

I-Introduction

The phytochemical investigation of a plant may involve the following: extraction of the plant material; separation and isolation of the constituents of interest; characterization of the isolated compounds; investigation of the biosynthetic pathways to particular compounds; and qualitative evaluation¹.

Phytochemicals are defined as substances found in edible fruits and vegetables that prevent chronic and degenerative diseases. Phenolics are defined as a class of polyphenols which are important secondary metabolites present in plants and are also responsible of their antioxidant action and various beneficial effects in a multitude of diseases .Polyphenols are characterized as: Phenolic compounds with at least one hydroxyl group attached directly to a benzene ring, these are hydroxylated derivatives of benzoic acids presents in form of esters and glycosides ².

1.1-Flavonoids

Flavonoids are natural products widely distributed in plant kingdom, and currently consumed in large amount in the daily diet³. Flavonoids are widely spread throughout the plant kingdom. They serve a variety of ecological and physiological function on plants ⁴. Flavonoids are one of the largest class of naturally –occurring polyphenolic compounds. This group of plant pigments is largely responsible for the colors of the fruits and the flowers, and over 4,000 flavonoid compounds have been characterized and classified according to chemical structure. The word flavonoids comes from the Latin *flavus* which means yellow; however some

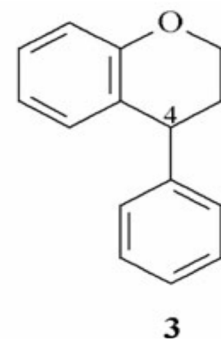
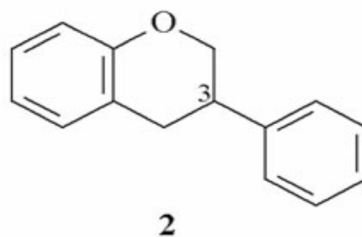
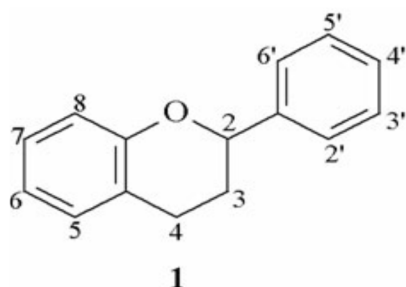
flavonoids are red, blue, purple or white. They were discovered, along with vitamin C, in 1928 by Albert Szent-Gyorgi who called them vitamin P⁵.

Flavonoids possess a potential pharmacological activities such as anti oxidant activity ,vitamin C sparing activity, Flvonoids have free radical scavenging and antioxidant properties , which are useful for their pharmacological activities including anticancer, and antiaging properties. Flavonoids show interaction with cytochrome P 450, which has antileukemic properties and mild vasodilator properties useful for the treatment of heart diseases ⁶. Of all aromatic compounds, flavonoids and tannins are the most common in plant organs. They are not only playing a major functional role in the plant , but also commercially significant in pharmacology, the food industry and in ornamental plants⁷. Flavonids are universal within the plant kingdom, where they afford the most common pigments next to chlorophyll and carotenoids. They generally occur in plant as glycosylated derivatives and their physiological role in the ecology of the plants are diverse . Due to their attractive colors , flavones, flavonols and anthocyanidins may act as visual signals for pollinating insects .In consideration of their astringency, catechins and other flavonols can represent a defense system against insects harmful to the plant ⁸.Moreover, flavonoids act as catalysts in the light phase of photosynthesis and/or as regulator of ion channels of phosphorylation .They also function as a stress protectant in plant cells by scavenging reactive oxygen spices produced by photosynthetic electron transport system. Because of their UV absorbing properties, flavonoids protect plants against UV radiation⁹.

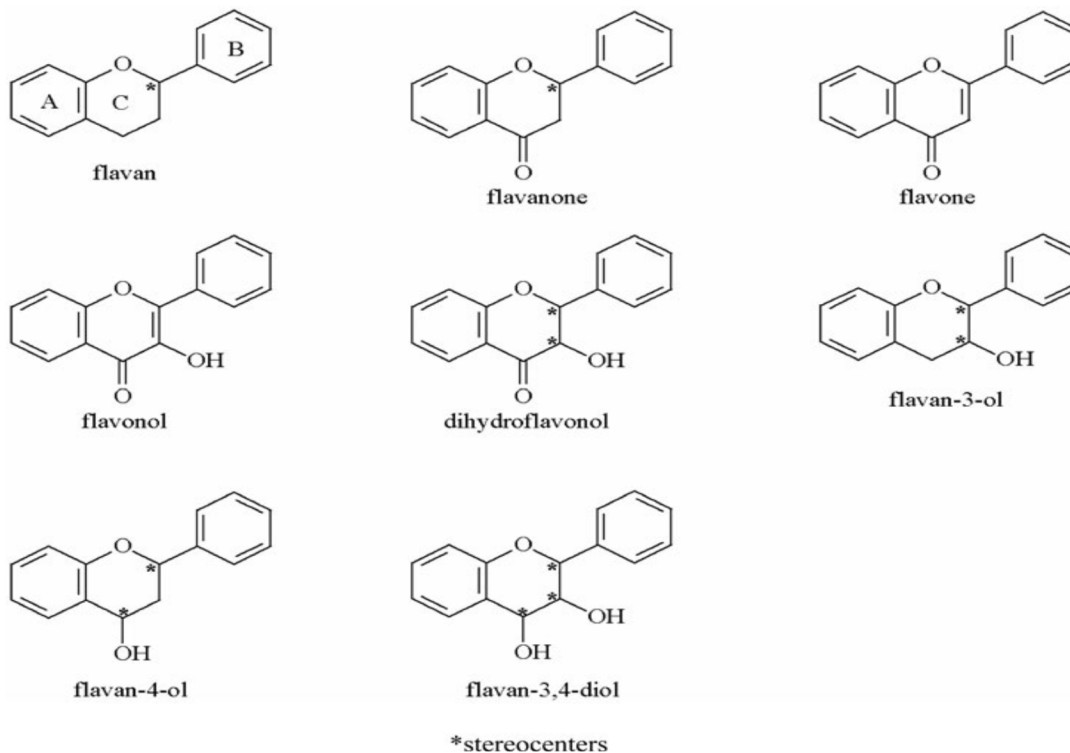
1.2- Classification of flavonoids

The study of flavonoid chemistry have emerged, like that of most natural products, from the search of new compounds of useful physiological properties. The term(

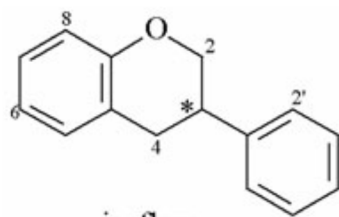
flavonoid) is generally used to describe a broad collection of natural products that include $C_6-C_3-C_6$ carbon frame work , or more specifically a phenylbenzopyran functionality .Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety .This group of natural products can be divided into three classes: The flavonoids (2-phenyl benzopyran)(1), isoflavonoids (3-benzopyrans) (2) and the neoflavonoids (4-benzopyrans)(3). These groups usually share common chalcone precursor, and therefore are biogenetically and structurally related ².



