhamnetin, isorhamnetin, luteolin, and apigenin)



(52)

Considerable amount of flavonoids and phenolics contents were found in all the fractions of *Launaea procumbens* (LP). Methanol and chloroform fraction exhibited efficient scavenging of 2,2- diphenyl-1-picrylhydrazyl (DPPH)[•], [•]OH, superoxide, lipid peroxide and nitric oxide free radicals. Significant correlation was found between 2,2- diphenyl-1-picrylhydrazyl (DPPH)[•], superoxide radical, β-carotene bleaching restraint and phosphomolybdenum assay with total flavonoids and phenolics contents. High performance chromatography (HPLC) of *Launaea procumbens* (LP) revealed the presence of vitexin(53), orientin(54), rutin(55), catechin(56) and myricetin(57)⁵⁰.



Epidemiologic studies suggest an inverse association of tea consumption with cardiovascular disease. The antioxidant effects of flavonoids in tea (fig 6) (including preventing

oxidative damage to LDL) are among the potential mechanisms that could underlie the protective effects ⁵¹. Other possible mechanisms include attenuating the inflammatory process in atherosclerosis, reducing thrombosis, promoting normal endothelial function, and blocking expression of cellular adhesion molecules. Cocoa and chocolate can also be rich sources of flavonoids (fig 6) ⁵¹. Flavanols and procyanidins isolated from cocoa exhibit strong antioxidant properties in-vitro. In acute feeding studies, flavanol-rich cocoa and chocolate increased plasma antioxidant capacity and reduced platelet reactivity. Based on limited data, approximately 150 mg of flavonoids is needed to trigger a rapid antioxidant effect and changes in prostacyclin. Some dose-response evidence demonstrates an antioxidant effect with approximately 500 mg flavonoids. Brewed tea typically contains approximately 172 mg total flavonoids per 235 ml (brewed for 2 min); hence, consumption of 1 and 3.5 cups of tea would be expected to elicit acute and chronic physiologic effects, respectively. Chocolate is more variable with some products containing essentially no flavonoids (0.09 mg procyanidin/g), whereas others are high in flavonoids (4 mg procyanidin/g). Thus, approximate estimates of flavonoid rich chocolate needed to exert acute and chronic effects are 38 and 125 g, respectively. Collectively, the antioxidant effects of flavonoid-rich foods may reduce cardiovascular disease risk ⁵¹.

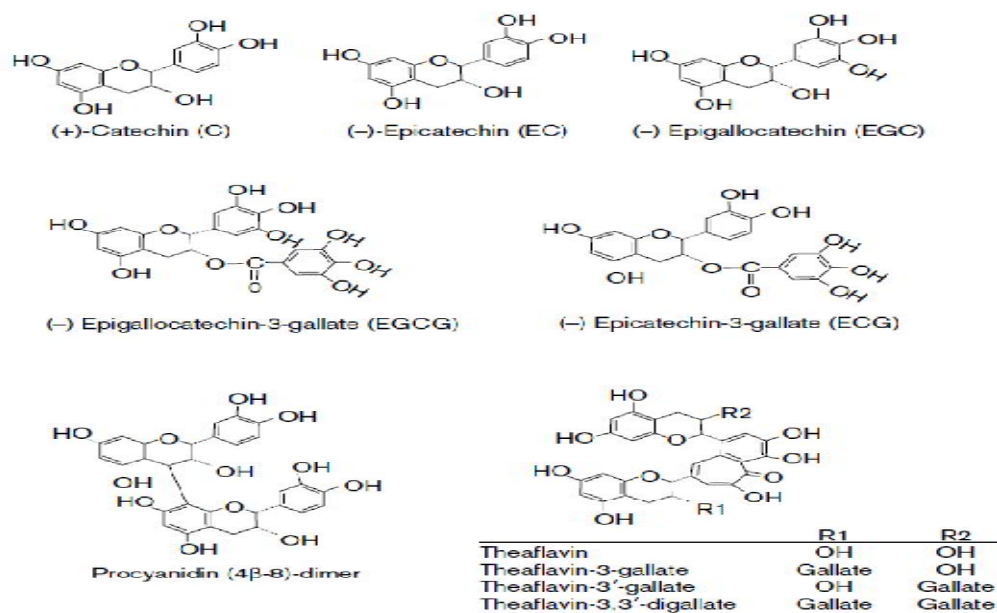


Figure 6. Chemical structures of flavanols in tea and cocoa

There are reports regarding beneficial health effects of flavonoids. Flavonoids are phenol substance isolated from a wide range of vascular plants, with over 8000 individual compound known. Flavonoids are secondary plant products. They are mainly found in fruits, vegetables and certain beverages that have diverse beneficial biochemical antioxidant effect. Flavonoids were originally referred to as “vitamin P”. Their dietary intake is quite high compared to other dietary antioxidants like vitamins C and E. The major actions of flavonoids are those against cardiovascular diseases, ulcers, viruses, inflammation, osteoporosis, diarrhea and arthritis. Brief description about the disease causing effect of free radicals is given and ways by which flavonoids neutralize free radicals has also been mentioned. The antioxidant activity of flavonoids

depends on their molecular structure, and structural characteristics of certain flavonoids found in hops and beer confer surprisingly potent antioxidant activity exceeding that of red wine, tea, or soy. Flavonoids and proanthocyanidins are often found in fruits and vegetables and they powerful anticancer agents. Antioxidants are the compounds that protects cell against the damaging effect of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals and peroxy nitrite. They also can protect LDL stickier and more likely to clog arteries. They also act as an anti-aging⁵².

The current knowledge about structure-activity relationships (SAR) can provide useful tools for revealing the nature of flavonoid antioxidant action. They may also help in the design of new and efficient flavonoids, which could be used as potential therapeutic agents. it seems that favourable general structural requirements for effective radical scavenging and/or the antioxidative potential of flavonoids as follow⁵³.

The o-dihydroxy (3',4'-diOH, i.e., catechol) structure in the B ring figure 7 , which confers high stability to the flavonoid phenoxyl radicals via hydrogen bonding or by expanded electron delocalization⁵³.

The C2-C3 double bond (in conjugation with the 4- oxo group) figure 7, which determines the coplanarity of the heteroring and participates in radical stabilization via electron delocalization over all three ring systems⁵³.

