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"Qualitative and Quantitative Analysis of Flavonoids"

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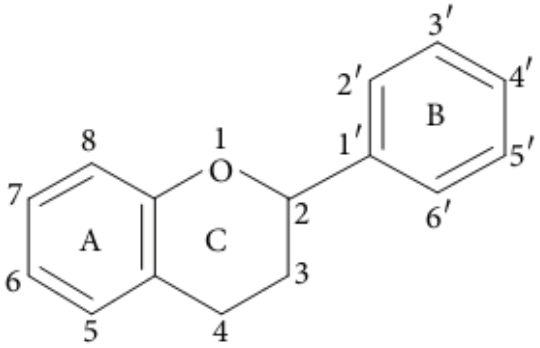
Abstract: *Flavonoids and Polyphenolic compounds have been given sizeable attention in this review; distinctively for the reason of their biological and physiological importance. This review emphasis on separation and revealing qualitative and quantitative estimation methods of flavonoids. This literature inspects various techniques used for quantitative and qualitative estimation of flavonoids. These techniques embrace, for example, HPLC, ultraviolet-visible spectroscopy, mass spectrometry, nuclear magnetic resonance spectroscopy. The inclusive topics are structural characterization by different techniques, conduct of sample with different solvents of different polarity, extraction, chromatography techniques such as liquid chromatography (LC), and gas chromatography (GC), electrophoresis. Emphasis will be on up to date developments and leaning*.

Keywords: *Flavonoids, Polyphenolic, Mass spectrometry, Chromatography.*

I. Introduction

Flavonoids and polyphenolic compounds embrace a huge group of water soluble secondary metabolites which are produced by plants. Afterward the core was found to be "FLAVONOIDS" (rutin) and till now numerous varieties flavonoids have been recognized. Polyphenolic compounds contain both phenolic rings with multiple hydroxyl groups, thus it is called polyphenolic. Flavonoids shield the plants from microbial infection. Due to antioxidant activities of the polyphenolic compounds scavenging free radicals impend health benefits from them. It also has protective enzyme systems which lead to perk up human health. Flavonoids can be classified into variety of classes such as flavone ((e.g., flavone, apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), flavanones (e.g., flavanone, hesperetin, and naringenin), and others. Their general structures are shown in Table 1. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings.



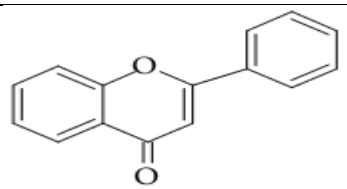
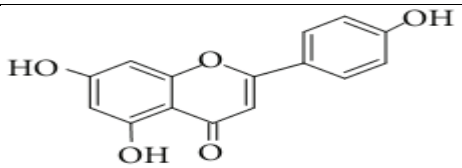
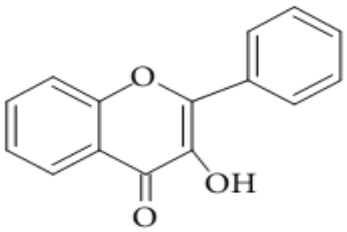
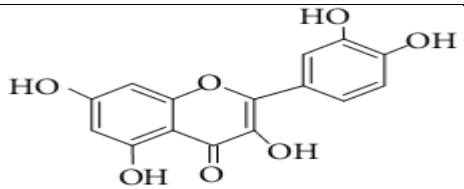
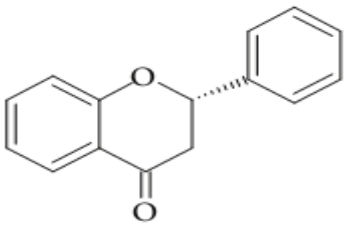
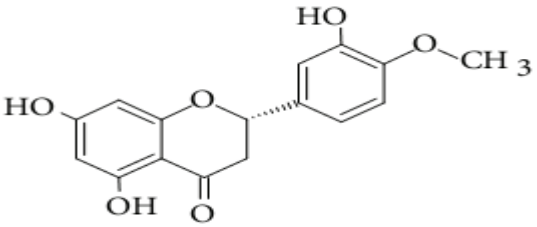
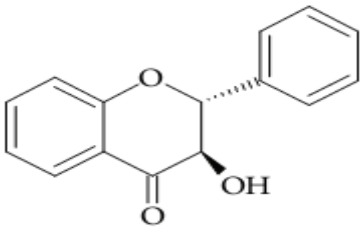
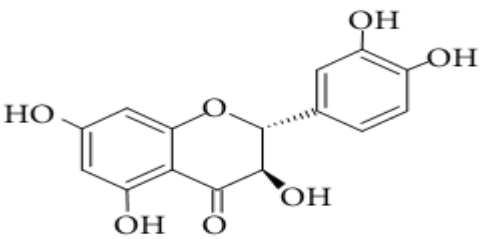
The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization . Recent interest in these substances has been stimulated by the potential health benefits arising fromthe antioxidant activities of these polyphenolic compounds. As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capacity both *in vivo* and *in vitro* systems . Flavonoids have ability to induce human protective enzyme systems. The number of studies has suggested protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases. Flavonoids also act as a secondary antioxidant defense system in plant tissues exposed to different abiotic and biotic stresses. Flavonoids are located in the nucleus ofmesophyll cells and within centers of ROS generation. They also regulate growth factors in plants such as auxin]. Biosynthetic genes have been assembled in several bacteria and fungi for enhanced production of flavonoids.

Flavonoid Rich Food and Medicinal Plants:-

Flavonoids in food are generally accountable for color, taste, preclusion of fat oxidation, and fortification of vitamins and enzymes. Particularly photosynthesizing plant cells contain flavonoids which are the most common and extensively distributed group of phenolic compounds in all plant parts. Only anthocyanin class of flavonoids imparts colures to flowering parts of plants. Flavonoids cannot be biosynthesized by humans and animals in their body so they are an vital part of human and animal diet. Most copious flavonoids in foods is flavonols. Soy isoflavones, flavonols, and the flavones also present in high amount in human diet. Although catechins mainly found in fruits and legumes, the levels diverge from 4.5 to 610 mg/kg. Flavonoid levels may varies on the behalf of processing of food material. For example, in a recent study, orange juices were found to restrain 81–200 mg/L soluble flavanones, while the content in the fruit as such was 206–644 mg/L which imply that the flavanones are strenuous in the cloud during processing and storage. Accurate estimation of the average dietary intake of flavonoids is difficult, because of thewide varieties of available flavonoids and the extensive distribution in various plants and also the diverse consumption in humans. Flavonoids have been consumed by humans since the advent of human life on earth, that is, for about 4 million years. They have extensive biological properties that promote human health and help reduce the risk of diseases.

Oxidative modification of LDL cholesterol is thought to play a key role during atherosclerosis. The isoflavan glabridin, a major polyphenolic compound found in *Glycyrrhiza glabra* (Fabaceae), inhibits LDL oxidation via a mechanism involving scavenging of free radicals.

Table 1: Classification of Flavonoids with Structure

Group of Flavanoid	Structure backbone	Examples
Flavones		Apigenin 
Flavonols		Quercetin 
Flavanones		Hesperetin 
Flavanonol		Taxifolin 

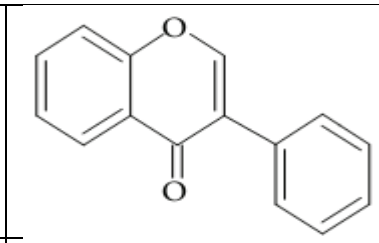
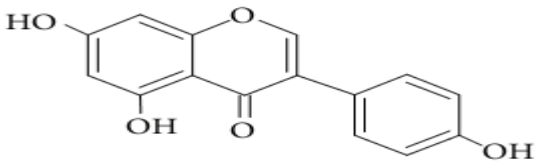
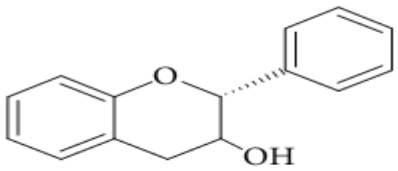
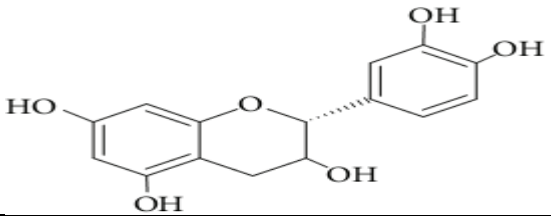
Isoflavones		Genistein 
Flavan-3-ols		Catechin 

Table 2 : Classification, Structure, and food sources of some dietary flavonoids

CLASS	FLAVONOID	DIETARY SOURCE	REFERENCES
Flavanol	(+)-Catechin (−)-Epicatechin Epigallocatechin	Tea	
Flavone	Chrysin, apigenin Rutin, luteolin, and luteolin glucosides	Fruit skins, red wine, buckwheat, red pepper, and tomato skin	
Flavonol	Kaempferol, quercetin, myricetin, and tamarixetin	Onion, red wine, olive oil, berries, and grapefruit.	
Flavanone	Naringin, naringenin, taxifolin, and hesperidin	Citrus fruits, grapefruits, lemons, and oranges	
Isoflavone	Genistin, daidzin	Soyabean	
Anthocyanidin	Apigenidin, cyanidin	Cherry, easberry, and strawberry	

Preparative methods for Flavonoids

High Performance Liquid Chromatography

HPLC is becoming by far the most popular technique for the separation of flavonoids, both on preparative and scales. Improvements in instrumentation, packing materials, and column technology are being introduced all the time, making the technique more and more attractive.