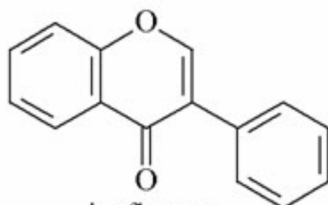
Flavonoids can also be divided into the following groups:



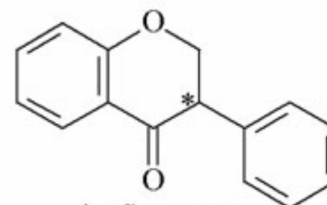
The isoflavonoids comprise a distinctive subclass of flavonoids. Such compounds possess a 3-phenyl chroman that are biogenetically derived by 1, 2 aryl migration in 2- phenylchroman precursors. Though isoflavonoids have a limited distribution in the plant kingdom, they are remarkably diverse as far as structural variations are concerned. This is a direct consequence of the number of complexity of substituents on the basic 3-phenylchroman system, different oxidation levels and presence of additional heterocyclic rings. Isoflavones are further divided into the following subgroup:



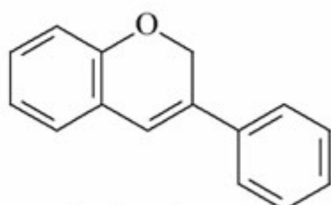
isoflavan



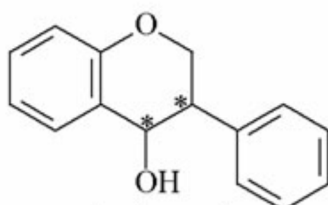
isoflavone



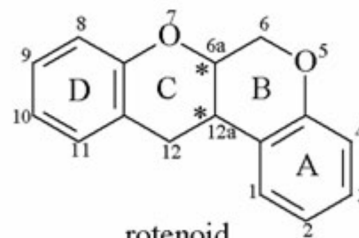
isoflavanone



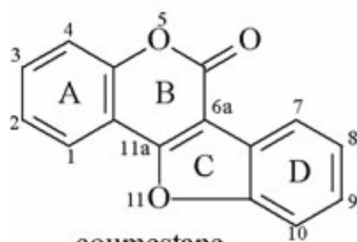
isoflav-3-ene



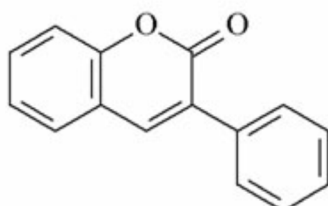
isoflavanol



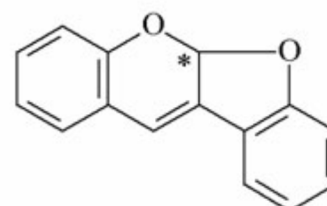
rotenoid



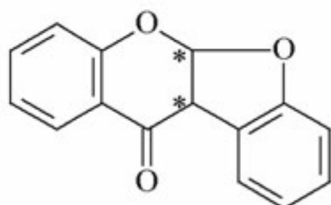
coumestane



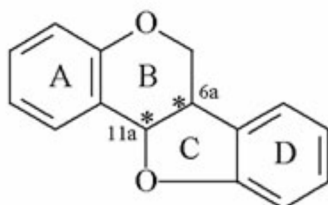
3-arylcoumarin



coumaronochromene

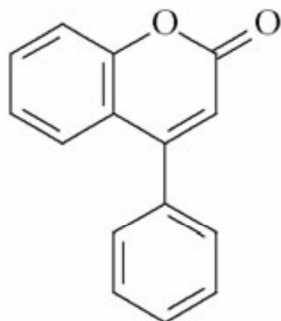


coumaronochromone

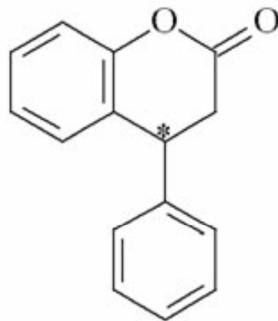


pterocarpan

The following flavonoids which are characterized by absence of a carbonyl function at C-4 are termed neoflavonoids:

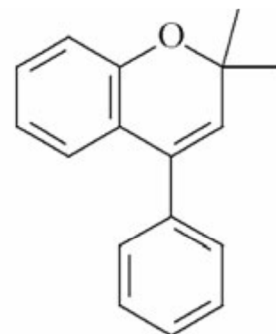


4-aryl coumarin



3,4-dihydro-4-aryl coumarin

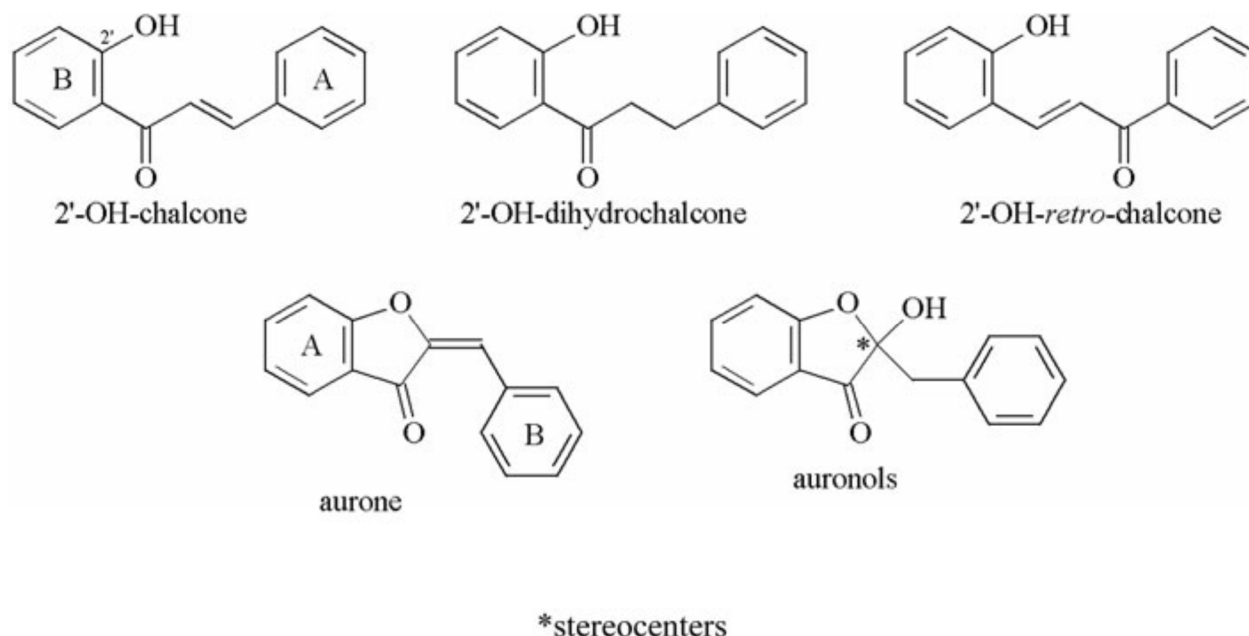
*stereocenters



neoflavene

Such phytochemicals are biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4- aryl coumarins (4-aryl-2H-1benzopyran-ones), 3,4 –dihydro -4- arylcoumarins and neoflafenes¹⁰.

Phytochemicals such as chalcones and aurones also contain a C₆-C₃-C₆ backbone and are termed minor flavonoids .Such compounds include: the 2-hydroxychalcones and the dihydroxychalcones,2-OH-retro –chalcones, aurones (2-benzylidenecoumaranone) and auranols¹¹.



1.3- Synthesis of flavonoids

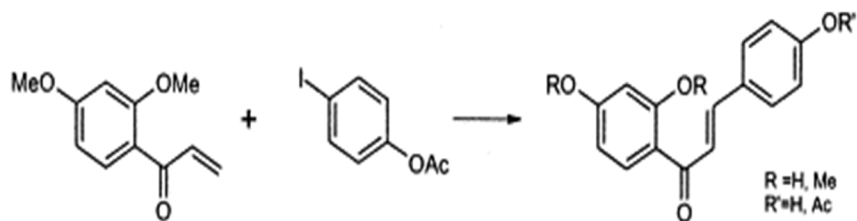
The synthesis of flavonoids has been subject of great number of studies. Although there are several types of skeletons (flavones, isoflavonoid, aurones, etc.), it is the flavones whose synthesis has been more widely studied. The most used strategy is the reaction of substituted acetophenones with corresponding substituted benzaldehydes either in basic or acidic media¹².