The presence of both 3-OH and 5-OH groups figure 7 for the maximal radical scavenging capacity and the strongest radical absorption.

Moreover, an additional criterion could be added: In the absence of o-dihydroxy structure in the B ring, hydroxyl substituents in a catechol structure on the A ring are able to compensate and become a larger determinant of flavonoid antiradical activity⁵³.

the basic flavonoid structure does not seem to be essential for good antioxidant activity. It becomes important only when the catechol moiety is not present. In addition, glycosylation of flavonoids mostly decreases their antioxidant activity. Blocking the hydroxy group at the C-3 position or removing the 3-OH group decreases antioxidative properties of flavonoids⁵³.

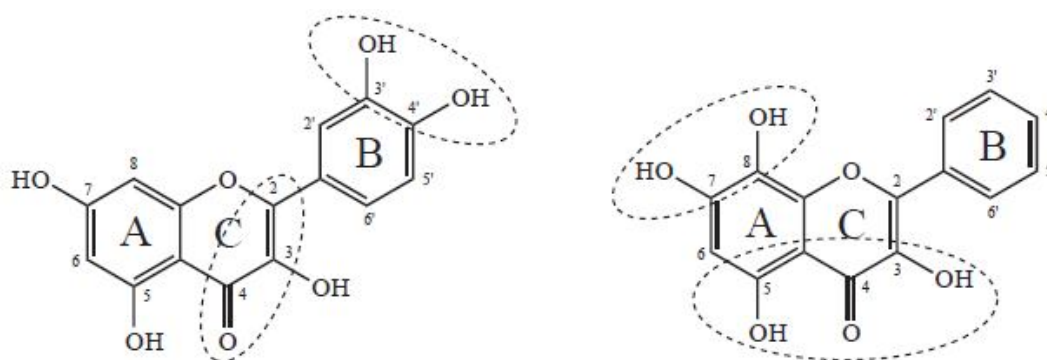


Fig. 7 summarizes the structural criteria that modulate the antioxidant activity of flavonoids.

Flavonoids are hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection⁵⁴, it

should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes⁵⁵.

All the flavonoids (58-62) showed in vitro antimicrobial activity against all the isolated strains of *K. pneumoniae*, similar to that produced by the control antibacterial Ofloxacin(63), on the other hand, another control, Ampicillin(64), had no activity. These flavonoids may be considered potential therapeutic compounds for infections that may be caused by extended spectrum β -Lactamase-producing bacteria in the future⁵⁶.

1.7- *Cassia fistula* Linn

Cassia fistula Linn (Caesalpinaceae) is a deciduous tree of middle size reaching up to 10m in height. Traditional claims include treatment of ulcers and other intestinal disorders⁵⁷. The plant is also used as laxative and tonic⁵⁸. *Cassia fistula* has also been used for haematemesis, diabetes, skin diseases and liver disorders⁵⁹. It has been reported that the plant has analgesic⁶⁰, antioxidant⁶¹, antifungal⁶² and hepatoprotective properties⁶³.

Phytochemical screening revealed the presence of flavonoids, tannins, saponins, alkaloids, terpenoids, anthraquinones, steroids and reducing sugars⁶⁴. Stem bark contains flavonols

beside xanthenes⁶⁵. In a study, the ethyl acetate fraction of the bark showed significant hypoglycemic activity and the mechanism underlying this action was addressed^{66,67}. Different in vivo studies documented the hepatoprotective effect of the seeds and fruits⁶⁸⁻⁷¹. Aqueous and methanol extracts showed significant radical scavenging capacity in the DPPH assay⁷²⁻⁷⁴. The methanol extract of buds exhibited potent antipyretic activity⁷⁵. The antifungal properties of leaf extracts was studied⁷⁶. It has been reported that the methanol extract of seeds significantly decreased tumor volume and increased the life span in some in vivo studies⁷⁷.

1.8-Acacia nilotica

Acacia is a genus of shrubs and trees belonging to the subfamily *Mimosoideae* of the family *Fabaceae*, first described in Africa by Swedish botanist Carolus Linnaeus in 1773. The plants tend to be thorny and pod-bearing, with sap and leaves typically bearing large amounts of tannins⁷⁸.

Acacia species is indigenous to Sudan, Ethiopia, East Africa, Angola, Iran, Afghanistan and India. The *Acacia* genus include more than 1,200 species⁷⁹ of flowering trees and shrubs, some of them are used in folkmedicine.

The traditional circumscription of the genus *Acacia* is not monophyletic. This discovery has led to the breaking up of *Acacia* into five genera along with the much debated re-

typification of the genus with an Australian species instead of the original African type species⁸⁰.

Nine sub-species are known for *Acacia nilotica*. Hybridization between the various *Acacia* species occurs and has been influenced by human's seed dispersal. Within the *Acacia nilotica* complex the pods are very variable⁸¹.

The bark and seeds are sources of tannins, while the gum, bark, roots and flowers are used for medicinal properties⁸². A 19th century Ethiopian medical text describes a potion made from an Ethiopian species of *Acacia* as a cure for rabies⁸³. An astringent medicine high in tannins, called catechu is procured from several species, but more especially from *Acacia catechu*.

The gum is used for toothache and for healing wounds. The pods are used for diabetes, leprosy, tuberculosis, cough, colds, diarrhea, dysentery, inflammations, ophthalmia, syphilitic ulcers, and fever^{84,85}.

It was demonstrated that the aqueous extract of *Acacia nilotica* possesses anti-inflammatory, analgesic and antipyretic activities⁸⁶. The aqueous and alcoholic extractives were also tested against human pathogenic bacteria. The extracts were more effective against Gram positive cocci than Gram negative bacilli. Though the aqueous and alcoholic extractives failed to show any detectable anti-fungal activity, the n-hexane extracts demonstrated a satisfactorily significant activity against *Candida albicans*^{87,88}.