TABLE 1.1
Preparative Separations of Flavonoids by HPLC

Sample	Column	Eluent	Referen
Phenolics from *Picea abies*	Nucleosil 100–7C_{18} 250 × 21 mm	MeOH–H_2O, gradient	21
Chalcones from *Myrica serrata*	LiChrosorb Diol 7 μm, 250 × 16 mm	MeOH–H_2O, 55:45	20
	Nucleosil 100–7C_{18} 250 × 21 mm	MeOH–H_2O, 76:24	
Flavones from *Tanacetum parthenium*	LiChrospher RP-18 250 × 25 mm	CH_3CN–H_2O, 3:7	22
Flavone glycosides from *Lysionotus pauciflorus*	LiChrosorb RP-18 250 × 10 mm	CH_3CN–H_2O, 1:4	23
Flavonoid glucuronides from *Malva sylvestris*	Spherisorb ODS-2 5 μm, 250 × 16 mm	CH_3CN–H_2O–THF–HOAc, 205:718:62:15	24
Flavonol galloyl-glycosides from *Acacia confusa*	Hyperprep ODS 250 × 10 mm	CH_3CN–H_2O, gradient	25
Flavanones from *Greigia sphacelata*	LiChrospher Diol 5 μm, 250 × 4.6 mm	Hexane–EtOAc, 7:3	26
Prenylated flavonoids from *Anaxagorea luzonensis*	Asahipack ODP-90 10 μm, 300 × 28 mm	CH_3CN–H_2O, 45:55	27
Prenylated isoflavonoids from *Erythrina vogelii*	μBondapak C_{18}10 μm, 100 × 25 mm	MeOH–H_2O, isocratic	28
Biflavones from *Cupressocyparis leylandii*	LiChrospher RP-18 7 μm, 250 × 10 mm	MeOH–H_2O, 72:28	29
Anthocyanin glycosides	Spherisorb ODS-2 10 μm, 250 × 10 mm	MeOH–5% HCOOH	30
Proanthocyanidins and flavans from *Prunus prostrata*	Eurospher 80 RP-18 7 μm, 250 × 16 mm	CH_3CN–H_2O (+0.1% TFA), 1:4, 3:17	31

The difference between the analytical and preparative methodologies is that analytical HPLC does not rely on the recovery of a sample, while preparative HPLC is a purification process and aims at the isolation of a pure substance from a mixture. Semipreparative HPLC separations (for 1 to 100 mg sample sizes) use columns of internal diameter 8 to 20 mm, often packed with 10 mm (or smaller) particles. Large samples can be separated by preparative (or even process-scale) installations but costs become correspondingly higher.\

Optimization can be performed on analytical HPLC columns before transposition to a semipreparative scale. The aim of this chapter is not a detailed description of the technique and instrumentation but to show applications of HPLC in the preparative separation of flavonoids. Approximately 95% of reported HPLC applications are on octadecylsilyl phases. Both isocratic and gradient conditions are employed.

Medium-Pressure Liquid Chromatography

The term ''medium-pressure liquid chromatography'' (MPLC) covers a wide range of column diameters, different granulometry packing materials, different pressures, and a number of commercially available systems. In its simplest form, MPLC is a closed column (generally glass) connected to a compressed air source or a reciprocating pump. It fulfills the requirement for a simple alternative method to open-column chromatography or flash chromatography, with both higher resolution and shorter separation times. MPLC columns have a high loading capacity — up to a 1:25 sample-to-packing-material ratio32 — and are ideal for the separation of flavonoids.

In MPLC, the columns are generally filled by the user. Particle sizes of 25 to 200 mm are usually advocated (15 to 25, 25 to 40, or 43 to 60 mm are the most common ranges) and either slurry packing or dry packing is possible. Resolution is increased for a long column of small internal diameter when compared with a shorter column of larger internal diameter (with the same amount of stationary phase).33 Choice of solvent systems can be efficiently performed by TLC34 or by analytical HPLC. Transposition to MPLC is straightforward and direct.

Centrifugal Partition Chromatography

Various countercurrent chromatographic techniques have been successfully employed for the separation of flavonoids.7 Countercurrent chromatography is a separation technique that relies on the partition of a sample between two immiscible solvents, the relative proportions of solute passing into each of the two phases determined by the partition coefficients of the components of

Separation of Flavonoids by Medium-Pressure Liquid Chromatography

Sample	Column	Eluent	Reference
Chalcones from *Piper aduncum*	Silica gel 800 × 36 mm	Hexane–TBME–CH_2Cl_2–EtOH, 99:0.4:0.3:0.3	36
Flavonoids from *Sophora moorcroftiana*	RP-18 20 μm	MeOH–H_2O, 3:1	37
Flavonol glycosides from *Epilobium* species	RP-18 15–25 μm 460 × 26 mm	MeOH–H_2O, 35:65	38
Dihydroflavonoid glycosides from *Calluna vulgaris*	Polyamide SC-6 460 × 26 mm	Toluene–MeOH	39
	RP-18 20–40 μm 460 × 15 mm	MeOH–H_2O	
Prenylflavonoid glycosides from *Epimedium koreanum*	RP-8 460 × 26 mm	MeOH–H_2O, 2:3	40
Prenylated isoflavonoids from *Erythrina vogelii*	RP-18 15–25 μm 500 × 40 mm	MeOH–H_2O, 58:42, 60:40	41
Biflavonoids from *Wikstoemia indica*	RP-18 300 × 35 mm	MeOH–H_2O, 55:45 → 95:5	42

the solute. It is an all-liquid method that is characterized by the absence of a solid support, and thus has the following advantages over other chromatographic techniques:

- No irreversible absorbtion of the sample.
- Quantitative recovery of the sample.
- Greatly reduced risk of the sample denaturation.
- Low solvent consumption
- Favourable economic.

It is obvious, therefore, that such a technique is ideal for flavonoids, which often suffer from problems of retention on solid supports such as silica gel and polyamide. Countercurrent distribution, droplet countercurrent chromatography, and rotation locular countercurrent chromatography are now seldom used but CPC, also known as centrifugal countercurrent chromatography, finds extensive application for the preparative separation of flavonoids. In CPC, the liquid stationary phase is retained by centrifugal force instead of a solid support (in column chromatography). Basically, two alternative designs of apparatus are on the market43: (a) rotating coil instruments; (b) disk or cartridge instruments.

Although most CPC separations are on a preparative scale, analytical instruments do exist.44 However, these are mostly used to find suitable separation conditions for scale-up.

There are numerous examples of preparative separations of flavonoids7,45 and some are listed in Table

The technique of CPC was also employed as a key step in the purification of 26 phenolic compounds from the needles of Norway spruce (Picea abies, Pinaceae). An aqueous extract of needles (5.45 g) was separated with the solvent system CHCl3–MeOH–i-PrOH–H2O (5:6:1:4), initially with the lower phase as mobile phase and then subsequently switching to the upper phase as mobile phase. Final purification of the constituent flavonol glycosides, stilbenes, and catechins was by gel filtration and semipreparative HPLC

Separation of Flavonoids by Centrifugal Partition Chromatography

Sample	Solvent System	Referenc
Flavonoids from *Hippophae rhamnoides*	$CHCl_3$–MeOH–H_2O, 4:3:2	46
Flavonol glycosides from *Vernonia galamensis*	$CHCl_3$–MeOH–*n*-BuOH–H_2O, 7:6:3:4	47
Flavonol glycosides from *Picea abies*	$CHCl_3$–MeOH–*i*-PrOH–H_2O, 5:6:1:4	20
Flavonol glycosides from	*n*-BuOH–EtOH–H_2O, 4:1.5:5	48
Polypodium decumanum	$CHCl_3$–MeOH–*n*-BuOH–H_2O, 10:10:1:6	
Flavone *C*-glycosides from *Cecropia lyratiloba*	$CHCl_3$–MeOH–H_2O, 46:25:29	49
	EtOAc–*n*-BuOH–MeOH–H_2O, 35:10:11:44	
Biflavonoids from *Garcinia kola*	*n*-Hexane–EtOAc–MeOH–H_2O, 2:8:5:5	50
Isoflavones from *Astragalus membranaceus*	EtOAc–EtOH–*n*-BuOH–H_2O, 15:5:3:25	51
	EtOAc–EtOH–H_2O, 5:1:5	
Isoflavones from *Glycine max*	$CHCl_3$–MeOH–H_2O, 4:3:2	52
	$CHCl_3$–MeOH–*n*-BuOH–H_2O, 8:6:1:4	
Anthocyanidins from *Ricciocarpos natans*	*n*-Hexane–EtOAc–*n*-BuOH–HOAc–HCl 1%, 2:1:3:1:5	53
Proanthocyanidins from	EtOAc–*n*-PrOH–H_2O, 35:2:2	54
Stryphnodendron adstringens		
Proanthocyanidins from *Cassipourea gummiflua*	*n*-Hexane–EtOAc–MeOH–H_2O, 8:16:7:10	55
Anthocyanins from plants	*n*-BuOH–TBME–CH_3CN–H_2O, 2:2:1:5	56
Polyphenols from tea	*n*-Hexane–EtOAc–MeOH–H_2O, 3:10:3:10	57

II. Chromatographic Techniques

Thin Layer Liquid Chromatography: - Thinly coated glass plates are taken (20x20 cm) with Silica Gel G and is kept at 100^0c for 30 minutes and cooled. Fractions of Ethyl ether and Ethyl acetate are applied separately 1cm above at the edge of the plates along with standard reference compound such as Quercetin , Kaempferol, Myricetin and the chromatograms are allowed to kept in a air tight saturated chamber containing about 200ml of solvent mixture (Butanol: acetic acid :water) (125:72:3). When the plates were completely developed, they were air dried and visualized under UV light in the presence of ammonia fumes, this ammonia fumes were kept in a concentrated chamber containing ammonium hydroxide for 10-15 seconds. Then the fluorescent spot which authenticated the quercetin was identified as quercetin blue. When these spots were exposed to the ammonium fumes the blue spots were changed into bright yellow in colour. Again the Rf values of the respected samples were calculated with an average of five replicates and were compared with the standard samples. Again the identification of the isolated flavonoids was done by mp,mmp performed in capillaries, Infra red spectrophotometer, Ultra violet and visible spectrophotometer according to their respective authentication.