In the field of microwave – assisted organic reactions, flavonoids have also been studied. However, in this field there was a lack of data for the two component reaction of acetophenones and benzaldehydes. Although synthesis of chalcones by reaction of these components¹³ were reported, all of the acetophenones studied, lacked the 2-OH-substituent that could allow the subsequent cyclization to close the pyrane ring present in flavanones. Otherwise there have been reported cyclizations of chalcones to 3-substituted-flavanones¹⁴, diphenyl - β - diketones to

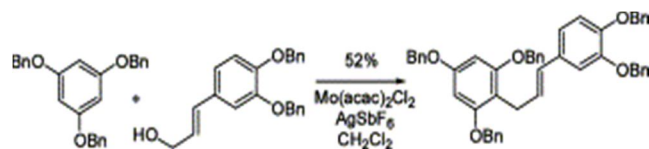
flavones¹⁵ and 2-aminochalcones to 2-aryl-1,2,3,4-tetrahydro-4-quinolones¹⁶. But it seemed that nobody had studied (or at least reported) the above mentioned approach of two component addition-cyclization without the addition of a second molecule of aldehyde to position 3 of the flavanone¹⁷.

Among the wide variety of reaction conditions for the classical reaction, Chang's reagent ($\text{SiO}_2/\text{H}_3\text{BO}_3/\text{piperidine}/\text{DMF}$) was attempted¹⁸. It was observed that shorter irradiation time lead to better yield¹⁹.

Many flavonoids have been synthesised following a new proposed method based on the use of the Heck reaction. The key step involves the coupling of an aryl vinyl ketone with an aryl iodide. This procedure affords the flavonoid moiety in a single step²⁰.



The above mentioned method deals with the formal total synthesis of flavonoids bearing the hydroxylation pattern of the catechin series based on an access to the fully functionalized skeleton via the alkylation of phloroglucinol tribenzyl ether by 3,4-dibenzyloxycinnamyl alcohol. This reaction was revealed to be most successful when catalyzed by the $\text{Mo}(\text{acac})_2(\text{SbF}_6)_2$ complex. In addition, the underlying concepts to the different ways that can be used in this $\text{C}_6\text{-C}_3\text{+C}_6$ strategy are discussed²¹.

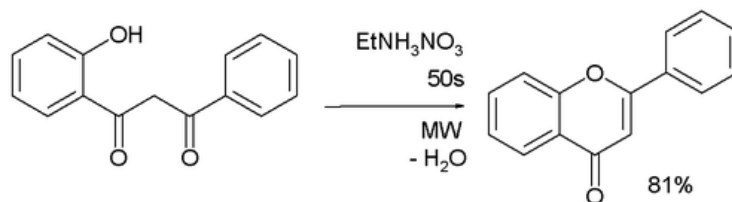


Interest in the biological properties of flavones has resulted in intense synthetic efforts towards the synthesis of various flavones. There are a number of methods reported for the synthesis of flavones.

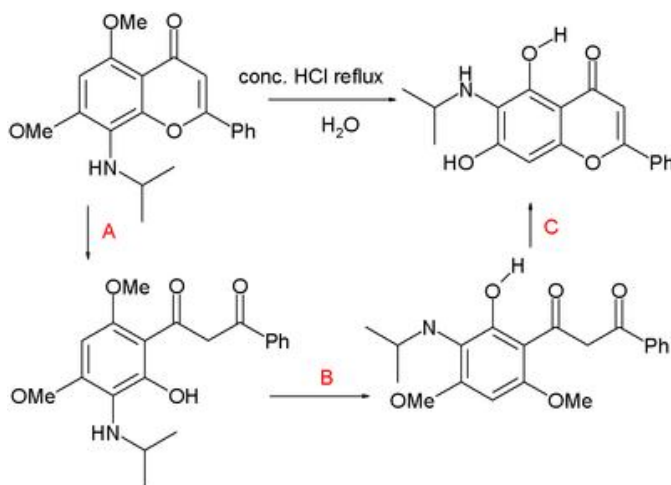
Several methods exist for the synthesis of flavones:

- [Allan-Robinson reaction](#)
- [Baker-Venkataraman rearrangement](#)
- [Algar-Flynn-Oyamada reaction](#)

The basic skeleton of flavonoids is also available via dehydrative cyclization of certain 1,3-diaryl diketones. The effect of an [ionic liquid](#) solvent and [microwave irradiation](#) on the yield of this process was studied²².

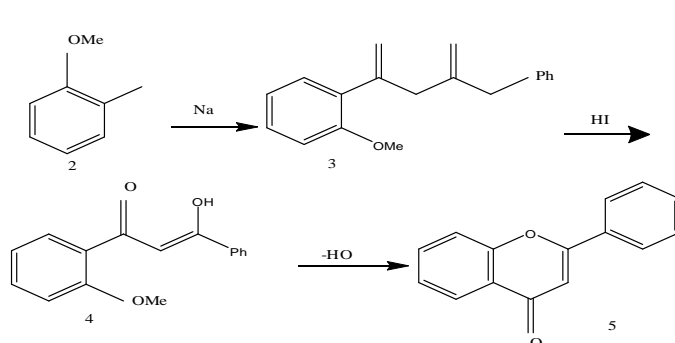


An important tool in structure elucidation of flavonoids is the Wessely-Moser rearrangement²³. It involves the conversion of 5,7,8-trimethoxyflavone into 5,6,7-trihydroxyflavone on hydrolysis of the methoxy groups to phenol groups. It also has synthetic potential for example²⁴:

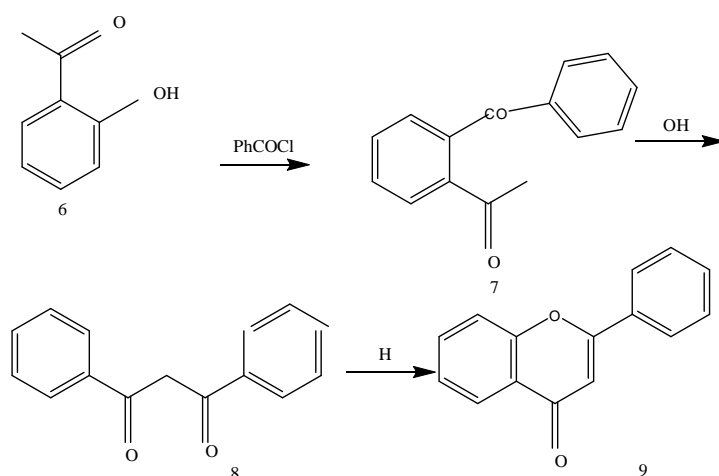


Such rearrangement takes place in several steps: (A) ring opening to the diketone, (B) bond rotation with formation of a favourable acetylacetonate (C) hydrolysis of two methoxy groups and ring closure²⁵.

A general method for synthesizing flavones is the Von-Konstanecki method which involves a reaction of o-methoxybenzoate and acetophenone in the presence of sodium to form (3). This is followed by treatment of (3) with an acid to form compound (4) followed by elimination of water in order to form the flavones (5)²⁶.



The most convenient route to the synthesis of flavones is the Baker-Venkataraman approach. In this reaction, 2-hydroxyacetophenone is converted to ester, which then undergoes rearrangement by intramolecular Claisen condensation in the presence of potassium hydroxide and pyridine to afford 1,3-diketone which is then cyclised to flavone under rather harsh conditions either by treatment with concentrated sulfuric acid or heating with glacial acetic acid²⁷.