1.9-*Eucalyptus camtaldulensis*

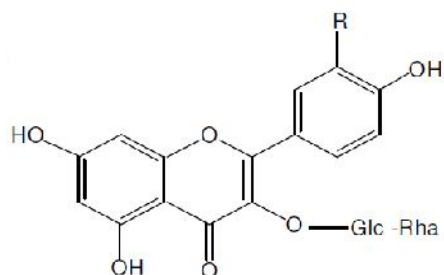
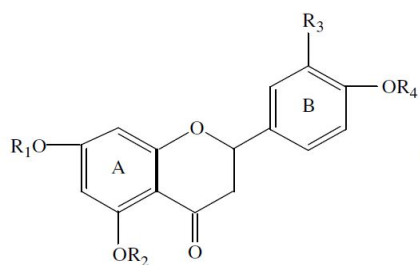
Eucalyptus camtaldulensis is a tree up to 50m in height. The genus *Eucalyptus* is a large genus comprising about 900 species indigenous to Australia , New Guinea and Tasmania. Now the genus is grown worldwide specially in temperate regions due to its economic value⁸⁹⁻⁹³(63,64,66,67).

Eucalyptus oil has been used traditionally against: kidney disorders,gastritis,diabetes,cystitis,ringworms,urethtitis,malaria,l eucorrhoea and laryngitis. It has also been used for asthma,bronchitis and inflammation of the respiratory tract⁹⁴⁻⁹⁸.Externally the oil is used for lung tuberculosis ,neuralgic pain and fever.Though it occurs in many parts of the plants , the oil is plentiful in leaves.

Aim of this study

This study was aimed to:

- Isolation and identification of flavonoids from the medicinally important species- *Acacia nilotica* subsp. *tomentosa*.
- Extraction of fixed oils from *Cassia fistula* and *Eucalyptus camaldulensis*.
- Biological activity of different fractions of *Acacia nilotica* subsp. *tomentosa* and target oils.



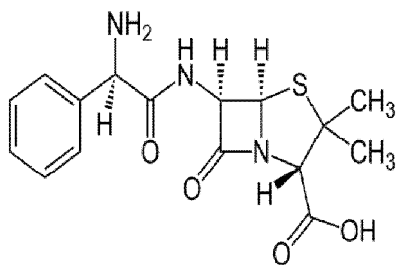
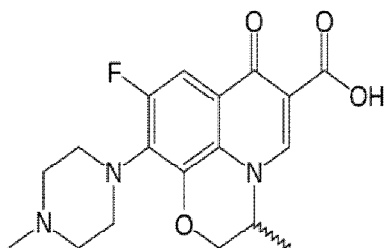
(58) R₁= R₂: - CH₃, R₃: -H, R₄-Glc:

(61)R: OH

(59) R₁= R₂: - H, R₃: -OH, R₄-Glc

(62)R: H

(60) R₁= R₂= R₃= R₄: -H

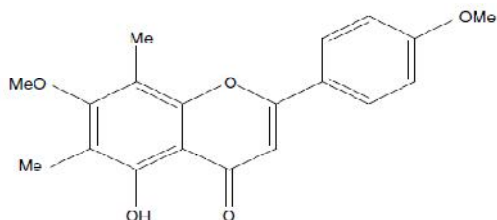
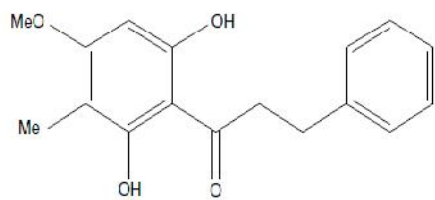


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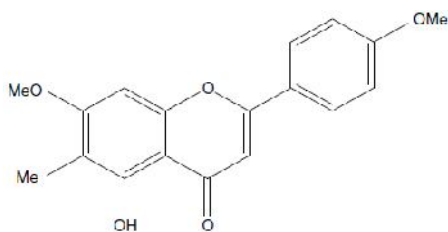
We have selected Eucalyptus clones possessing potent antibacterial activities, and have developed antibacterial agents containing the leaf extracts. After extraction we make structure elucidation and give compounds (65-67). This antibacterial agent is currently used in the formulation of wet tissues and other commercial products. Finally, the eucalyptus extracts were found to be effective against pathogens causing food poisoning, acne, and athlete's foot, and a wide range of commercial

applications (kitchen, restaurants, ingredients of cosmetics, sanitary products, etc.) can be anticipated⁵⁷.



(65)

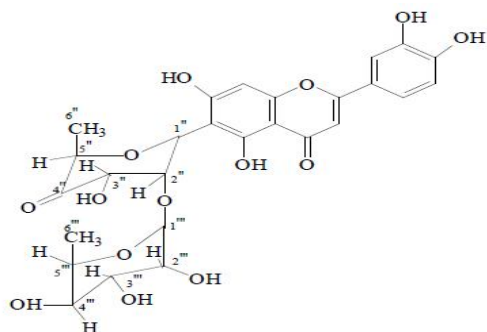
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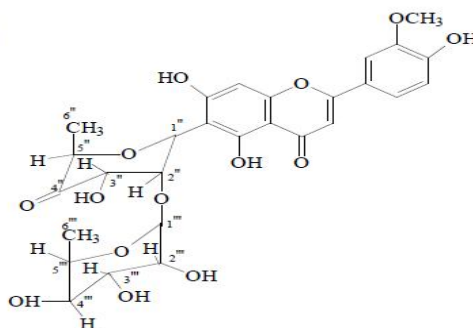
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Isolated compounds (68) (maysin) and (69) (maysin-3'-methyl ether) from the n-butanol fraction of methanol extract of corn silk. Flavonoid glycosides showed wider range of activity towards gram-positive and gram-negative bacteria. Comparatively, compound (68) exerted highest antibacterial activity towards gram positive bacteria than (69). In comparison with gentamycin, compound (68) showed significantly higher activity against the tested bacteria. Maysin-3'-methyl ether (69) showed comparatively lower activity than I, it seems the presence of methoxyl substitution on C-3' position slightly decreases the sensitivity towards bacteria. The presence of

phenolic hydroxyl groups was essential for higher antibacterial activity⁵⁸.