Preparative thin layer chromatography (PTLC) :- Glass plates (20 x 20 cm) thickly coated (0.4-0.5 nm) with silica gel 'G' (45 gm/80 ml water) dried at room temperature, activated at 100°C for 30 minutes and cooled at room temperature were used for preparative thin layer chromatography (PTLC). The various extracts along with the standard samples of kaempferol, quercetin, catechin and myricetin were applied 1cm above the edge of the glass plate. These glass plates developed in an air tight chromatographic chamber containing about 200 ml of organic solvent mixture of n-butanol,acetic acid and water (4:1:5 upper layer). The developed glass plates were dried at room temperature. The developed plates were dried and visualized under UV light. Florescent spots coinciding with those of the standard reference compounds of kaempherol and quercetin were marked. The marked spots were scrapped and collected separately along with the adsorbent and eluted with ethyl acetate. Each of the elutes was obtained out of both diehyl ether and ethyl acetate extracts were dried over sodium sulphate, reconstituted in chloroform and crystallized. The same process was followed alike for both the ethyl ether and ethyl acetate fractions from each of the extracts. The substances thus isolated were purified, crystalised , weighed and percentage calculated separately on dry weight basis. Each of the substances was then subjected to mp, Ultra violet and IR spectral studies using nuzol or kBr pallets 9,10,11. This purified material was also subjected to HPLC studies (Water associates, column - microporasil, 80% hexane and 20% ethyl acetate, chart spectra 1 cm/min, 0.5 ml/min UV detector at 254 nm).

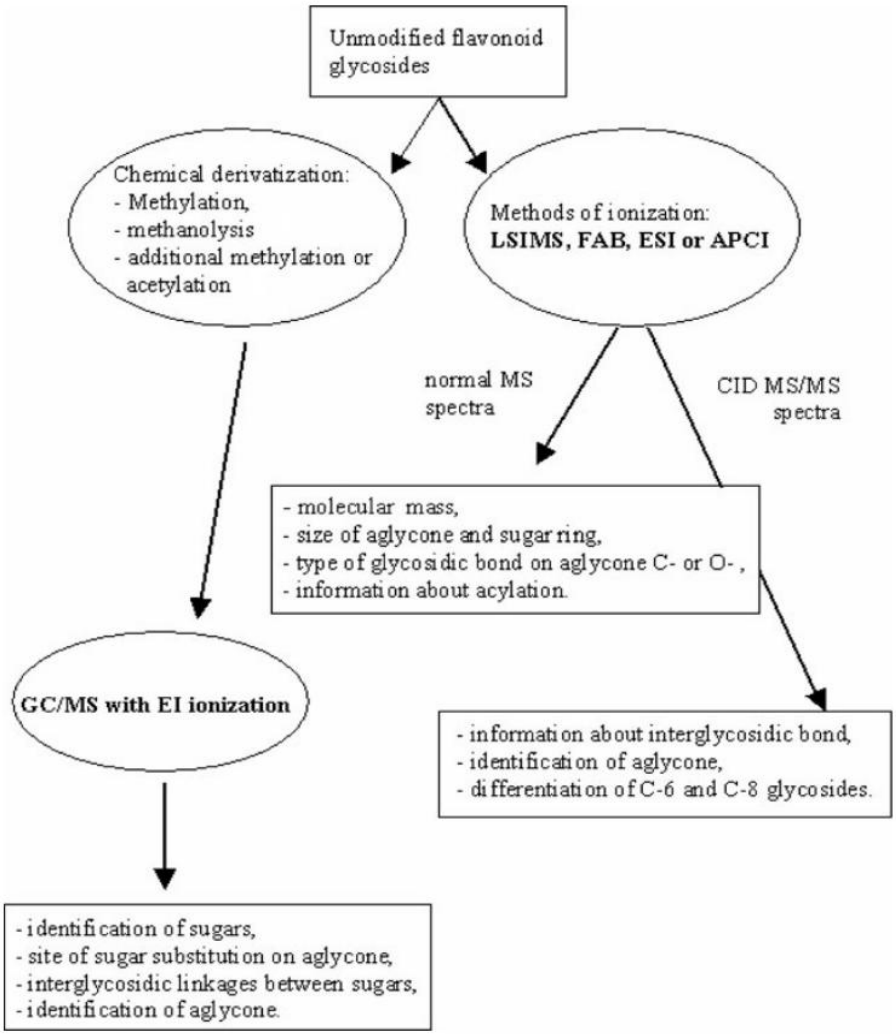
Gas chromatography:- Many volatile compounds are directly amenable to analysis by gas chromatography, a technique of unsurpassed separation capacity. In particular, when combined with mass spectrometry it offers high sensitivity and selectivity. One chemical characteristic of the OH group in phenolic compounds is the hydrogen bonding capability, which increases the melting point. Consequently, the significant concern with this technique is the low volatility of phenolic compounds. Gas chromatography is a major chromatographic technique employed for the analysis especially of phenolic acids in plants. Preparation of samples for GC may include the removal of lipids from the extract, and as mentioned earlier, liberation of phenolics from ester and glycosidic bonds by alkali, acid, or enzymatic hydrolysis. Traditionally, analysis in the gas phase requires a chemical derivatisation step, in addition to sample extraction, isolation and clean-up. Hyphenation of chromatographic and spectroscopic methods is important in analytical chemistry and is of great value in modern natural product analysis. But early work with derivatised phenolics was typically performed with flame ionisation detection (FID). Mass spectrometry later became widespread. Most of the GC–MS work is performed in the electron impact ionisation mode, with the ionisation voltage set to a standard 70 eV. The spectra are collected up to m/z 650 in the scan mode.

There are a variety of reagents used to modify and generate volatile derivatives via converting hydroxyl groups to ethers or esters. Prior to chromatography, phenolics are usually transformed into more volatile derivatives by methylation, conversion into trimethylsilyl (TMS) derivatives, or derivatisation with N-(tert-butyldimethylsilyl)-Nmethyltrifluoroacetamide Typically, in GC analysis, flaivonoids are hydrolysed and converted into their derivatives, injected onto a non-polar column (1% phenyl– 99% methyl polysiloxane or 5% phenyl–95% methyl polysiloxane) in the split or splitless mode, and separated with a linear 30–90 min temperature programme up to 3008C. GC in the identification of aglycones as silylated derivatives completed by mass selective detection can be regarded as fairly acceptable in the identification of phenolics. Care is taken to ensure anhydrous conditions during the preparation and derivatisation process because of the high sensitivity of TMS derivatives to moisture. The early GC–MS study of perdeuteromethylated flavonoid aglycones is of theoretical importance. Methylation was carried out with C2H3I and NaH in dimethylformamide. This method provided information about the sugar sequence, their interglycosidic linkages, and the sugar attachment to the aglycone. Diazomethane is often used for the generation of methyl esters. Although solutions of diazomethane react efficiently with carboxylic acids, it must be generated in the laboratory, and is explosive and harmful. HuDek made use of ethyl and methyl chloroformate for the formation of ethyl and methyl esters, respectively. Dimethyl sulphoxide with methyl iodide in an alkaline medium is another alternative to methylation. However, methyl esters can lead to some confusion, as they are naturally occurring in some plant-based material.

Mass Spectrometry

MS is a very sensitive analytical method used to identify flavonoid conjugates or to perform partial structural characterization using microgram amounts of sample (Cuyckens and Claeys, 2004). Indeed, significant structural data can be obtained from less than 1 mg of the analyzed compound when different MS techniques are used in combination with chemical derivatization of the characterized compounds (Franski et al., 1999, 2002, 2003). A strategy for the combined application of different MS techniques and chemical derivatization are presented in fig1.

Flavonoid glycosides are thermally labile compounds and the evaporation without decomposition of the analyte is impossible, even in the ion source of a MS, where high vacuum exists (about 3 × 10-5 torr). In this situation, soft ionization methods need to be applied for the analysis of this group of compounds, and the analyte molecules are ionized without evaporation in high vacuum (FAB or LSIMS, MALDI) or under atmospheric pressure (ESI, APCI). From normal mass spectra, information can be obtained about the molecular weight of the whole conjugate, the size of the sugar moieties attached to the aglycone, and the molecular weight of the aglycone (Stobiecki, 2000; Cuyckens and Claeys, 2004).



Structural information obtainable with different mass spectrometric methods.

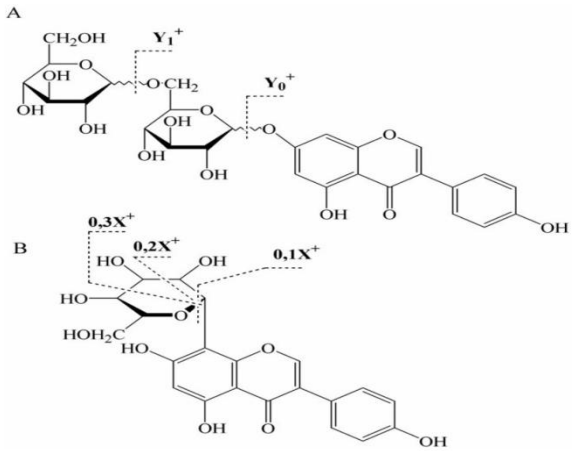
With FAB or LSIMS ionization, the desorption of the analyte molecule ions from the liquid matrix may be improved when the interactions of the polar groups of the analyte with the matrix decrease. Improved efficiency of ion desorption may be further achieved after the methylation of the analyzed compounds. In addition, the methylation of a flavonoid glycoside may help to elucidate the glycosylation pattern of the aglycone hydroxyl groups (Stobiecki et al., 1988).