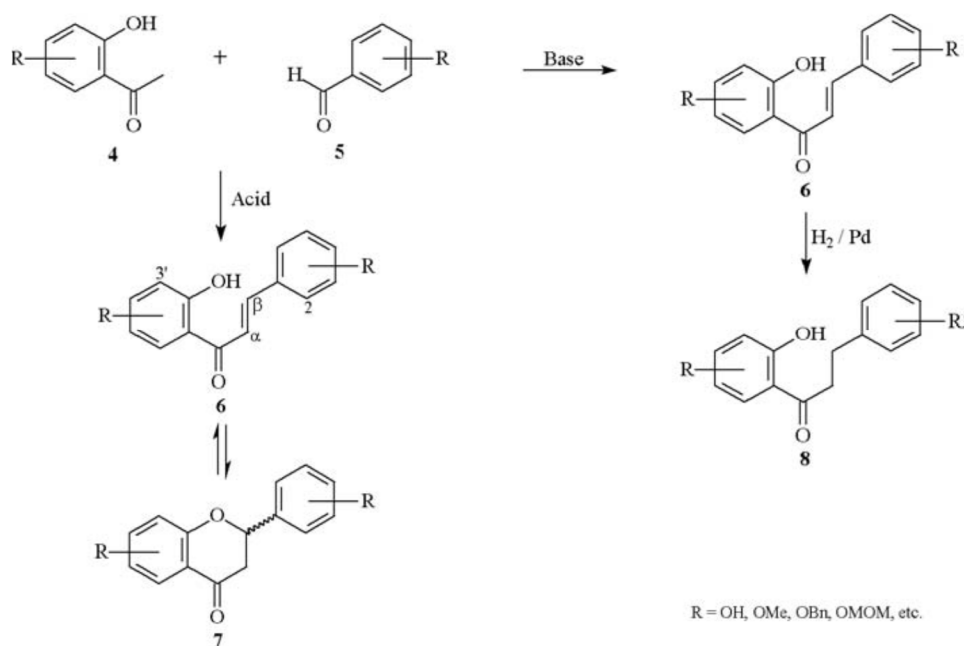
1.4- Chalcones

Chalcones and dihydrochalcones are considered to be the primary C6-C3-C6 precursors and constitute important intermediates in the synthesis of flavonoids².

Chalcones are readily accessible via two well-established routes comprising a base-catalyzed aldol condensation or acid-mediated aldolization of 2-hydroxyacetophenones and benzaldehydes¹⁵. The base-catalyzed aldol condensation is usually the preferred route toward chalcone formation, since under acidic conditions cyclization of the ensuing chalcone leads to formation of

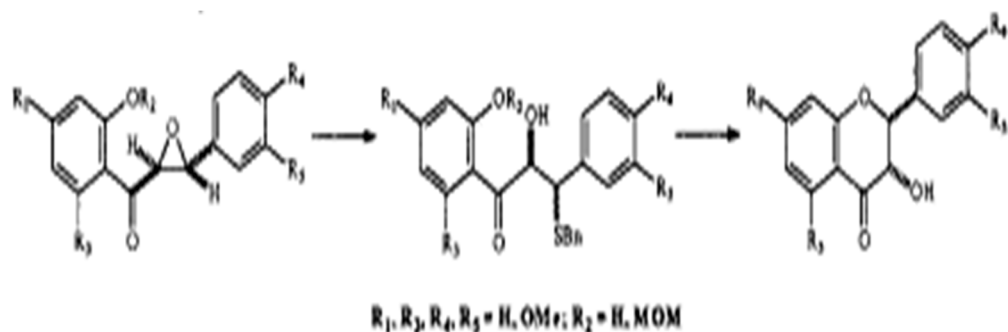
corresponding racemic flavanones. Dihydrochalcones are generally obtained via reduction (H_2/Pd) of the preceding chalcones².

Conventional base-catalyzed aldol condensation usually employs NaOH or KOH, but other bases like NaH have also been utilized to produce chalcones in up to 89 % yield.⁽⁹³⁾ These compounds can also be obtained in high yields (75 - 96 %) by Lewis acid catalysis, e.g. borontrifluoride-etherate²⁸.



1.5- *Trans*- and *cis*-dihydroflavonols

Epoxidation of a series of poly-oxygenated chalcones with H_2O_2 in the presence of poly- α -aminoacids yields chiral aromatic oxygenated oxiranes in moderate to high optical yields. Lewis acid-catalysed phenylmethanethiol ring opening of the epoxide functionality and subsequent formation of the pyranone heterocycle, afforded *trans*- and *cis*-dihydroflavonols in moderate to high enantiomeric excess and yield²⁹.



1.6-Isolation Techniques of flavonoids

Isolation of flavonoid compounds involves the separate procedure: (a) extraction and (b) isolation and separation into pure compounds.

Solvents used for extraction are chosen according to the polarity of the flavonoids being studied. The less polar solvents are particularly useful for the extraction of flavonoids aglycone, whilst the more polar solvents are used if flavonoids glycosides, sulphates or anthocyanins are involved³⁰⁻³²

In general 70-80% ethanol is the solvent of choice for the extraction of the dried plant material of all classes of flavonoids. 50% ethanol is recommended when water soluble derivatives (glucuronides or potassium bisulphates) are expected to be present. For anthocyanins, hydrochloric acid (0.1%) should be present to prevent their conversion to the pseudo base form³³. The crude extract is then subjected to two dimensioned papers or thin layer chromatography. This is followed by the separation of the flavonoids using suitable methods of each class^{34,35}.

Sequential extraction with a number of solvents of varying polarity can lead to the separation of glycosides from aglycones and the separation of polar from non polar aglycones. Although a certain degree of overlap could occur. More conventional methods are the use of column, paper and thin layer chromatography. High pressure liquid chromatography (HPLC) and counter current chromatography (CCC) ³⁶.

So far, column chromatography remains the single most useful method for the isolation of large quantities of flavonoids from crude extracts. The absorbents of choice have generally been silica gel, cellulose, polyamide and sephadex.

Silica gel column has been used for the separation of isoflavones, flavanones, dihydroflavonols and highly methylated flavones and flavonols using chloroform and chloroform-methanol for the separation of flavonoids glycosides.

Column chromatography using cellulose is considered as a scale-up from paper chromatography. It is suitable for the separation of all classes of flavonoids and their glycosides. In principle the full range of solvent developed for use in paper chromatography is available for partition column chromatography and may have been used with success ³⁷.

Polyamide chromatography is suitable for the separation of all types of flavonoids ³⁸. It has capacity for phenolic materials, which form strong hydrogen bonds with phenolic hydroxyl groups via their amide carbonyl function ³⁹. Another suitable absorbent is the special sephadex LH-20 which can be used with organic solvents or water solvent mixtures. Sephadex is highly cross linked dextran on which separation is ideally obtained on the basis of the molecular size ⁴⁰.

Column chromatography often yields pure flavonoids or simple mixtures which may be further separated by paper chromatography. Chromatography on paper

usually involves either partition or adsorption chromatography. Paper chromatography is suitable for the separation of all types of flavonoids and their glycosides. Most flavonoids appear as colored spots on paper chromatograms viewed in UV light, and fuming with ammonia often produces significant changes in their colors. For the majority of flavonoids, separation is first affected by the use of n-butanol – acetic acid – water (4:1:5) (BAW) which produce a separation based largely on partitioning. The second dimension is commonly run using an aqueous solvent such as water and 2-60% aqueous acetic acid.

Flavonoid aglycone and highly methylated flavonoids are best separated by (BAW), chloroform- acetic acid -water (13:6:1), phenol –water (4:1), or acetic acid- water- conc. HCl (30:10:3). Solvents containing HCl or acetic acid are required for the chromatography of anthocyanins and anthocyanidins^{40,41}.