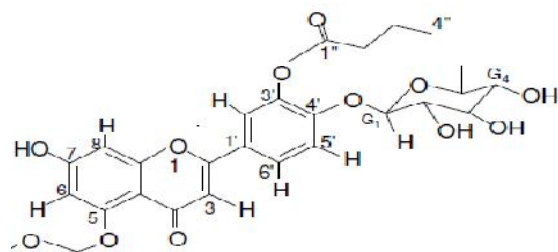


(68)



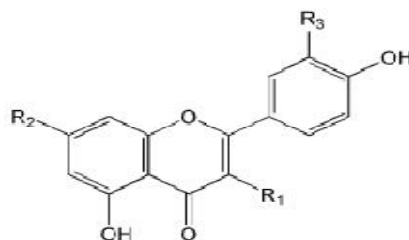
(69)

Flavonoids are secondary metabolites and their biological activities have an impact on human health so that they serve as target molecules to develop new drugs. From methanolic extract of aerial parts of *Chaerophyllum macropodum* (Boiss.), a diglycosylated flavonoid derivative (70) have been isolated by column chromatography (CC) and preparative TLC. The antimicrobial activity of the methanol extracts of aerial part were determined against seven Grampositive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), as well as three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The bioassay showed that the extract exhibited moderate antimicrobial activity⁵⁹.



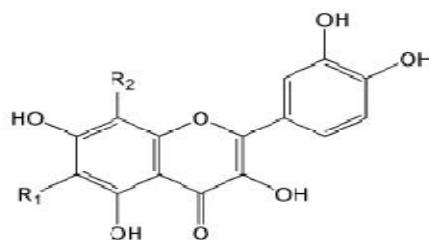
(70)

Phytochemical investigation of the whole *Morettia philaena*. Plant led to the identification of nine flavonoids isolated using chromatographic techniques. These are kaempferol(71), kaempferol 3-O- β -Glucopyranoside(72), kaempferol 3, 7-di-O- β -glucopyranoside(73), kaempferol 3-O- β -sophoroside-7-O- β -glucopyranoside(74), quercetin(75), quercetin 3-O- β glucopyranoside(76), quercetin 3-O- β -gentobioside(77), orientin(78) and isoorientin(79). Their structures were established through chemical and spectral analysis. The antimicrobial activity of the isolated flavonoids and the aqueous methanol extract against six bacteria and one fungus species was studied. Among them, the isolated aglycones, mono-O-glycosides and C-glycosides exhibited interesting high activity against most of the tested organisms⁶⁰.



| Compound | R1 | R2 | R3 |
|----------|----|----|----|
|----------|----|----|----|

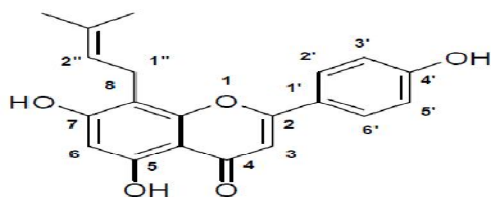
| | | | |
|------|----------------------|----------------------|----|
| (71) | OH | OH | H |
| (72) | O- glucopyranosid | OH | H |
| (73) | O- glucopyranosid | O- glucopyranosid | H |
| (74) | O-sophrosid | O- glucopyranosid | H |
| (75) | OH | OH | OH |
| (76) | O- glucopyranosid | OH | OH |
| (77) | O-gentobioside | OH | OH |



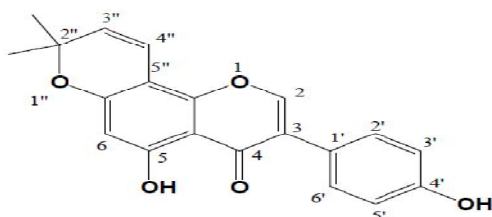
| Compound | R1 | R2 |
|----------|-----------------------|-----------------------|
| (78) | H | C- glucopyranoside |
| (79) | C- glucopyranoside | H |

We have investigated the antibacterial and antifungal activities of two flavonoids isolated from *Retama raetam* flowers. The compounds licoflavone C (80) and derrone (81) were active

against *Pseudomonas aeruginosa* and *Escherichia coli* and showed important antifungal activity. Strong antifungal activity against *Candida species* was for example found with compound (81). These two compounds may be interesting antimicrobial agents to be used against infectious diseases caused by many pathogens⁶¹.



(80)



(81)

1.1.9.3 Anti-inflammatory activity.

Cartilage erosion in inflammatory joint diseases occurs predominantly from the lateral aspects of the joint at the junction between the invading synovium and the cartilage. Over time, there is a breakdown of the cells in joints. Age, trauma, genetic predisposition and general wear and tear stress cause the release of membrane components into joint tissue. The liberated phospholipids are then converted to arachidonic acid by phospholipase A2. Arachidonic acid can be produced in the body and plays an important role in many metabolic pathways. However, when joints are damaged, the excess of arachidonic

acid is converted by the Cyclooxygenase and lipoxygenase pathways into powerful inflammatory substances known as prostaglandins and leukotrienes, respectively^{62, 63}.

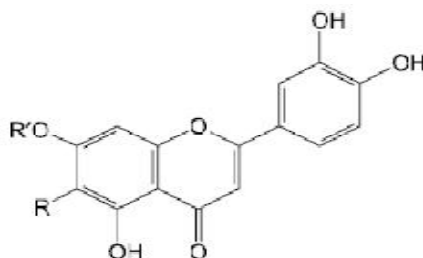
Most clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related diseases. Though these have potent activity, long-term administration is required for treatment of chronic disease. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics⁶⁴.

Epidemiological and experimental studies reveal a negative correlation between the consumption of diets rich in fruit and vegetables and the risks for chronic diseases, such as cardiovascular diseases, arthritis, chronic inflammation and cancers. These physiological functions of fruits and vegetables may be partly attributed by their abundance of phenolics⁶⁵.

There has been a growing interest in phenolic components of fruits and vegetables, which may promote human health or lower the risk for disease. Recent studies have focused on health functions of phenolics, including flavonoids from fruit and vegetables⁶⁵.

Luteolin(82) and its derived glycosides such as a cynaroside(83), cesioside(84), isoorientin(85) and stereolensin(86) have been isolated and identified from different

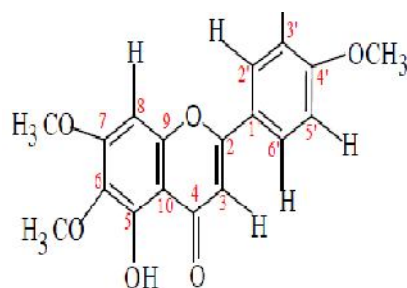
kinds of plant species. The results demonstrated that the reactivities of luteolin and its related glycosides against arachidonic acid synthesis and hydrogen peroxide scavenging are dependent on their molecular structures. The presence of ortho-dihydroxy groups at the B ring and OH substitution pattern at C-5 position of the A ring could significantly contribute to the antiinflammatory and antioxidant activities of flavonoids⁶⁶.