The *O*-glycosides of flavonoids give positive ion mass spectra containing intense [M+H]+ ions as well as fragment ions created after the cleavage of glycosidic bonds between sugar moieties or sugar and aglycone, in this case Yn + type ions (for the ion different situation is observed in the negative ion mass spectra mode, where much lower fragmentation of the deprotonated molecule ions [M-H]− occurs. In the case of flavonoid *O*-glycosyl-glycosides, the rearrangement of sugars may take place during the fragmentation process, and the sequence of sugar losses does not correspond to the sequence of the sugar moieties in the intact molecule (Cuyckens et al., 2001, 2002). This situation was observed for the first time in flavonoid rutinosides α(1-6) and neohesperidosides α(1-2), in which rhamnose occurs on the nonreducing end of the diglucoside moiety and glucose is bound to the aglycone (Ma et al., 2001). A different fragmentation pathway may be observed in the flavonoid *C*-glycosides, in which rupture of the sugar ring takes place and strong [M+H-120]+ ions are created. The nomenclature of the fragment ions in mass spectra of *C*-glycosides was introduced by Becchi and Fraisse (1989). The cleavage of the sugar moiety in all types of flavonoid *C*-glycosides is observed in both positive and negative mode mass spectra. The possible fragmentation patterns ofm flavonoid *O*- and *C*-glycosides are presented on Figure 2.

Structural information obtainable from the mass spectra may be achieved whentandem mass spectrometric techniques are applied. It is possible to increase the degree of fragmentation by applying CID MS/MS technologies; however, in LC/MS instruments with atmospheric pressure ionization (APCI or ESI) the increase of potential between the entry slit to the analyzer and the skimmer also promotes the fragmentation of the molecule ions, especially in the positive ion mode. The analysis of metastable ions is also possible in MS with electromagnetic analyzers equipped with collision cells in the field-free regions (McLafferty, 1983; Stobiecki et al., 1988; Becchi and Fraisse, 1989). When a full CID MS/MS system is used, the identification of the isomeric aglycones, for example kaempferol and luteolin or apigenin and genistein, is also possible (Ma et al., 1997). An important advantage of some CID MS/MS systems is the possibility of discrimination between *C*-6 and *C*-8 flavonoid glycosides. There are several reports showing that the differentiation ofboth isomers is possible on the basis of the relative intensities of the fragment ions obtained after CID fragmentation of the [M+H]+ ions (Becchi and Fraisse, 1989; Li et al., 1992; Waridel et al., 2001; Bylka et al., 2002; Cuyckens and Claeys, 2004).

The analysis of the CID mass spectra of flavonoid *O*-diglucosides permits one to distinguish between α(1-2)- and α(1-6)-linked sugars in the investigated molecules (Cuyckens et al., 2000; Ma et al., 2001; Franski et al., 2002). Mass spectrometers with laser desorption ionization combined with the time of flight analyzer (MALDI ToF) also were used for the analysis of isoflavone glycosides in soy products (Wang and Sporns, 2000a), and [M+H]+ and Yn+ type ions were observed in the mass spectra. The same authors tried to apply this MS technique for the analysis of flavonol glycosides in food (Wang and Sporns, 2000b).

Studies on the application of the MALDI ToF technique for the quantitative analysis of flavonoid glycosides also were performed in the same laboratory; in this case the proper choice of the matrix and the deposition of samples in target wells was very important (Frison and Sporns, 2002). MALDI ToF also has been used for the analysis of condensed tannins isolated from plant material. In the first paper on this subject (Ohnishi-Kameyama et al., 1997), the MALDI and FAB ionization for the identification of flavan-3-ol oligomers in apples was compared. The presence of up to pentadecamer polymers composed of catechins was reported. However, only tetramers were identified in the same plant source when FAB ionization was used in earlier experiments (Self et al., 1986).



Fragmentation pattern and daughter ion nomenclature of different glycosides of isoflavonoids. (A) Genistein-7-O-glucosyl-glucoside. (B) Genistein-8-C-glucoside.

This comparison of results obtainable with both ionization methods shows the advantages of the MALDI technique in studies of polymers and other compounds of high molecular weight. The efficiency of the ion desorption from the ion source depends on the compound used for the matrix cocrystalized with the analyte. Different compounds, mainly organic acids such as trans-3-indoleacrylic acid or dihydroxybenzoic acid, are used.

However, the signal-to-noise ratio may vary substantially depending on the matrix utilized. Generally, MALDI instruments are used for analysis of compounds with molecular weights above 500 Da. MALDI ToF MS studies of polygalloyl polyflavan-3-ols in grape seeds and wine were performed by several groups (Wang and Sporns, 1999; Yang and Chien, 2000; Krueger et al., 2000; Perret et al., 2003).

Reed and coworkers (2003) identified procyanidines in sorghum. The isolation of target compounds from plant extracts with different solvents permitted one to distinguish two groups of compounds: oligomers of flavan-3-ols and a series of their 5-*O*-glucosylated derivatives. It was demonstrated that the fragmentation obtainable in

ToF analyzers due to the postsource decay (PSD) technique permits the achievement of fragment-protonated molecules of flavan-3-ol trimers and tetramers together with sequence information (Behrens et al., 2003).

Nuclear Magnetic Resonance

NMR is a well-established and the most commonly used method for natural product structure analysis. The studies of flavonoid structures using 1H-NMR were initiated in 1960s (Markham and Mabry, 1975) and along with 13C-NMR have became the method of choice for the structure elucidation of these compounds. The chemical shifts and multiplicity of signals corresponding to particular atoms and their coupling with other atoms within the molecule allow for easy identification of the aglycone structure, the pattern of glycosylation, and the identity of the sugar moieties present. The literature of this topic is abundant and rapidly growing (Markham and Geiger, 1994; Albach et al., 2003; Kazuma et al., 2003; Francis et al., 2004).

Functions of Flavonoids in plants

Anthocyanin pigment present in flowers provide colour to it contributing to pollination [8, 10, 20]. Flavonoids present in leaves promote physiological survival of plant by protecting it from fungal infections and UV radiations. In addition, flavonoids are involved in photosensitization, energy transfer, respiration and photosynthesis control, morphogenesis, sex-determination, energy transfer.

S. No.	Flavonoid subclass	Food source	Representative flavonoids
1.	Flavonol	Onion, kale, broccoli apples, cherries, berries, tea, red wine	Kaempherol, myricetin, quercetin, rutin
2.	Flavones	Parsley, Thyme	Apigenin, chrysin, luteolin
3.	Flavonones	Citrus	Hesperitin, erodictyol, naringen
4.	Catechins	Apple, tea	Catechin, galocatechin
5.	Anthocyanidins	Cherries, Grapes	----------
6.	Isoflavones	Soya beans, Legumes	Daidzen, genistein, glyciten, formanantine

Occurrence of flavonoids in food [9, 19]

III. Pharmacology of Flavonoids

Reported Pharmacological activities of flavonoids

Flavonoids have been reported to exert wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic [10, 11, 15], cytotoxic antitumour, treatment of neurodegenerative diseases, vasodilatory action [4,11,13,39]. 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 [15, 39, 40]. These are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase [41].

Flavonoids as antioxidants:

Mechanism of flavonoids as antioxidants

Flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers [42]. This activity is attributed to their hydrogen-donating ability. 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 structure [1]. Free radical scavenging capacity is primarily attributed to high reactivities of hydroxyl substituents that participate in the reaction [8] as shown in fig (IV):

F-OH + R**.** F-O**.** + RH

Fig 1: Scavenging capacity of free radical (R.)

Flavonoids inhibit lipid peroxidation in vitro at an early stage by acting as scavengers of superoxide anion and hydroxyl radicals. They terminate chain radical reaction by donating hydrogen atom to a peroxy radical as in fig (V), thus, forming flavonoids radical, which, further reacts with free radicals thus terminating propagating chain [15, 6].

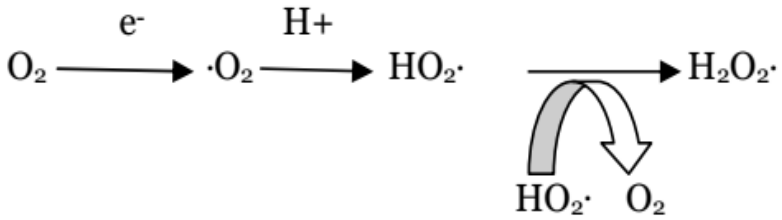


Fig 2: Formation of peroxy radical

Naturally, the organism has developed a defence against toxic substances such as peroxynitrite and nitrous acid. An important mechanism is catalyzed by the enzyme superoxide dismutase (SOD), which converts two superoxide anions to H2O2 and O2 [16] as shown in fig 2

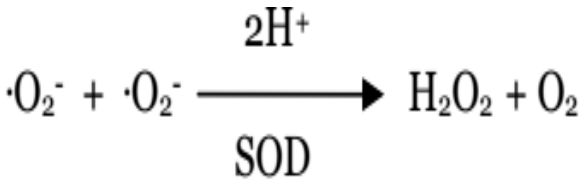


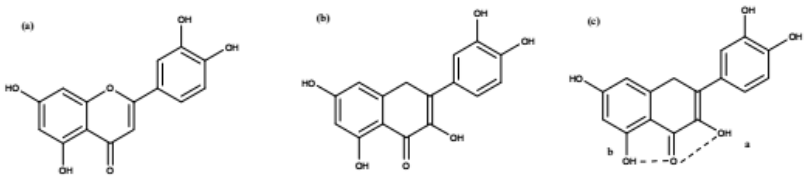
Fig 3: Mechanism catalysed by SOD

According to kinetic studies of aroxyl radical formation and decomposition reactions, the antioxidant capacity of a flavonoid is linked to its three structural groups as shown in fig (VII).