Thin layer chromatography is commonly used for the analysis of mixtures and /or the isolation of pure flavonoids. The adsorbents of choice for the separation of flavonoids are silica gel, polyamide, cellulose and sephadex. The greater speed of TLC due to the more compact nature of the adsorbent when spread on a plate is an advantage when working with labile compounds.

This method is perfectly used : a) when the compounds are obtained from small quantity of plant material, b) resolving mixtures of glycosides and acylated glycosides which will not separate on paper, d) resolving the bioflavonoids which do not separate on paper chromatography, e) test the purity of glycoside sample and f) identification by chromatography.

The highly methylated flavones and flavonols are separated on cellulose, polyamide and silica gel plates using solvent system : t- butyl alcohol – acetic acid –water (upper phase) (4:1:5), benzene- petroleum ether - methylated ketone –

methanol ratio (60:26:7:7) , toluene –methyl ethyl ketone –methanol – acetyl acetone (40:20:30:10) and (13:3:3:1) ; chloroform – formic acid – acetone (9: 1:2); benzene – pyridine – formic acid (36:9:5) and benzene - dioxane – acetic acid (90:25:4) ⁴².

Paper electrophoresis is a technique of limited application in flavonoid analysis science. To be mobile; flavonoid must be in an ionized states at the P^H of the electrolyte. Thus at P^H 2.2 (formate, acetate buffer) on Whatman 3mm, flavonoid bisulphates migrates towards the anode whereas other flavonoids do not⁴³.

HPLC is a kin to GLC except that the carrier gas is replaced by a solvent or a solvent mixture. In principle it is ideally suited to chromatographic analysis, qualitative and quantitative analysis of nonvolatile compounds⁴⁴ .Advantages claims for HPLC analysis include: a) short analysis time, b) no risk of thermal decomposition, c) no derivatization, d) high resolution and e) easy quantification ⁴⁵ . HPLC of flavonoids has been described previously in several publications utilizing both reversed phase and silica column ⁴⁶.

Counter current chromatography (CCC) is a support – free partition chromatography and has the advantage over liquid chromatography of eliminating all complications arising from the use of solid support. Counter current distribution method (CCD) and counter current chromatography (CCC) can yield pure fraction at high reproducibility rate without the risk of adsorptive loss of samples, while it permits continuous elution, monitoring and fractions are performed as in liquid chromatography ⁴⁷.

A counter current extraction scheme with a coil planet centrifuge, based on the dual counter current system, has the capability of both clean up and sample enrichment in one step operation ⁴⁸.

Recently high speed counter current chromatography has been successfully developed by utilizing a new coil configuration called a multi – layer coil, which compactly accommodates a number of coil layers around a pool-shaped holder^{49,50}. The isolation of water soluble and high polar derivatives is also achieved by the application of counter current techniques⁵¹.

1.7-Identification technique of flavonoids

The identification technique of flavonoids involves both chemical methods and physical tools.

1.7.1 Chemical methods

If the pure flavonoid is suspected to be a glycoside, the standard procedure is to hydrolyze it, normally acidic and/or enzymatic hydrolysis. Complete acidic hydrolysis is carried out by 2N hydrochloric acid, while mild acid hydrolysis is carried out by 0.2N hydrochloric acid. Enzymatic hydrolysis is carried out with specific enzymes such as *B .glucuroidase*.

The sugar moiety obtained by hydrolysis of a flavonoid glycosides is identified by paper and thin layer chromatography. Identification of the aglycone obtained by the hydrolysis of a flavonoid glycosides involve such data as RF values, spot color under UV light, UV data, nuclear magnetic resonance (H^1NMR and $C^{13}NMR$) and mass spectrometry.

Demethylation is also applied in case of a methylated flavonoid suspected of being present. This is carried out with anhydrous $AlCl_3$, pyridinium chloride alkaline degradation could also be used to identify the basic nucleus of the flavonoid under study⁴⁴.

1.7.2 Physical methods

Complete elucidation of the flavonoids should include physical methods such as ultra violet UV, nuclear magnetic resonance ^1H NMR, ^{13}C NMR and mass spectrometry⁴⁰.

1.7.2.1 Ultra violet and visible spectrometry

UV spectrometry has become a major techniques for the structural analysis of flavonoids for two main reasons. The first is : only trace of material is required. The second reason is that the amount of structural information gained from UV spectrum is considerably enhanced by the use of specific reagents which react with one or more functional groups on the flavonoids nucleus. The reagents used are sodium methoxide, aluminum chloride, aluminum / hydrochloric acid, sodium acetate and sodium acetate / boric acid.

The UV spectrum of most flavonoids consist of two major absorption maxima one of which occurs in the range 240-285nm (band II) and the other in the range 300-400nm (band I) .In general terms, the bandII absorption may be considered as originated from the A ring benzoyl system, and bandI absorption originates from the B ring cinnamoyl system.

Band I of flavones absorbs between 304-350 nm, while that of flavonols absorbs in the range 352-384nm, so that the position of bandI absorption provides to the type of the flavonoids been examined.

Isoflavones, flavanones and dihydroflavonols are grouped together because they all lack conjugation between the A and B rings. Their spectra are readily distinguished from those of flavones and flavanols in that they normally exhibit a low intensity band I absorption which often appears a shoulder to band II.

In chalcones, band II appears in the range 220-270nm region, while band I is usually in the range 340-390nm, although a minor peak or inflection often occurs at 300-320nm. In aurones, band I appears in the range 385-415nm region. Anthocyanidin (and anthocyanins) have a band I absorption maxima in the range 465-550 nm; with band II being represented by less intense peak in 270-280 nm region⁴⁵.

1.7.2.2-Proton magnetic resonance spectroscopy

Until about 1964, PMR studies were confined to the relatively non-polar flavonoids such as isoflavones and highly acetylated or methylated flavones which are soluble in solvents such as deuteriochloroform $CDCl_3$ or carbon tetrachloride CCL_4 ⁵². However most flavonoids and their glycosides are insoluble in these solvents, and it was dimethyl sulphoxide ($DMSO-d_6$) as a solvent for flavonoids^{53,54} that initiated the use of this method in elucidation of structure of flavonoids.

Proton magnetic resonance of flavonoids normally occurs between 0-9ppm, and within this range, signals may be assigned tentatively to structural features⁵⁵.

1.7.2.3- Mass spectrometry

Mass spectrometry is a powerful tool for structural study of flavonoid compounds. It enables not only the determination of precise molecular weight but the characterization of structure types by analysis of the fragmentation patterns. It was observed that most flavonoid aglycones yield intense peaks for the molecular ion (M^+) and this is often the base peak. In addition to the molecular ion, flavonoid aglycones usually afford major peaks for $(M-1)^+$. And when methylated $(M-CH_3)^+$. A useful fragmentation for flavonoid identifications are those involving

cleavage of intact A-and B ring fragments .Such fragments usually originate from the retro Diels-Alder fission⁵⁵.

1.8- Isolation of flavonoids

The analysis of flavonoids and their conjugates helps greatly in solving problems in biological and medical sciences. Different methods of isolation are available, but strategies is dependent on the origin of the biological material from which the products are to be extracted (plant or animal tissue or body fluid). In the case of polyphenolic compounds, it is often important to 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 materials investigated. This second approach most often takes place during the nutritional studies of different foods or fodder mainly of plant origin. The presence of carbohydrates and / or lipophylic substances may influence the profile of the qualitative and quantitative composition of the flavonoids and their derivatives in the obtained extract.