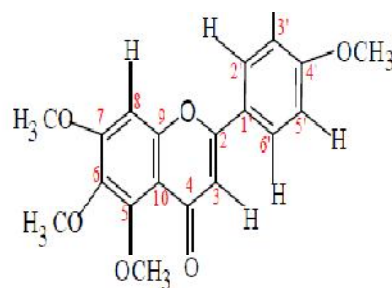
| Compounds | R | R' |
|------------------|--------------------------|----------------------------|
| Luteolin(82) | H | H |
| Cynaroside(83) | H | β -D-Glucoside |
| Cesioside(84) | H | β -D- Primeverose |
| Isoorientin(85) | β -D- Glucoside | H |
| Stereolensin(86) | β -D- Glucoside | β -D-Glucoside |

The anti-inflammatory activity of *Orthosiphon stamineus* leaf is largely attributable to the presence of some bioactive compounds such as the flavonoids sinensetin(87), and

eupatorin(88). Such anti-inflammatory properties may be ascribed to inhibition of prostaglandin synthesis and reduced NO production⁶⁷.

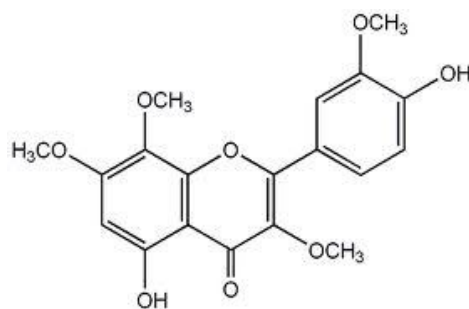


(87)



(88)

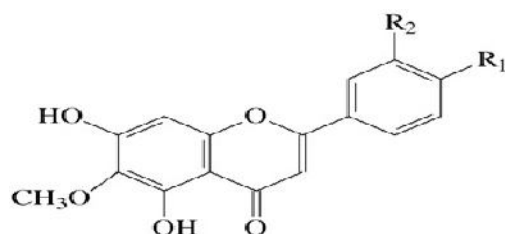
Ternatin(89), a tetramethoxyflavone extracted from the Brazilian medicinal plant *Egletes viscosa L.* The study confirms the anti-inflammatory actions of ternatin is an effective anti-inflammatory agent in acetic acid-induced rat colitis and suggest that its anti-inflammatory action may at least in part involve an antioxidant mechanism⁶⁸.



(89)

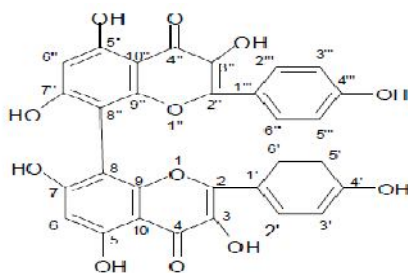
Three anti-inflammatory compounds identified as nepetin(90), jaceosidin(91) and hispidulin(92) have been isolated from *Eupatorium arnottianum* dichloromethane active extract on the

ear edema assay. Among them, nepetin was the most active one. These findings are in accordance with the results previously reported for these compounds in some in vivo models of inflammation⁶⁹.



| Compound | R ₁ | R ₂ |
|----------------|------------------|------------------|
| Nepetin(90) | OH | OH |
| Jaceosidin(91) | OH | OCH ₃ |
| Hispidulin(91) | OCH ₃ | OCH ₃ |

The biflavonoid (92) with bimolecular kaempferol structure has been found in the shells of *Camellia oleifera*. The separation procedure includes reflux extraction, hydrolysis and crystallization, which is simple and proper for industrial production. The biflavonoid(92) has obvious anti-inflammatory effect. It can control pain induced by heat and chemicals indicating its central and peripheral analgesic effects. The anti-inflammatory and analgesic mechanism may be attributed to inhibition of the synthesis or action of prostaglandins, which is related to its ability of eliminating free radical in vivo⁷⁰.



(92)

1.1.9.4 Anticancer activity.

Cancer is the second most abundant cause of death in the United States and in many other western countries. According to the annual report of German cancer research center, over 450,000 new cases are diagnosed in Germany with 270,000 cancer-related deaths each year in females alone. The prognosis for a patient with metastatic carcinoma of the lung, colon, breast, or prostate remains a concerning issue and accounts for more than half of all cancer deaths ⁷¹.

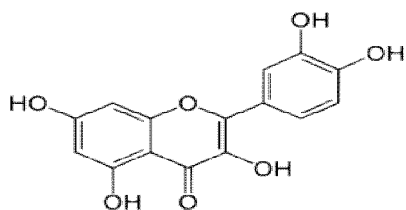
Cancer may be controlled by a variety of means, including suppression, blockage, and transformation. Suppressing agents prevent the formation of new cancers from procarcinogens; blocking agents prevent carcinogenic compounds from reaching critical initiation sites; and transformation agents act to facilitate the metabolism of carcinogenic components into less toxic materials or prevent their biological actions. Flavonoids can act in all three ways ⁷².

Flavonoids may act at the different development stages of malignant tumors by protecting DNA against oxidative damage,

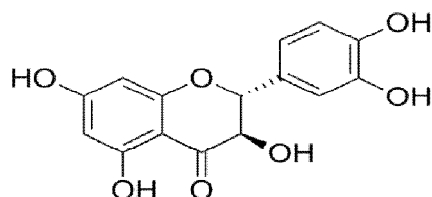
inactivating carcinogens, inhibiting the expression of the mutagenic genes and enzymes responsible for activating procarcinogenic substances, and activating the systems responsible for xenobiotic detoxification ⁷³.

Although most flavonoids appear to be nontoxic to humans and animals, they have been demonstrated to inhibit proliferation in many kinds of cancerous cell lines. For instance, it has been reported that flavonoids quercetin(93) and taxifolin(94) have antiproliferative effects on squamous cell carcinoma ⁷⁴.