1.The ortho-dihydroxy (catechol) structure in the B-ring, which confers greater stability to aroxyl radicals, possibly through hydrogen bonding, and which participates in electron dislocation.

2. The 2,3-double bond, in conjugation with a 4-oxo function, responsible for electron dislocation from the B-ring.

3. The presence of both 3-(a)-and 5-(b)-hydroxyl groups **(Fig.4)**



Structural groups responsible for antioxidant activity

S. 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_2O_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_2O_2 is amplified in presence of heme proteins
4.	OH (Hydroxy radical)	Three electrons reduction products of O2 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.	$^1O_2$	Singlet Oxygen

Reactive oxygen species that can be scavenged or their formation can be inhibited by flavonoids[43]

3',4'-catechol structure in B-ring strongly enhances lipid peroxide inhibition and this arrangement is an important characteristic of most potent scavengers of peroxyl, superoxide and peroxynitrite radicals [8] and its

absence decreases antioxidant activity. The absence of the hydroxyl group at position 3 in flavanones and flavones decreases their antioxidant ability[1].

Ghasemzadeh et al., reported that high level of total phenolic and flavonoid in Halia Bara variety possessses potent antioxidant activities [44]. Bitis et al., isolated diosmetin, kaempherol, quercetin, kaempherol 3-glucoside (astragalin), quercetin 3-rhamnoside (quercitrin), quercetin 3-xyloside and quercetin 3-galactoside (hyperoside) from *Rosa agrestis* leaves and reported it to possess antioxidant activity [45].

Shariffer et al., reported antioxidant Shariffer et al., reported antioxidant activity of methanolic extract of *Teucrium polium* and rutin and apigenin were found to be potent inhibitors of lipid peroxidation and oxidation of beta-carotene [46].

Braca et al., isolated several flavonoids from the leaves of *Licania licaniaeflora* and reported quercetin derivatives to possess strongest antioxidant activity and flavonone 8-hydroxy-naringen and kaempferol 3-O-α-rhamnoside possesses lowest antioxidant activity.

Salucci et al., reported that dietary flavonoids like epicatechin, epigallocatechin, galate, gallic acid, quercetin-3-glucoside possess strong antioxidant activity [48].

Gulati et al., reported that quercetin at a dose of 15 mg/ 100g p.o. produced significant hepatoprotection [49].

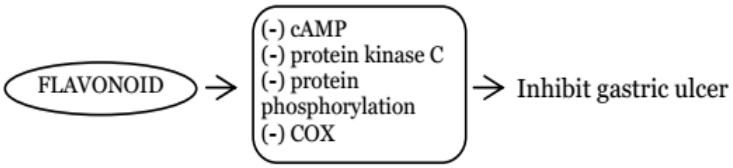
IV. Effect of flavonoids on gastric ulcer

Ulcer is a commonly occurring disease in developed countries and its occurrence is emerging with increase in modernisation of living standards.

Etiology of gastric ulcer: The stress hormones like epinephrine, norepinephrine, and ACTH, causes a vasoconstriction in the integument and the periphery, whereas, they dilate the vessels of muscles, heart, and brain. But, if the duration of this state gets prolonged, then blood supply to major organs, e.g., stomach, intestine, liver, kidneys, and skin get reduced and thus failing to satisfy demand for oxygen, antibodies and other agents that are required to maintain a healthy condition.

As a result, the stress hormones increases the glandular secretion which denatures proteins in plasma membranes and catalyses the hydrolysis of polysaccharide moieties of proteoglycans in the protective mucous coat covering the luminal surface of the stomach and the upper intestine to a perilous extent during prolonged stress. 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 in which eicosanoids, e.g., PGs, participate [16].

Flavonoids in treatment of gastric ulcer: Flavonoids inhibit regulation of protein phosphorylation. Inhibition of P-kinase signalling influence protein phosphatase which reverses the action of protein P-kinase as shown



Effect of flavonoids on gastric ulcer

Flavonoids are favourable, effective, and usually innocuous substitutes for the classical therapeutic agents. It has also been reported that flavonoids protect against gastric cancer. Similar to aspirin, acylated flavonoids may transfer their acyl group to the side chain hydroxyl group of serine in the active site of COX [16]. Flavonoids glycosides of *Ocimum basilicum* decreased ulcer index and thus inhibit gastric acids in aspirin-induced ulcers. Quercetin, Kaempferol, rutin when administered intraperitoneally (25-100 mg/kg) inhibited dose-dependent gastric damage produced by ethanol in rats [43].

Effect of flavonoids on inflammation:

Etiology: Inflammation is the integrated response of many defence systems of the body to the invasion of a foreign body. Inflammation involves action of the complement system, blood coagulation, humoral and cellular immunity, cytokines, tissue hormones, angiogenesis, and repair processes. It is both a free radical generating and free-radical producing process An important mechanism in inflammation is the recruitment of macrophages to participate in the battle against invasion by microorganisms or their toxins which are recognised by specific antibodies mounted on the surface of the macrophages in a receptor (FcR). Fc receptor is non-covalently associated with a second messenger producing enzymes i.e. phosphatidylinositol lipase which liberates inositol

phosphates and diacylglycerol (DAG) from the plasma membrane. Several inositol phosphates activate specific protein phosphokinases and DAG stimulates PKC. The toxin-antibody complexes are endocytised, and raise the alarm in the cell, with the result that the latter emits IL-1. This substance induces the expression of the COX gene, which encodes the PG COX that produces the eicosanoids, i.e., signal substances for the pain pathway (fig IX), chemotaxis, and smooth muscle contraction [16].

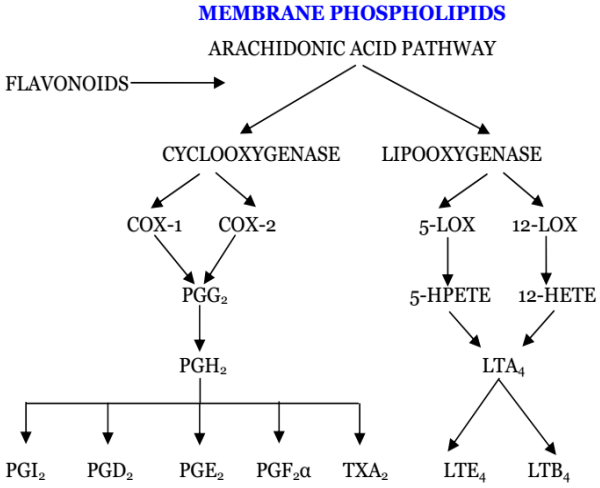
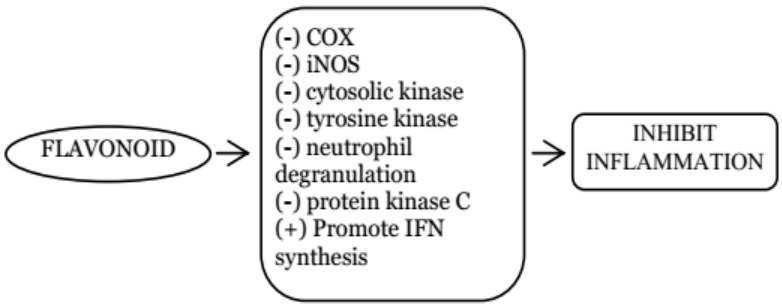


Fig IX: Arachidonic acid pathway [51]

Flavonoids in treatment of inflammation: Flavonoids have been found to be prominent inhibitors of COX or LOX [42, 45]. Flavonoids prevent synthesis of PGs that suppress T-cells. The immune cells communicate with chemical signals called cytokines which are controlled by flavonoids. Flavonoids inhibit the activity of PKC at ATP-binding site. Flavonoids also promote IFN synthesis [16].Various flavonoids (e.g., quercetin, apigenin, tea catechins) have also been shown to have anti-inflammatory activity by inhibiting cycloxygenase-2 (COX2) and inducible nitric oxide synthase [52], which is related to antioxidant activity [50]. Flavonoids also inhibit cytosolic and tyrosine kinase [40, 53] and also inhibit neutrophil degranulation [40]. The figure (X) represents the effect of flavonoids in the treatment of inflammation.

Effect of flavonoids on Inflammation



Citrus flavonoids, hesperidin is known to possess anti-inflammatory and analgesic effects. Apigenin, luteolin, quercetin are known to possess anti-inflammatory activity [43].
Guardia et al., reported quercetin and hesperedin given at a daily dose of 80 mg/kg inhibit both acute and phase of inflammation while rutin was found to be effective only in chronic case [54].
Kaempferol, quercetin, myricetin, fisetin are reported to possess COX and LOX inhibitory activities [43].

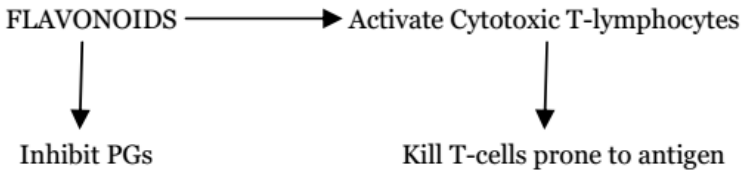
Effect of flavonoids on rheumatic disease:
Etiology of Rheumatic disease: Autoimmunity and inflammation is an important component of rheumatoid arithritis. Since rheumatic disease is associated with painful joints, it is believed that the antigen specificity of the T-cells is directed against an epitope characteristic of the cartilage in a joint, e.g., a part of chondroitin sulphate.

IL-1 is produced by macrophages. It acts as a growth hormone on T-lymphocytes, which are induced to proliferate and secrete IL-2, which, in turn, is a mitogen to T and B-lymphocytes, macrophages, granulocytes, and blood vessel endothelial cells. Osteoclasts resembles macrophages because both produce and secrete IL-1

and are phagocytic. If osteoclasts respond to IL-2 secreted from immune cells, then, the enhanced osteoclastic activity leads to the destruction of the heads of the long bones in the joints.