The utilization of dried material for extraction may cause substantial decrease in the yield of the flavonoid conjugate. Acylated flavonoid glycosides are especially labile at elevated temperature and are frequently thermally degraded during the process of drying plant tissue. This is important during the profiling of this class of natural products in research directed towards the investigation of their physiological and biochemical roles in plants under the influence of environmental factor, or in studies of genetically modified plants for the elucidation of changes in metabolic pathways⁵⁵.

Free flavonoid aglycones exuded by the plant tissues (leaf or root) may be washed from the surface with non polar 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 materials (plant or animal tissues and different food products). The extraction efficiency may be enhanced by the application of ultrasonic action^{56,57} or pressurized liquid extraction (PLE), a procedure performed at elevated temperature ranging from 60°C to 200°C⁵⁸. Supercritical fluid extraction with carbon dioxide also may be used⁵⁹. However, the temperature condition during the extraction has 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 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 which are structurally similar to the studied analyte but not present in the sample. Compounds labeled with stable isotopes (¹H 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 and consecutive components of the analyzed flavonoid mixture need reference standard compounds necessary for the preparation of the calibration curve 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 organ tissue; cellular or even sub cellular level is of special interest in some projects. In these situations, the amount of biological material of the natural

products may be extremely small, and the application of micro-extraction techniques is necessary⁶⁰. In many cases it is necessary to avoid the chemical and/or enzymatic degradation of the metabolites. This is of very special importance in the profiling flavonoids glycosides in research, in plant functional genomics or during physiological or biological studies that need information about all classes of flavonoids⁴⁷.

1.9-Biological activity of flavonoids

Flavonoids are known to exert wide spectrum of biological activities. including: anti-inflammatory, antibacterial, antiviral, antiallergic⁶¹⁻⁶³, cytotoxic, antitumour, treatment of neurodegenerative diseases, vasodilatory action⁶⁴⁻⁶⁷. In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation^{63,68,69}. They are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatase, arylsulphatase, cAMP phosphodiesterase, lipase, α -glucosidase, kinase⁷⁰.

Flavonoids are potential antioxidants against free radicals⁶⁹. This activity is due to the hydrogen-donating ability of flavonoids. Indeed, the phenolic groups of flavonoids serve as a source of a readily available ‘‘H’’ atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure⁷⁰.

Flavonoids , by acting as scavengers of superoxide anion and hydroxyl radicals, inhibit *in vitro* lipid peroxidation at an early stage. They terminate chain radical reaction by donating hydrogen atom to a peroxy radical ; thus, forming flavonoids radical, which, further reacts with free radicals thus terminating a propagating chain^{63,71} .

The following table shows the free radical scavenging capacity of flavonoids.

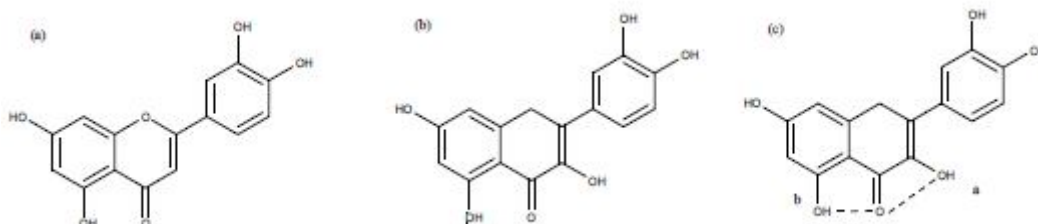
Table 1: Reactive oxygen species that can be scavenged by flavonoids⁷²

No.	Reactive species	Mechanism
1.	O ₂ (Superoxide anion)	One-electron reduction product of O ₂ . Produced by phagocytes, formed in autoxidation reaction and generated by oxidases (heme proteins)
2.	HO ₂	Protonated form of O ₂
3.	H ₂ O ₂ “-“ (Hydrogen peroxide)	Two electron reduction product of O ₂ formed from O ₂ by “-“ dismutation or directly from O ₂ . Reactivity of O ₂ and H ₂ O ₂ is amplified in presence of heme proteins
4.	OH (Hydroxy radical)	Three electrons reduction products of O ₂ generated by Fenton’s reaction, transition metal (iron, copper)-catalysed Haber-Weiss reaction; also formed by decomposition of peroxynitrite produced by reaction of O ₂ with nitric oxide radical.
5.	RO. (Alkoxy radical), ROO. (Peroxyl radical)	Lipid peroxy radical (LOO.) produced from organic hydroperoxide, ROOH by hydrogen abstraction.
6.	¹ O ₂	Singlet Oxygen

Now it is known that the antioxidant capacity of flavonoids is related to its structural features:

- The 2,3-double bond, in conjugation with a 4-oxo function is responsible for electron dislocation from the B-ring.
- The presence of both 3 α - and 5 β -hydroxyl groups may contribute to the free radical scavenging capacity

- The ortho-dihydroxy structure in the B-ring confers greater stability to aroxyl radicals, possibly through hydrogen bonding, and which participates in electron dislocation. Structural features responsible for antioxidant activity are shown below:



Structural features responsible for antioxidant activity

It is reported that a 3',4'-catechol moiety in B-ring strongly enhances lipid peroxide inhibition and this arrangement is an important characteristic of most potent scavengers of peroxy, superoxide and peroxy nitrite radicals and its absence decreases antioxidant activity. It was observed that the absence of the hydroxyl group at position 3 in flavanones and flavones decreases their antioxidant ability⁷⁰.

The flavonoids : diosmetin, kaempferol, quercetin, kaempferol 3-glucoside (astragalins), quercetin 3-rhamnoside (quercitrin), quercetin 3-xyloside and quercetin 3-galactoside (hyperoside) were reported from leaves of some species well known for their potential antioxidant activity. Rutin and apigenin were found to be potent inhibitors of lipid peroxidation and oxidation of β -carotene⁷³.

Several flavonoids were isolated from the leaves of *Licania licaniaeflora* and it was reported⁸⁹ that quercetin derivatives possess strongest antioxidant activity and 8-hydroxy-naringenin and kaempferol-3-O- α -rhamnoside gave the lowest antioxidant activity.

Dietary flavonoids like epicatechin, epigallocatechin galate, gallic acid, quercetin-3-glucoside possess strong antioxidant potency⁷⁴. Quercetin at a dose of 15 mg/100g p.o.is reported to produce significant hepatoprotection⁷⁵.

When the walls of the blood vessels supplying oxygen, nutrients, and protective substances to this area gets sufficiently weakened, then slight mechanical insults easily cause ruptures, resulting in leakage of blood into the tissue. Such events start inflammation and repair processes are needed⁷⁶.

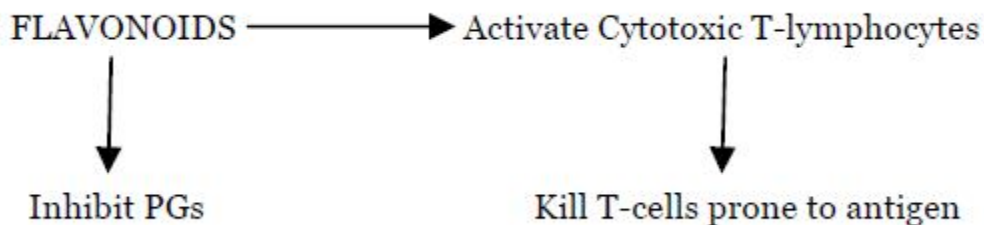
Flavonoids are favourable, effective, and usually innocuous substitutes for the classical therapeutic agents. It has also been reported⁷⁶ that flavonoids protect against gastric ulcer. Similar to aspirin, acylated flavonoids may transfer their acyl group to the side chain hydroxyl group of serine in the active site of COX⁷⁶.