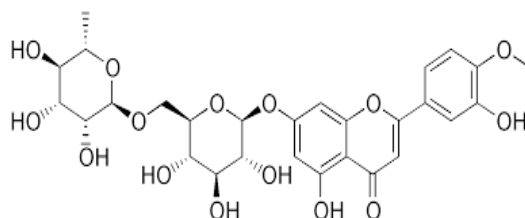
Diosmin(95), another important Citrus flavonoid, which is on the market as a venotonic, has shown antiproliferative activity in colon cancer cell lines although with less efficacy than quercetin(93) ⁷⁵.



(93)



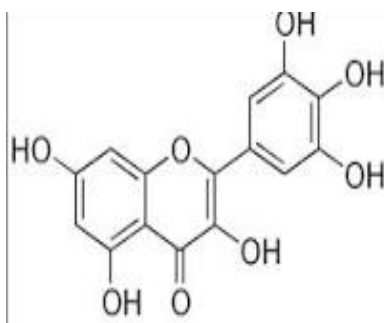
(94)



(95)

Comparison to the structure of the flavonoids shows that the presence of a double C2-C3 bond in polyhydroxylated

flavonoids increases the antiproliferative activity against certain cancer cell lines ⁷⁶. Another structural element that may influence antiproliferative activity is the number and position of the substituents in the flavonoid base structure. Taking quercetin(93) and myricetin(96) as an example, the presence of one additional hydroxyl group in the B-ring of myricetin(96) leads to greater activity of this flavonol compared to quercetin(93) when tested as anticancer⁷⁷.

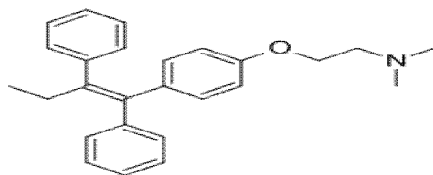


(96)

A particularly interesting influence on cell proliferation is observed in breast cancer. This type of cancer is the most common cancer among women in the Western world, with about 40,000 deaths from breast cancer in the US alone in 2000 ⁷⁸.

Around 94 % of breast cancer cases are diagnosed at the early stage of the disease. The primary aim of treatment for early breast cancer is to maximize local control and to prevent the progression of the disease to metastatic sites as metastatic breast cancer is currently incurable ⁷⁹.

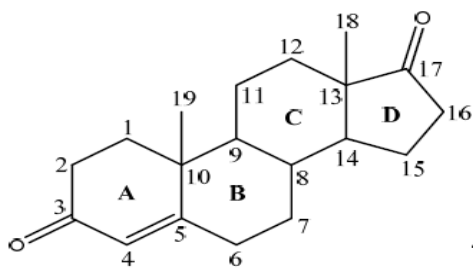
Approximately 75 % of breast cancers are positive for the estrogen receptor (ER) and/or progesterone receptor (PgR). As estrogen is the main stimulant in the development and growth of these tumors, the deprivation of estrogenic signaling has been the main form of hormonal therapy for patients with ER-positive and/or PgR-positive disease. Tamoxifen(97), which works by blocking the tumor's ability to respond to estrogen stimulation, has been the main hormonal therapy used. Furthermore, aromatase inhibitors help to prevent the growth of these tumors by lowering the amount of estrogen in the body. This approach has recently attracted considerable attention ⁷⁹.



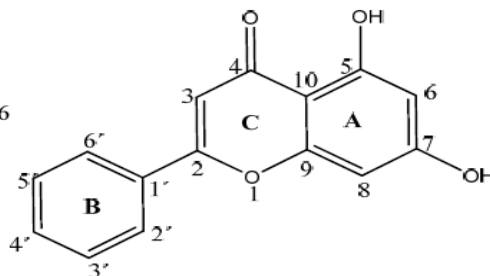
(97)

Flavonoid based aromatase inhibitors and several flavonoids demonstrate inhibitory activities against the aromatase enzyme, thus lowering estrogen biosynthesis and circulating estrogen levels ⁸⁰. Strong evidence for the binding of chrysin(98) to the active site of aromatase has been obtained by different spectral absorption studies ⁸¹, with 7,8-benzoflavone displacing androstenedione(99) from the aromatase active site and inducing a spectrum consistent with the low-spin state of iron.

Binding of flavonoids to aromatase requires certain structural features. Reduction of the flavone 4-keto group for instance is detrimental to aromatase inhibition by these compounds⁸². Based on data obtained from site-directed mutagenesis studies and ligand docking into a homology model of the aromatase protein, a binding orientation has been predicted in which the A and C rings of the chrysin(98) the C and D rings of the steroid substrate, respectively. The 2-phenyl substituent (ring B) is orientated in a region similar to that occupied by the A ring of the steroid. This analysis places the flavone 4-keto functionality in the same position as the steroid 19-angular methyl group with respect to the heme iron⁸³.



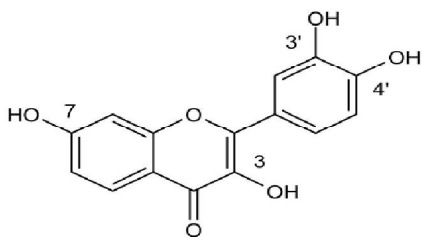
(99)



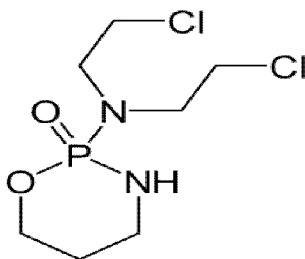
(98)

The natural flavonoid fisetin(99) was recently identified as a lead compound that stabilizes endothelial cell microtubules. In this study we investigated the antiproliferative and antiangiogenic properties of fisetin(99) in vitro and in vivo . fisetin not only displays in vitro and in vivo antiangiogenic properties, but that it can also markedly improve the in vivo antitumour effect of cyclophosphamide(100). We propose that

this drug combination associating a non-toxic dietary flavonoid with a cytotoxic agent could advantageously be used in the treatment of solid tumours⁸⁴.