In rheumatic arithritis, such a destruction, such a destruction is followed by repair processes, which are uncoordinated with the mechanical requirements defined by the stress gradients. The topological activation pattern of the osteocytes leads to the formation of bony spikes in the head of the bone. These spikes cause tissue lesions, bleeding, inflammation, and pain [16].

Flavonoids in treatment of Rheumatic diseases: 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 unidentified [16] as shown in fig .



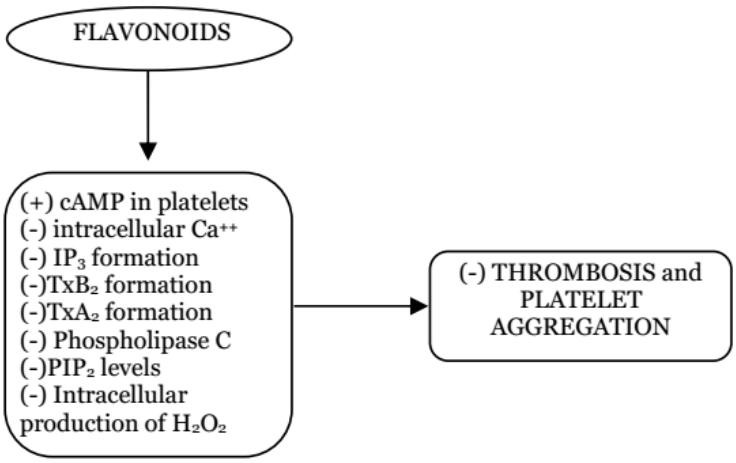
Effect of flavonoids in Rheumatoid arithritis

Kang et al., reported that apigenin inhibits autoantigen-presenting and stimulatory functions of APCs necessary for activation and expansion of autoreactive Th1 and Th17 cells and B cells and reported that it could suppress inflammation in rheumatoid arithritis [53].Guardia et al., reported rutin to be most effective plant flavonoids for the treatment of inflammation in chronic phase [54].

Flavonoids in treatment of thrombosis:

Etiology of Thrombosis: Arachidonic acid released by in inflammatory conditions is metabolized by platelets to form prostaglandin, endoperperoxides and thromboxane A2 thus contributing to platelet activation and aggregation. Platelet aggregation further contributes to atherosclerosis and acute platelet thrombus formation. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals which inhibit endothelial function of prostacyclin and nitric oxide.

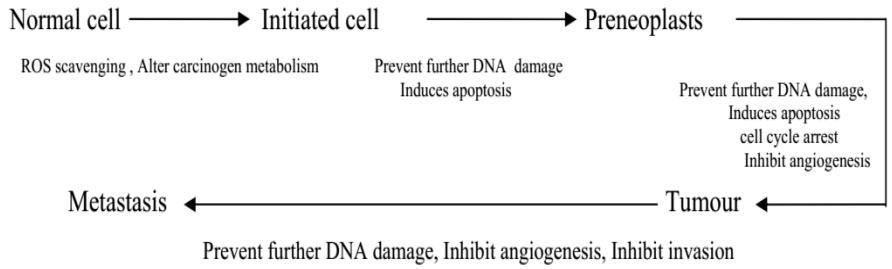
Flavonoids in treatment of thrombosis: Flavonoids are used as antithrombotic due to their ability to scavenge free radicals. They inhibit cyclooxygenase and lipooxygenase pathway. The main antiaggregatory effect is by the inhibition of thromboxane A2 formation. Flavonoids like quercetin, kaempferol and myricetin are known to possess antiaggregatory properties [40, 43]. The fig XII represents mechanism of flavonoid in thrombosis.



Effect of flavonoids on thrombosis

Effect of flavonoids on cancer-related pathways:

Etiology of Cancer: Cancer is a growth of diseases caused by disturbance in growth metabolism



Multistage of carcinogenesis and potential effects of polyphenols on cancer progression

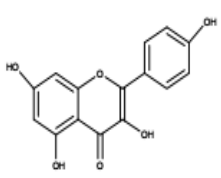
Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which are possibly involved in DNA damage and tumor promotion. Flavonoids may also have a beneficial effect through their impact on the bioactivation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

The flavonols quercetin (fig XV), kaempferol (fig XIV) and galangin, and the flavones apigenin (fig XVI) have been reported to inhibit cytochrome P450 enzymes of the CYP1A family. Cancer cells manifest to varying degree of uncontrolled proliferation, dedifferentiation and loss of function, invasiveness and metastasis that differ it from normal cells.

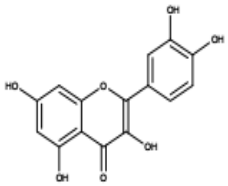
Flavonoids in treatment of Cancer: Flavonoids for a long time have been part of the herbal treatment by lay practitioners, but they were recognised only recently as effector substances. Examples of herbal preparations owing their growing recognition as effective anticancer drugs to flavonoids are propolis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

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 metastasis [55] as shown in fig (XIII).

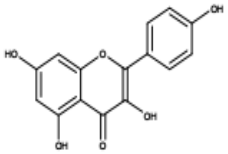
Flavonoids have emerged as potential chemopreventive candidates for cancer treatment due to their ability to induce apoptosis[55].



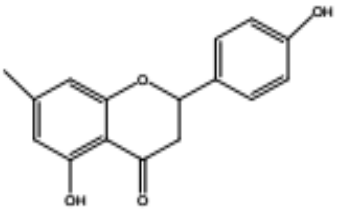
1. Kaempferol



2. Quercetin



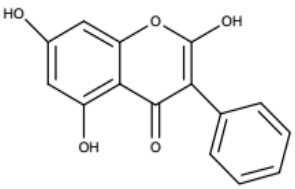
3, Apigenin



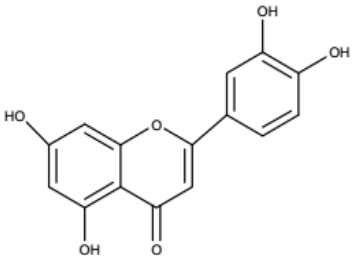
4.Naringin

Quercetin (fig XIV) and naringin (fig XVII) 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 [16]. 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 [55]. In addition, animal and 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 [16, 55]. In estrogen-dependent tumor cells or animal models, this anti-proliferative effect has been related to the antiestrogenic properties of certain flavonoids (e.g., isoflavonoids, quercetin). In other in vitro models, flavonoids have also been to affect cell-signaling and cell cycle progression. For example, tea flavonoids inhibit signal transduction pathways mediated by epidermal growth factor and platelet-derived growth factor, favorably affecting downstream events such as angiogenesis.

Genistein (fig XVIII) and quercetin inhibit protein tyrosine kinase which is also involved in cell proliferation [16, 55, 56]. Finally, apigenin, luteolin (fig XIX) and quercetin have been shown to cause cell cycle arrest and apoptosis by a p53-dependent mechanism.



1. Genistein



2. Luteolin

In a nutshell, multiple mechanisms have been identified for the anti-neoplastic effects of flavonoids, including antioxidant, anti-inflammatory and anti-proliferative activities, inhibition of bioactivating enzymes, and induction of detoxifying enzymes [52].

Ghasemzadeh et al., studies have shown that some flavonoids components such as quercetin had anticancer activities and were able to inhibit cancer cell growth.

Effect of flavonoids on memory:

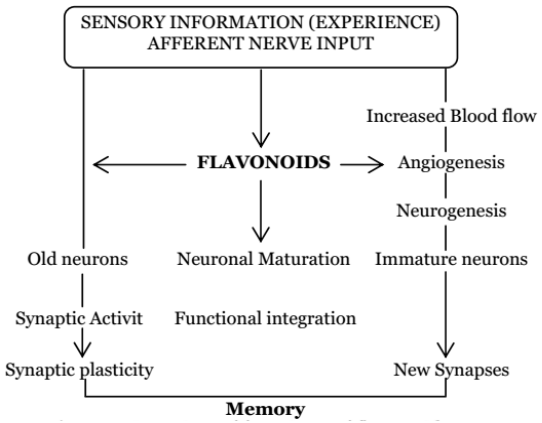
Flavonoids in treatment of memory cognition: Historically, the biological actions of flavonoids to their ability to exert antioxidant actions. However, it has been speculated that this classical hydrogen-donating antioxidant activity cannot account for the bioactivity of flavonoids in the brain as they are present in very low concentrations. Instead, it has been postulated that their effects in the brain is mediated by an ability to protect vulnerable neurons, enhance existing neuronal function, stimulate neuronal regeneration and induce neurogenesis.

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 memory. 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 stimulants [14]. 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. Researches revealed that isoflavones results in positive effects on neuro-cognitive functions which is apparent in post-menoupausal women. Activation of both synaptic plasticity and new neural growth may act together to enhance memory and cognition as shown in fig

It has been found that flavonoids subclasses flavonols, flavanols, flavanones, flavones and anthocyanins do not exert exert oestrogen like effects and thus cannot influence memory and cognition via similar mechanism.

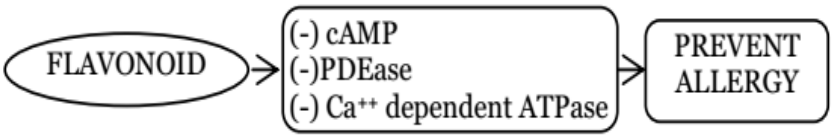
Jung et al., showed that quercetin at a dose of 10, 20, 40 mg/kg p.o. impairs cognitive function by suppressing pAkt and pCaMKII thus decreasing pCREB expression in hippocampus [49].

Maher et al., reported fisetin to facilitate memory by activating ERK and inducing cAMP response element-binding protein phosphorylation.