Flavonoid of *Ocimum basilicum* decreased ulcer index and thus inhibited gastric acids in ulcers. Quercetin, Kaempferol, rutin when administered intraperitoneally (25-100 mg/kg) inhibited dose-dependent gastric damage produced by ethanol in rats⁷².

Inflammation is the integrated response of many defence systems of the body to the invasion of a foreign body. Inflammation is a free radical generating process⁷⁷. Various flavonoids (e.g., quercetin, apigenin, tea catechins) have also been shown to have anti-inflammatory activity by inhibiting cyclooxygenase-2 (COX2) and inducible nitric oxide synthase, which is related to antioxidant activity. Flavonoids also inhibit cytosolic and tyrosine kinase^{67,78} and also inhibit neutrophil degranulation⁶⁷ which are involved in inflammation. Citrus flavonoid, hesperidin is known to possess anti-inflammatory and analgesic effects. Apigenin, luteolin, quercetin are also known to possess anti-inflammatory activity⁷².

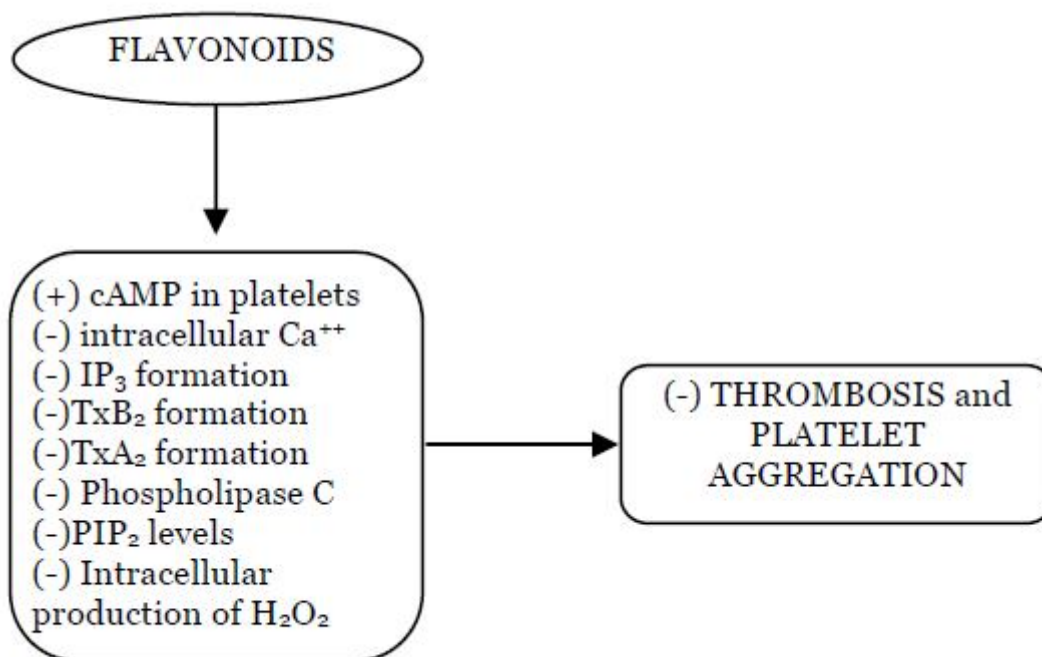
Quercetin and hesperedin given at a daily dose of 80 mg/kg inhibit both acute and chronic phase of inflammation while rutin was found to be effective only in chronic case. Kaempferol, quercetin, myricetin, fisetin are reported to possess COX and LOX inhibitory activities⁷². Also flavonoids possess antirheumatic activity. It is known that inflammation is an important component of rheumatoid arthritis..

The beneficial effect of orally consumed flavonoids includes the elimination of the PGs which mediate the pain. An additional effect of the flavonoids may be to activate cytotoxic T-lymphocytes, which kill cells presenting the harmful foreign antigen. Flavonoids do activate cytotoxic T-lymphocytes, but the antigen, against which the lymphocytes are directed, remains unidentified⁶⁷:



Effect of flavonoids in rheumatoid arthritis

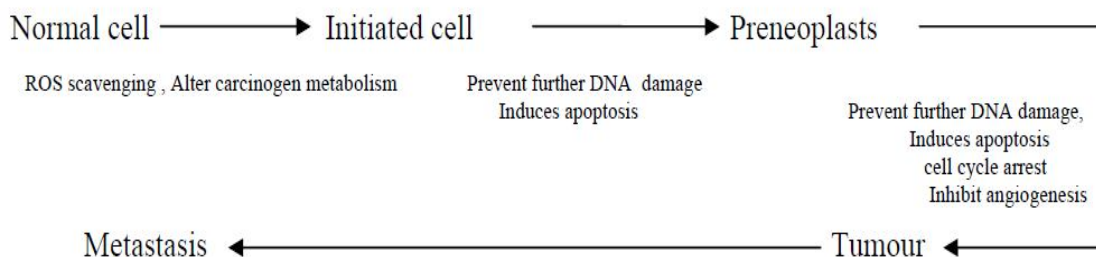
Flavonoids are used as antithrombotic due to their ability to scavenge free radicals. They inhibit cyclooxygenase and lipoxygenase pathway. The main antiaggregatory effect is by the inhibition of thromboxane A2 formation. Flavonoids like quercetin, kaempferol and myricetin are known to possess antiaggregatory properties^{67,73}. The following scheme represents mechanism of flavonoid in thrombosis:



Many reports indicate the role of flavonoids as anticancer nutrients. Cancer is a growth of diseases caused by disturbance in growth metabolism. Cancer cells manifest to varying degree uncontrolled proliferation, loss of function, invasiveness such events are not reflected by normal cells⁷⁹.

Flavonoids for a long time have been part of the herbal treatment by lay practitioners, but they were recognised only recently as effective substances. Examples of herbal preparations owing their growing recognition as effective anticancer drugs to flavonoids are Propolis and Essiac⁷⁶.

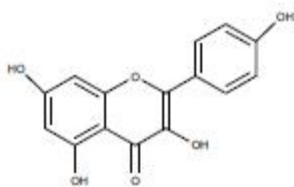
Flavonoids are potent bioactive molecules that possess anticarcinogenic effects since they can interfere with the initiation, development and progression of cancer by the modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis⁸⁰ as shown in scheme below:



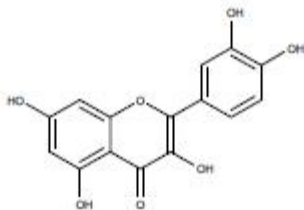
Flavonoids have emerged as potential chemopreventive candidates for cancer treatment due to their ability to induce apoptosis⁸⁰.

Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which are possibly involved in DNA damage and tumor promotion. Flavonoids may also have a beneficial effect through their impact on the bioactivation of carcinogens.

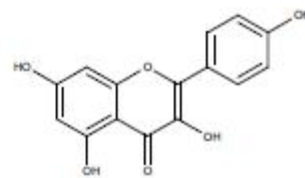
The flavonols kaempferol (**12**) quercetin (**13**), and galangin, and the flavone apigenin (**14**) have been reported to inhibit cytochrome P450 enzymes of the CYP1A family.