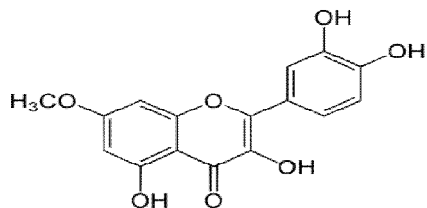
(99)



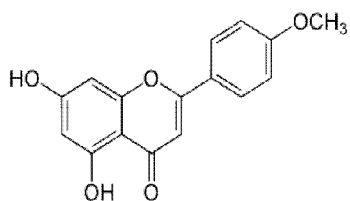
(100)

Flavonoids are common components of the human diet and appear to be of interest in cancer prevention or therapy, but their structure-activity relationships remain poorly defined. In this study, were compared 24 flavonoids for their cytotoxicity on cancer cells, and their morphological effect on endothelial cells that could predict antiangiogenic activity. Ten flavonoids presented inhibitory concentrations for 50% of cancer cells: rhamnetin(101), luteolin(82), acacetin(102), apigenin(103), quercetin(93), baicalein(104), fisetin(99), and galangin(105). Important SAR for cytotoxicity included the C2-C3 double bond and 3',4'-dihydroxylation. Concerning the morphological effects on endothelial cells, only fisetin(99), quercetin(93), kaempferol(106), apigenin(103), and morin(107) could induce the formation of cell extensions and filopodias at non cytotoxic concentrations. In conclusion, this study disclosed several

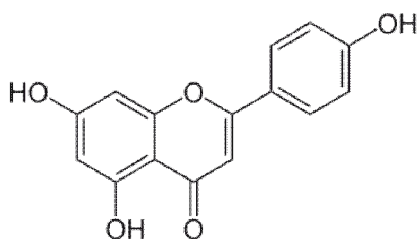
structure-activity relationships that could guide the choice or the rational synthesis of improved flavonoids for cancer prevention or therapy⁸⁵.



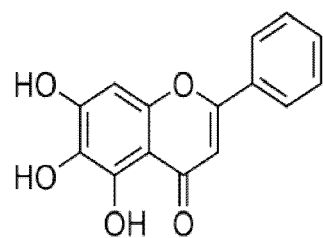
(101)



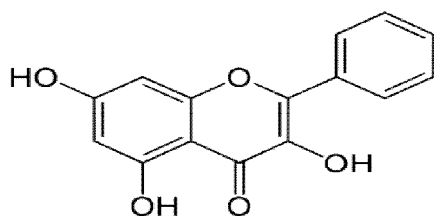
(102)



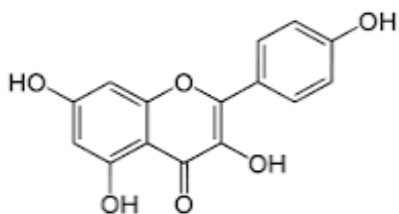
(103)



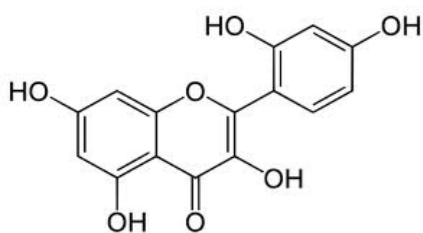
(104)



(105)



(106)



(107)

1.1.9.5 Thrombolytic agent

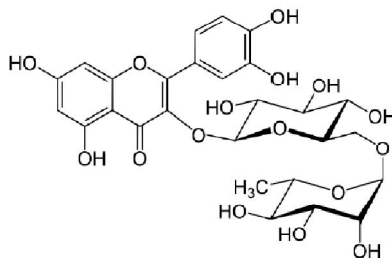
Thrombosis is the process of formation of solid mass or thrombus in circulation from the constituents of flowing blood. A blood clot is the mass of coagulated blood formed in vitro e.g. in a test tube. The extra-vascular accumulation of blood clot e.g. into the tissues is known as Haematoma while the blood clots formed in healthy individuals at the site of bleeding e.g. in injury to the blood vessel are called Haemostatic plugs. In other words, haemostatic plug at the cut end of a blood vessel may be considered the simplest form of thrombosis. Haemostatic plugs are useful as they stop the escape of blood and plasma, whereas

thrombi developing in the unruptured cardiovascular system may be life threatening by causing ischaemic injury and Thromboembolism⁸⁶. Thrombosis or blood clot formation and its consequences remain a leading cause of morbidity and mortality, and recurrent thrombosis is common despite current optimal therapy⁸⁷.

Thrombolytic drugs rapidly lyse thrombi by catalyzing the formation of plasmin from plasminogen. These drugs create a generalized lytic state when administered intravenously. Thus, both protective hemostatic thrombi and target thromboemboli are broken down⁸⁸.

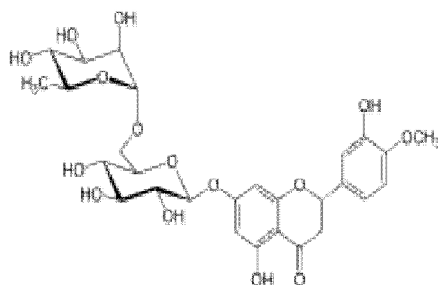
The formation of blood clots is a cause not only of heart attacks and strokes, but of deep venous thrombosis and pulmonary embolism as well. The number one killer of Americans is a blood clot that blocks blood flow to the heart or to the brain and approximately half of all morbidity and mortality in the United States can be attributed to heart attack or stroke. All the blood clot related conditions are life-threatening, and so there is a need for safe, effective and preventive treatment⁸⁹. A natural substance rutin(108), also called rutoside, is a citrus flavonoid glycoside found in *Fagopyrum esculentum* (buckwheat), the leaves and petioles of *Rheum* species, and *Asparagus*. This flavonoid compound has shown effective thrombolytic activity (prevents the formation of blood clots) by blocking the enzyme

protein disulfide isomerase found in all cells involved in blood clotting. Food and Drug Administration has established that rutin(108) is safe and, thus provides a safe and inexpensive drug that could reduce recurrent clots and help save thousands of lives⁸⁹.



(108)

Two flavonoids, rutin(108) and hesperidin(109), were investigated in vitro for anticoagulant activity through coagulation tests: activated partial thromboplastin time, prothrombin time and thrombin time. Only an ethanolic solution of rutin prolonged the partial thromboplastin time, while thrombin time were unaffected. In order to evaluate whether the prolongation of partial thromboplastin time was due to the decrease of coagulation factors, the experiment with deficient plasma was performed, showing the effects on factors VIII and IX. An effort was made to correlate stability of complexes with their anticoagulant properties⁹⁰.