Effect of flavonoids on allergy:

Flavonoids in treatment of allergy: Flavonoids inhibit cyclic AMP phosphodiesterase and calcium-dependent ATPase which are responsible for histamine release from mast cells and basophils.

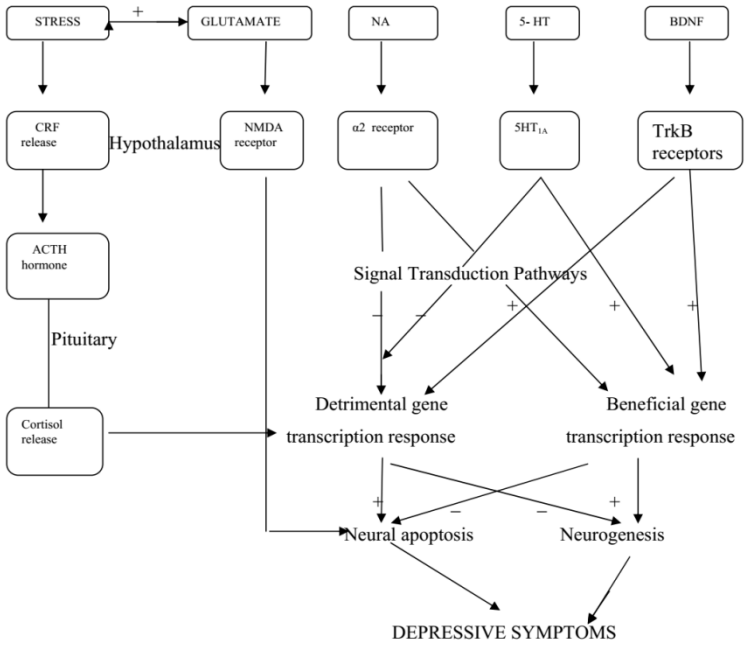


Effect of flavonoids on allergy

The fig depicts the mechanism of flavonoids in allergy. Quercetin prevents immune cells [59] and inhibits both the production and release of histamine and is useful in allergic conditions like asthma, hayfever.

Effect of flavonoids on depression:

Etiology of Depression: Depression is caused by functional deficiency of monoamine transmitters at certain sites in brain [51] as shown in fig.



Pathophysiology of depression

Flavonoids in treatment of depression:

Flavonoids have found to be ligands for GABAA receptors in the central nervous system and it led to hypothesis that they act as benzodiazepine-like molecules. Many flavone derivatives were found to be ligands for the GABAA receptors in the CNS; and thus they bind to the benzodiazepine binding site with resulting depressant actions in mice [7]. These were also found to possess sedative action, tranquilizers, anticonvulsant. Considering the sedative, the spontaneous locomotor activity and thiopental-induce sleeping time effects obtained with the flavonoid glycosides, the following decreasing order of action results:

2S-hesperidin>linarin>rutin>diosmin\cong2S-neohesperidin> gossypin≅2S-naringin.

Position of the sugar on the flavonoids nucleus seems relevant as well and position-7 is the most effective 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 CNS [7].

Yi et al., reviewed that dietary flavonoids possess multiple neuroprotective actions in Central nervous pathophysiological conditions including depression and it was reported that naringenin possess potent antidepressant-like property via central seotonergic and noradrenergic system. It was further suggested that dietary flavonoids possess a therapeutic potential in disorders especially where monoaminergic system is involved.

Effect of flavonoids on antimicrobial activity:

Flavonoids have been used extensively since centuries for the treatment of various diseases. Propolis has been used referred even in old testament for its healing properties. The antimicrobial activity of propolis has been attributed to its high flavonoids content. Galangin is a flavonol commonly found in propolis. It has been reported to possess inhibitory actions against Aspergillus tamarii, Aspergillus flavus, Cladosporium sphaerospermum, Pencillium digitatum, Penicillium italicum [10].

5,7,4'-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-2S-flavonone isolated from shrub Eysenhardtia texana and flavonoids 7-hydroxy-3',4'-(methylenedioxy) flavan isolated from Termanalia bellerica possess antifungal activity against Candida albicans. 6,7,4'-trihydroxy-3',5'-dimethoxyflavone and 5,5'-dihydroxy-8,2',4'-trimethoxyflavone are effective against Aspergillus flavus [10].

Nobiletin and langeritin isolated from peelings of tangerine orange showed fungistatic action towards Deuterophoma tracheiphila while hesperidin stimulate fungal growth slightly [43]. Quercetin, naringenin are reported to be inhibitors of Bacillus subtilis, Candida albicans, Escherichia coli, Staphylococcus nervous, Staphylococcus epidermis, Saccharomyces cerevisiae.

Rattanachaikunsopon et al., isolated morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin, quercetin-3-O-arabinoside from Psidium guajava leaves and reported that these four possess bacteriostatic action action against all foodborne pathogenic bacteria including Bacillus stearothermophilus, Brochothrix thermosphacta, Escherichia coli, Listeria monocytogenes, Pseudomonas fluorescens, Salmonella enteric, Staphyloccus aureus, Vibrio cholera/

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 [43].

Flavonoids in treatment of cardiovascular diseases:

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

Etiology of Cardiovascular Diseases: Atherosclerosis involves modification of LDL particles by oxidative stress with subsequent induction of inflammation which is caused by increased leucocyte adherence [16].

Endothelial dysfunction with increased platelet aggregation facilitates procoagulation, which may induce a thrombosis resulting in an acute myocardial infarction. In the ischemic phase of AMI platelet aggregation, the activation of neutrophils, an increase in cellular free redox active iron, and the transformation of xanthine dehydrogenase into ROS producing xanthine oxidase (XO) play important role [16].

Common consequences of AMI are heart failure and arrhythmias. Here again, ROS can mediate the cardiac hypertrophy and patients with heart failure have an increased production of ROS. Similarly, ROS, and in particular the superoxide radical, may play an important role in the genesis of some arrhythmias. Patients with arterial hypertension have an increased oxidative stress status [64].

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

Table 5: Proposed positive effects of flavonoids on CVS:

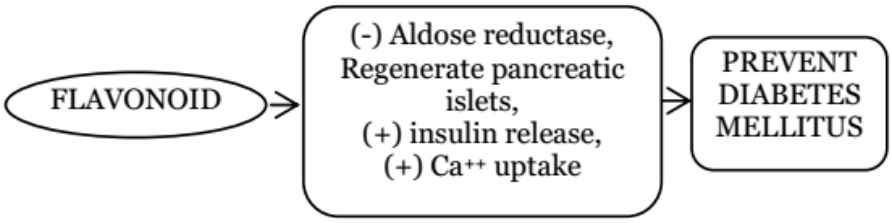
S. No.	Cardiovascular diseases	Influence of flavonoids
1.	Atherosclerosis	Decrease in LDL oxidation by LOX inhibition and attenuation of oxidative stress, inhibition of leucocyte leucocyte adhesion, myeloperoxidase, decreased expression of iNOS and COX-2
2.	Acute myocardial infarction	Decrease in ROS burst, inhibition of platelet aggregation
3.	Heart Failure	Decrease in oxidative stress (direct ROS scavenging) inhibition of metalloproteinase
4.	Arrhythmia	Decrease in oxidative stress
5.	Hypertension	Vasodilatory properties, inhibition of NADPH oxidase, recovery of NO due to inhibition of superoxide production

Flavonoid consumption prevent many cardiovascular diseases including hypertension and atherosclerosis. Quercetin protects LDL against oxidative modifications effect. 7-monohydroxyethylrutoside and 7', 3', 4'-trihydroxyethylrutoside are reported to be cardioprotective [43].

Effect of flavonoids on diabetes mellitus:

Etiology of diabetes mellitus: 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 patients [65].

Flavonoids in treatment of diabetes mellitus: All 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 mellitus [16]. Flavonoids have been identified to be good inhibitors of aldose reductase [66]. The fig depicts the mechanism of flavonoids in diabetes mellitus.



Effect of flavonoids on diabetes mellitus

It has been reported by several researchers 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 Ca2+ uptake from isolated islet cells thus suggesting it to be effective even in non-insulin dependent diabetes

Li et al., indicated that flavonoids in *Ipmoea batalas* leaf possesses antidiabetic activity against alloxan-induced diabetes at a dose of 100 mg/kg .

Sriram et al., reported fisetin to be a therapeutic agent for treatment of diabetes mellitus at a dose of 10 mg/kg .

Effect of flavonoids in treatment of hepatotoxicity:

Role of flavonoids in treatment of hepatotoxicity: Flavonoids bind to subunit of DNA-dependent RNA polymerase I, thus activating the enzyme. As a result, protein synthesis gets increased leading to regeneration and production of hepatocytes [11].

Silymarin, apigenin, quercetin, naringenin are reported to be potent therapeutic agents against microcrystin LR-induced hepatotoxicity. Rutin and venoruton are reported to show regeneration and hepatoprotective effects in experimental cirrhosis [43].

Gulati et al., studied hepatoprotective studies on *Phyllanthus emblica* and quercetin and quercetin and found that if the extract is producing hepatoprotection at a dose of 100 mg/ 100 g p.o., then quercetin is producing

hepatoprotection at a dose of 15 mg/ 100 g p.o.; thus concluding that quercetin is a potent hepatoprotective agent.

Kim et al., isolated isovitexin, hirustin, trifolin, avicularin and quercetin. It was observed that hirustrin, avicularin, quercetin possess hepatoprotective action against t-BHP in HepG2 cells, whereas isovitexin and trifolin possess no protective effect.

Oh et al., 2004 reported that among various flavonoids i.e. apigenin, luteolin, kaempherol-3-O-glucoside and quercetin-3-O-glucoside isolated from *Equisetum arvense*, onitin and luteolin exhibited hepatoprotective activity against tacrine-induced cytotoxicity in human liver-derived Hep G2 cells.