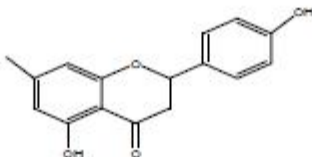
(12)



(13)

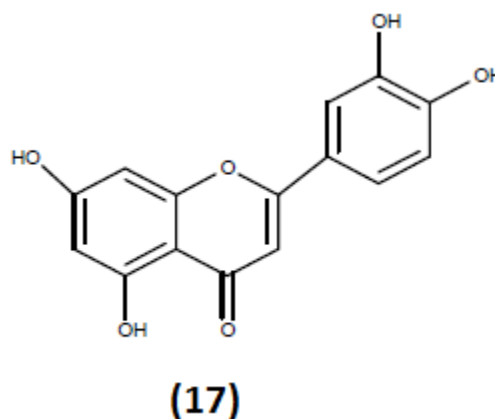
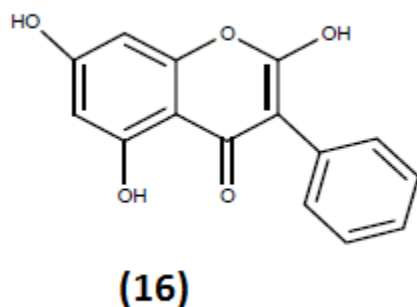


(14)



(15)

Quercetin and naringin have also been shown to inhibit CYP3A4, which is the most abundant P450 enzyme in the liver and beneficial in metabolizing a significant number of carcinogens and medications. Quercetin is abundantly found in human diet and it gets extensively metabolized during absorption in the small intestine and in the liver, and thus exerts a dose-dependent inhibitory effect on cell proliferation. In addition, *in vitro* studies have shown that tea catechins increase the activity of several detoxifying and antioxidant enzymes, such as glutathione reductase, glutathione peroxidase, glutathione S-reductase, catalase, and quinone reductase^{76,80}. Genistein (**16**) and quercetin inhibit protein tyrosine kinase which is also involved in cell proliferation^{76,80,81}. Finally, apigenin, luteolin (**17**) and quercetin have been shown to cause cell cycle arrest and apoptosis⁸².



Studies have shown⁸³ that some flavonoids components such as quercetin had anticancer activities and were able to inhibit cancer cell growth.

The biological actions of flavonoids is historically related to their ability to act as antioxidants. However, this classical hydrogen-donating antioxidant activity cannot account for the physiological effects of flavonoids in the brain as they exist in very low concentrations. Thus, it has been suggested that the effect of flavonoids in the brain is mediated by an ability to protect vulnerable neurons, enhance existing neuronal function, stimulate neuronal regeneration and induce neurogenesis.

Early indications regarding the ability of flavonoids to impact upon brain function were reported in the 1950s, with flavones reported to act as novel brain-stem stimulants⁸⁴. Studies suggest that flavonoids, in particular isoflavones such as genistein might be detrimental to memory processes in the brain due to their ability to act as tyrosine kinase inhibitors.

Indeed, it has become evident that flavonoids are able to exert neuroprotective actions even at low concentration via their interactions with critical neuronal intracellular signalling pathways pivotal in controlling neuronal survival and differentiation, long-term potentiation and memory⁸⁵: It was shown⁴⁵ that

quercetin at a dose of 10, 20, 40 mg/kg p.o. positively influence memory function and it has been reported⁸⁶ that fisetin to facilitate memory.

Flavonoids inhibit cyclic AMP phosphodiesterase and calcium-dependent ATPase which are responsible for histamine release from cells and basophils⁶². Quercetin inhibits both the production and release of histamine and is useful in allergic conditions like asthma, hayfever etc⁸⁷.

It was reported that⁸⁸ flavonoids possess multiple neuroprotective actions in central nervous pathophysiological conditions including depression and it was claimed that naringenin possess potent antidepressant-like property .Depression is caused by functional deficiency of monoamine transmitters at certain sites in brain⁷⁹.

As far as structure is concerned , the position of the sugar on the flavonoid nucleus seems relevant to the antidepressant property of the glycosylated flavonoids. The 7-glycosyl derivative was found to exert significant antidepressant activity. the most , but the presence of a double bond between carbons: 2 and 3, resulting in flavone derivatives with planar configuration (i.e. linarin) does not appear to be critical for activity. Flavonoid glycosides form the newest group within the growing family of flavonoids with activity on the central nervous system⁸².

For centuries ,flavonoids have been used extensively for the treatment of various diseases. Propolis has been referred even in old testament for its healing properties. The antimicrobial activity of propolis has been attributed to its high flavonoid content. Galangin is a flavonol commonly found in propolis. It has been reported⁶¹ to possess inhibitory actions against *Aspergillus tamaris*, *Aspergillus flavus*, *Cladosporium sphaerospermum*, *Pencillium digitatum*, *Penicillium italicum*⁶¹ .

The flavonoid :5,7,4'-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-2S-flavonone isolated from shrub *Eysenhardtia texana* and flavonoid: 7-hydroxy-3',4'-methylenedioxyflavan isolated from *Termanalia bellerica* possess antifungal activity against *Candida albicans* while, 6,7,4'-trihydroxy-3',5'-dimethoxyflavone and 5,5'-dihydroxy-8,2',4'-trimethoxyflavone are effective against *Aspergillus flavus*⁷².

Nobiletin and langeritin isolated from peelings of tangerine orange showed fungistatic action towards *Deuterophoma tracheiphila* while hesperidin stimulate fungal growth slightly⁶⁹.

Quercetin, naringenin are reported to be inhibitors of *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Staphylococcus nervous*, *Staphylococcus epidermis*, *Saccharomyces cerevisiae*⁸⁹.

Morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin, quercetin-3-O-arabinoside were isolated from *Psidium guajava* leaves and reported to possess bacteriostatic action against all foodborne pathogenic bacteria including *Bacillus stearothermophilus*, *Brochothrix thermosphacta*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Salmonella enteric*, *Staphylococcus aureus*, *Vibrio cholera*⁹⁰.

Those flavonones having sugar moiety showed antimicrobial activity while none of the flavonols and flavonolignans showed inhibitory activity on microorganisms. Quercetin has been reported to completely inhibit growth of *Staphylococcus aureus*⁷².

Cardiovascular diseases which include: atherosclerosis, coronary heart disease, arterial hypertension, and heart failure are today the principal cause of death in both developing and developed countries.. The major reason behind such diseases

is oxidative stress. Oxidative stress is a condition of imbalance between endogenous oxidants and reactive oxygen/nitrogen species (RONS) with predominance of reactive species.

Atherosclerosis involves modification of LDL particles by oxidative stress with subsequent induction of inflammation which is caused by increased leucocyte adherence⁷⁶.

Studies ensure that long-term administration of flavonoids can decrease, or tend to decrease the incidence of cardiovascular diseases and their consequences.

Flavonoid consumption prevent many cardiovascular diseases including hypertension and atherosclerosis. Quercetin protects LDL against oxidative modifications effect. 7-mono-hydroxyethylrutoside and 7', 3', 4'-trihydroxyethylrutoside are reported to be cardioprotective⁷².

The role of flavonoids in diabetes mellitus has been investigated. Diabetes mellitus is a serious chronic disease. Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both types 1 and 2 diabetic patients⁹¹. Flavonoids cannot cure diabetes mellitus because most types of this disease are basically genetic and no single drug can correct an inborn error. However, flavonoids can ameliorate some of the consequences of diabetes mellitus⁷⁶. Flavonoids have been identified to be good inhibitors of aldose reductase⁹² and regenerate pancreatic islets.

Several researchers claimed that quercetin possess antidiabetic activity and it has been found that it brings about regeneration of pancreatic islets and increases insulin release in streptozotocin-induced diabetes. Also, it has been reported to stimulate Ca^{2+} uptake from isolated islet cells thus suggesting it to be effective even in non-insulin dependent diabetes⁸⁸. It was claimed that⁹³ flavonoids in

Ipomoea batatas leaf possess antidiabetic activity against alloxan-induced diabetes at a dose of 100 mg/kg and it was reported⁹⁴ that fisetin is a therapeutic agent for treatment of diabetes mellitus at a dose of 10 mg/kg.



Fig1: *Geigeria alata* (DC) Benth



Fig 2: flowers and leaves of *geigeria alata* (DC)