(109)

1.1.9.6 Hypolipidimic Effect

Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, and is also identified as dyslipidemia, to describe the manifestations of different disorders of lipoprotein metabolism. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk⁹¹.

flavonoids quercetin(93), rutin(108), and morin(107) show a hypolipidemic effect either alone or in association. These results suggest the potential use of flavonoids in drug treatment or prevention of cardiovascular disease e⁹².

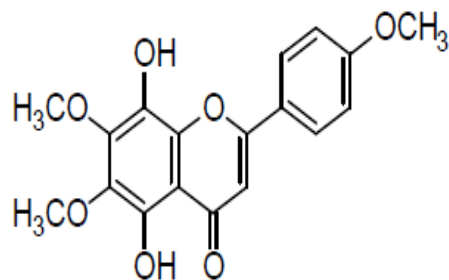
Hypolipidimic effect of total flavonoids of *PerillaFrutescens* leaves in hyperlipidemia rats induced by high-fat-diet was studied, where they found out that total flavonoids of *PerillaFrutescens* consisted of apigenin(103) with a small amount of luteolin(82) which was highly effective in decreasing the level of serum total cholesterol, triaglycerols, low density lipoprotein cholesterol and adipose tissue lipid accumulation⁹³.

1.1.9.7 Anti Diabetes Effect

Diabetes mellitus is a metabolic disorder which arises from complex interactions between multiple genetic and environmental or lifestyle factors. This chronic disease is characterized by the presence of hyperglycemia due to defective insulin secretion, insulin action, or both. Long-term diabetes is associated with several comorbidities, such as erectile dysfunction, blindness, poor wound healing, kidney failure, heart disease, etc; as a result of considerable damage, dysfunction, and failure of various organs that develop as the disease progresses⁹⁴. The incidence of diabetes worldwide is now estimated to be around 366 million, far beyond the 285 million projected by the World Health Organization (WHO) for 2010 from global statistics gathered in 2008⁹⁵. This means that there may have been more than 4 million deaths or 6.8% of global mortality in 2010 that could be attributed directly or indirectly to diabetes⁹⁶. And explains why global diabetes health expenditure in 2010 was estimated around \$320 billion or 12% of total global health care costs⁹⁷.

Citrus sinensis is native to Asia and throughout the Pacific and warm areas of the world. The ethyl acetate extract of the roots of *Citrus sinensis* yielded a flavonoid. The compound was characterized as 5, 8-dihydroxy-6, 7, 4'-trimethoxyflavone (110) *Citrus sinensis* is one of the important medicinal plants that is

found broadly in the district of Shahjahanpur. This plant is prescribed as a traditional medicine for the treatment of various ailments. It has been used as an anti-diabetic⁹⁸ .



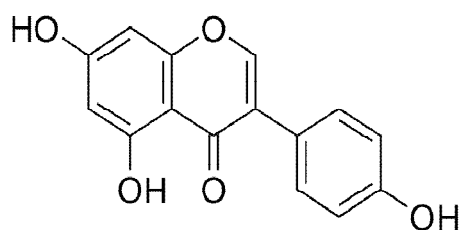
(110)

Intraperitoneal administration of pruning(111) produces a significant hypoglycemic effect in diabetic rats. Anti-hyperglycemic effects have also been demonstrated for various flavonoids including chrysin(98) and its derivatives⁹⁹. Long-term studies carried out with rutin(108) orally administered to diabetic rats showed that it decreased the plasma glucose levels by up to 60% when compared to the control group. However, oral administration of rutin(108) to normal rats did not show any significant effect on fasting plasma glucose levels¹⁰⁰.

The investigators also observed that apigenin(103) , and luteolin(82), both lacking the 6-hydroxyl substituent, showed negligible activity (12% and 22% inhibition respectively) in the α -glucosidase inhibitory assay. From their study, the present investigators suggested that 5,6,7-trihydroxyflavone skeleton is crucial for high α -glucosidase inhibitory activity regardless of B-ring hydroxylation, in addition, glycosation of 7-hydroxyl

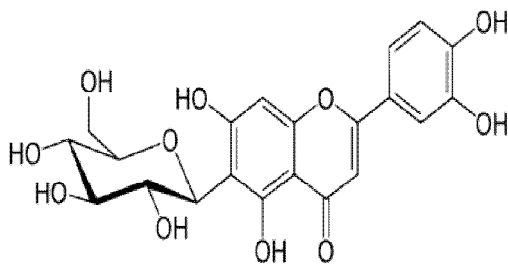
substituent as well as acylation of the sugar reduces the enzyme inhibitory activity¹⁰¹.

The genistein(111) isolated from an EtOAc-soluble partition of the MeOH extract of a branch of *Tetracera scandens* were evaluated to possess promising activities on Type-2 diabetes mellitus treatment since the test compounds significantly stimulated the uptake of glucose, adenosine monophosphate-activated kinase, glucose transport protein-4 and GLUT1 mRNA expressions and protein tyrosine phosphatase 1B inhibition¹⁰².

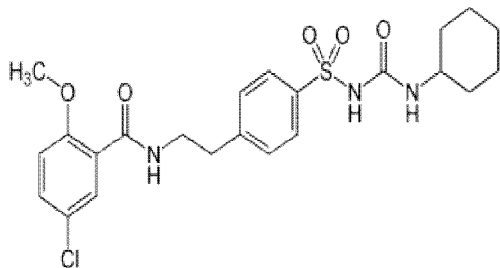


(111)

Isoorientin (102), isolated from the water and butanolic extracts of *Cecropia obtusifolia*, exhibited potent hypoglycemic activity comparable to that of glibenclamide(103) at a dose of 3 mg/kg body weight in diabetic rats¹⁰³.

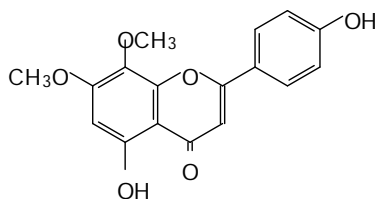


(102)



(103)

A flavone xylopyranoside, (104), isolated from the roots of *Euphorbia leucophylla*, was found to reduce the blood glucose levels and increase the serum insulin levels in normal and diabetic rats¹⁰⁴.



(104)