Toxicological Profile of flavonoids:

Flavonoids are widely distributed in edible plants and beverages and have been previously used in traditional medicines, so they are believed to be non-toxic [10, 15]. However, this family of compounds possess a diverse range of activities in mammalian cells. So, in-vivo confirmation of their side effects would be necessary for full evaluation of their practical usefulness in the field of modern medicine. Given that the selectivity of flavonoids for eukaryotic enzymes appears to vary from compound to compound, a study regarding assessment of its toxicity is required to be done on these phytochemicals on an individual basis [10]. Flavonoids are found to be toxic to cancer or immortalized cells but are less toxic to normal cells [40]. Due to the low solubility of flavonoid aglycones in water, to the short residence time of flavonoids in the intestine, and to the low coefficient of absorption, it is not possible for humans to suffer acute toxic effects from the consumption of flavonoids, with the exception of a rare occurrence of allergy. The margin of safety for the therapeutic use of flavonoids in humans, therefore, is very large and probably not surpassed by any other drug in current use [45].

V. Conclusion

Flavonoids constitute a wide array of biological active compounds that are found abundantly in plant kingdom and dietary intake. They are gaining interest due to their wide variants and number of members. These are reported to be effective in pathogenesis of majority of disases. Antioxidant activity is the foundation of many actions which lead to its beneficial effects in majority of the diseases.

References

[1]. Tripoli, E, Guardia, ML, Giammanco, S, Majo, DD, Giammanco, M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chemistry 2007; 104: 466-479.

[2]. Slade, D, Ferreira, D, Marais, JPJ. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. Phytochemistry 2005. 66; 2177 2215.

[3]. Hotta, H, Nagano, S, Ueda, M, Tsujino, Y, Koyama, J, Osakai, T. Higher radical scavenging activity of polyphenolic antioxidants ascribed to chemical reactions following oxidation. Biochimica et Biophysica Acta 2002; 1572: 123-132.

[4]. Markham, K.R., Techniques of Flavonoid Identification, Academic Press, London, 1982.

[5]. Mabry, T.J., Markham, K.R., and Thomas, M.B., The Systematic Identification of Flavonoids, Springer-Verlag, New York, 1970.

[6]. Harborne, J.B., Mabry, T.J., and Mabry, H., Eds., The Flavonoids, Chapman & Hall, London, 1975.

[7]. Bohm, B.A., Introduction to Flavonoids, Harwood Academic Publisher, Singapore, 1998.

[8]. Waterman, P.G. and Mole, S., Analysis of Phenolic Plant Metabolites, Blackwell, Oxford, 1994.

[9]. Peterson, J, Dwyer, MSJ, RD, DSc. Flavonoids: Dietary occurrence and biochemical activity. Nutrition Research 1998; 18: 1995-2018.

[10]. Niranjan, A, Tewari, SK, Lehri, A. Biological activities of Kalmegh (*Andrographis paniculata*) and its active principles: A Review. Indian Journal of Natural Products and Resources 2010; 1: 125-135.

[11]. Haslam, E. *Chemistry and Pharmacology of Natural Products. Plant Polyphenols: Vegetable Tannins Revisited*; Cambridge University Press: Sydney, Australia, 1989.

[12]. Corder, R.; Mullen, W.; Khan, N.Q.; Marx, S.C.; Woods, E.G.; Carrier, M.J.; Crozier, A.Oenology: Red Wine Procyanidins and Vascular Health. *Nature* **2006**, *444*, 566.

[13]. Taylor, L.P.; Grotewold, E. Flavonoids as Developmental Regulators. *Curr. Opin. Plant Biol.* **2005**, *8*, 317-323.

[14]. *Santos-Buelga, C. and Williamson, G., Eds., Methods in Polyphenol Analysis, Royal Society of Chemistry, Cambridge, 2003.*

[15]. *Markham, K.R. and Geiger, H., 1H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide, in The Flavonoids: Advances in Research Since1986, Harborne, J.B., Ed., Chapman & Hall, London, 441, 1993.*

[16]. *Markham, K.R. and Chari, V.M., Carbon-13 NMR spectroscopy of flavonoids, in The Flavonoids:*

[17]. *Advances in Research, Harborne, J.B. and Mabry, T.J., Eds., Chapman & Hall, London, 19, 1982.*

[18]. Exarchou, V. et al., LC–UV–solid-phase extraction–NMR–MS combined with a cryogenic flow probe and its application to the identification of compounds present in Greek oregano, Anal Chem., 75, 6288, 2003.

[19]. Sannomiya, M., Fonseca, VB, Silva, MAD, Rocha, LRM, Santos LCD, Hiruma-Lima, CA, Brito, ARMS, Vilegas, W. Flavonoid and antiulcerogenic activity from *Brysonima crassa* leaves extract. Journal of Ethnopharmacology 2005; 97: 1-6.

[20]. Pal, RS, Ariharasivakumar, G, Girhepunjhe, K, Upadhay, A. In-vitro antioxidative activity of phenolic and flavonoids compounds extracted from seeds of *Abrus precatorius*. International Journal of Pharmacy and Pharmaceutical Sciences 2009; 1: 136-140.

[21]. Chebil, L, Humeau, C, Falcimaigne, A, Engasser, J, Ghoul, M. Enzymatic acylation of flavonoids. Process Biochemistry 2006; 41: 2237-2251.

[22]. De P. Emerenciano, V. et al., Application of artificial intelligence in organic chemistry. Part XIX. Pattern recognition and structural determination of flavonoids using 13C-NMR spectra, Spectroscopy,13, 181, 1997.

[23]. Garcia-Martinez, C. et al., Further nuclear magnetic resonance studies of flavone, Spectroscopy, 12, 85, 1994.

[24]. Pal, RS, Ariharasivakumar, G, Girhepunjhe, K, Upadhay, A. In-vitro antioxidative activity of phenolic and flavonoids compounds extracted from seeds of *Abrus precatorius.* International Journal of Pharmacy and Pharmaceutical Sciences 2009; 1: 136-140.

[25]. *Shirwaikar A, Shirwaikar A, Rajendran K, Punitha ISR: In vitro antioxidant studies on the benzyl tetra tsoquinoline alkaloid berberine. Biol Pharm Bull. 2006, 29: 19061910 10.1248/bpb.29.1906.*

[26]. *Cragg GM, Newman DJ, Weiss RB: Coral reefs, forests, and thermal vents: the worldwide exploration of nature for novel antitumor agents. Semin Oncol. 1997, 24: 156163.*

[27]. *Gebick L, Banasiak E: Role of medicinal in oxidative stress. Acta Biochim Pol. 2009, 56: 509513.*

[28]. Aksnes, D.W., Standnes, A., and Andersen, Ø.M., Complete assignment of the 1H and 13C NMR spectra of flavone and its A-ring hydroxyl derivatives, Magn. Reson. Chem., 34, 820, 1996.

[29]. Nilsson, M. et al., high-resolution NMR and diffusion-ordered spectroscopy of Port wine, J. Agric. Food Chem., 52, 3736, 2004.

[30]. Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol 1998;439:175–82.

[31]. de Groot H. Reactive oxygen species in tissue injury. Hepatogastroenterology 1994;41:328–32.

[32]. Grace PA. Ischaemia-reperfusion injury. Br J Surg 1994;81:637–47.

[33]. Korkina LG, Afanas'ev IB. Antioxidant and chelating properties of flavonoids. Adv Pharmacol 1997;38:151–63.

[34]. Miller, AL. Antioxidant flavonoids: Structure, function and Clinical Usage. Alternative Medicine Review 1996; 1: 103-111.

[35]. Zhou, T, Luo, D, Li, X, Luo, Y. Hypoglycaemic and hypolipidemic effects of flavonoids from lotus (*Nelumbo nuficera*) leaf in diabetic mice. Journal of medicinal Plants Research 2009; 3: 290-293.

[36]. Friesenecker B, Tsai AG, Allegra C, Intaglietta M. Oral administration of purified micronized flavonoid fraction suppresses leukocyte adhesion in ischemia-reperfusion injury: in vivo observations in the hamster skin fold. Int J Microcirc Clin Exp 1994;14:50–5.

[37]. Ross, J. A.; Kasum, C. M. Dietary Flavonoids: Bioavailability, Metabolic Effects, and Safety. *Annu. Rev. Nutri.* **2002,** *22*,19–34.

[38]. Bravo, L. Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. *Nutri. Rev.* **1998,** *56*, 317– 333.

[39]. Li, F, Li, Q, Gao, D, Peng, Y. The optimal extraction parameters and anti-diabetic activity of flavonoids from *Ipmoea batatas* leaf. African Journal of Traditional, Complementary and Alternative Medicines 2009; 6: 195-202.

[40]. Kim, SM, Kang, K, Jho, EH, Jung, Y, Nho, CW, Um, B, Pan, C. Hepatoprotective effect of flavonoids glycoside from *Lespedeza cuneata* against oxidative stress induced by tert-Butyl hyperoxide. Phytotherapy Research 2011.

[41]. http://www.umm.edu/altmed/articles/quercetin-000322.htm .

[42]. Maher, P, Akalshi, T, Abe, K. Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory. Proceedings of National academy of Sciences United States of America 2006; 103: 16568-16573.

[43]. Lamson, DW, Brignall, MS. Antioxidants and Cancer III: Quercetin. Alternative Medicine Review 2000; 5: 196-208.

[44]. Murray, MT. Quercetin: Nature's antihistamine. Better Nutrition 1998. Schels, H.; Zinsmeister, H. D.; Pfleger, K. Mass Spectrometry of Silylated Flavonol O-Glycosides. *Phytochemistry* **1977,** *16*,1019–1023.

[45]. Prey, JO, Brown, J, Fleming, J, Harrison, PR. Effect of dietary flavonoids on major signal transduction pathways in human epithelial cells. Biochemical Pharmacology 2003; 66: 2075-2088.