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Recent Updates on Free Radicals Scavenging Flavonoids: An Overview

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Abstract: Flavonoids are low molecular weight, polyphenolic compounds present in majority of vascular plants, possessing many therapeutic activities vis a vis antioxidant activity. The present review discuss the chemical nature, mechanism of action, current status, pharmacodynamic/pharmacokinetic studies, industrial significance, nutritive value in health system and analysis of flavonoids with the recent technology.

Key words: Flavonoids, chemical nature, dietary antioxidants

INTRODUCTION

Reactive Oxygen Species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions or from exogenous factors (Cerutti, 1991). These ROS may be very damaging and attack lipids in cell membranes and also attack DNA, inducing oxidation that causes membrane damage such as membrane lipid peroxidation (Cerutti, 1994; Pietta, 2000; Kumar and Sharma, 2006). Lipid peroxidation has been implicated in the pathogenesis of a number of diseases like arthritis (Naik, 2003), diabetes (Yagi, 1987), cancer (Rekha *et al.*, 2001), atherosclerosis (Tiwari, 2001), neurodegenerative diseases (Thomas and Kalyanaraman, 1997), etc. Definitely, many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention onto the naturally occurring antioxidants (Gupta and Sharma, 2010a,b; Kumar and Sharma, 2006). Flavonoids and their synthetic analogues have been intensely investigated and found the prominent role in the treatment of ovarian, breast, cervical, pancreatic and prostate cancer, in recent years. Their use has mainly centred on prevention and the maintenance of health (Aruoma and Cuppet, 1997). The recognized dietary antioxidants are vitamin C, vitamin E, selenium, carotenoids (beta carotene), etc. However, recent studies have demonstrated that flavonoids found in fruits and vegetables may also act as antioxidants. Like alpha-tocopherol (vitamin E), flavonoids contain chemical structural element that may be responsible for their antioxidant activities (Di Carlo *et al.*, 1999). Flavonoids generally occur in plants as glycosylated derivatives and impart different color shades (blue, scarlet and orange in

leaves, flowers and fruits (Brouillard and Cheminat, 1988). Flavonoids are major components of citrus fruits and several other medicinal plants and have been used in traditional medicine around the world (Winston, 1999; Di Carlo *et al.*, 1999; Kadarian *et al.*, 2002; Pascual *et al.*, 2001; Samuelsen, 2000). Many families have been reported to have isoflavonoids in addition to Leguminosae. The spectrum of isoflavonoid producing taxa includes the representatives of four classes of multicellular plants, namely the Bryopsida, the Pinopsida, the Magnoliopsida and the Liliopsida. Isoflavonoids in non-leguminous families provided listing of 164 isoflavonoids altogether reported in 31 non-leguminous angiosperm families (Mackova *et al.*, 2006).

CHEMICAL NATURE OF FLAVONOIDS

Flavonoids are polyphenolic compounds are ubiquitous in nature and categorized into many classes according to their chemical structure. Over 4000 flavonoids have been identified, many of which occur in the fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks) (Aruoma and Cuppet, 1997). The flavones apigenin (3b) and luteolin (3a) are common in cereals grains and in aromatic herbs viz., rosemary, thyme, parsley etc. (Pietta *et al.*, 1995). The flavonols quercetin (4b) and kaempferol (4c) are usually present in vegetables and fruits. Flavonoids are formed in the plants from the aromatic amino acids phenylalanine and tyrosine and malonate. Isoflavones are found mostly in legumes (soybeans, black beans, green beans and chick peas) (Herman, 1976). Flavan oligomers (proanthocyanidins) are found in apples, grapes, berries, barley grains etc. (Franke *et al.*, 1994). Anthocyanidins and their glycosides

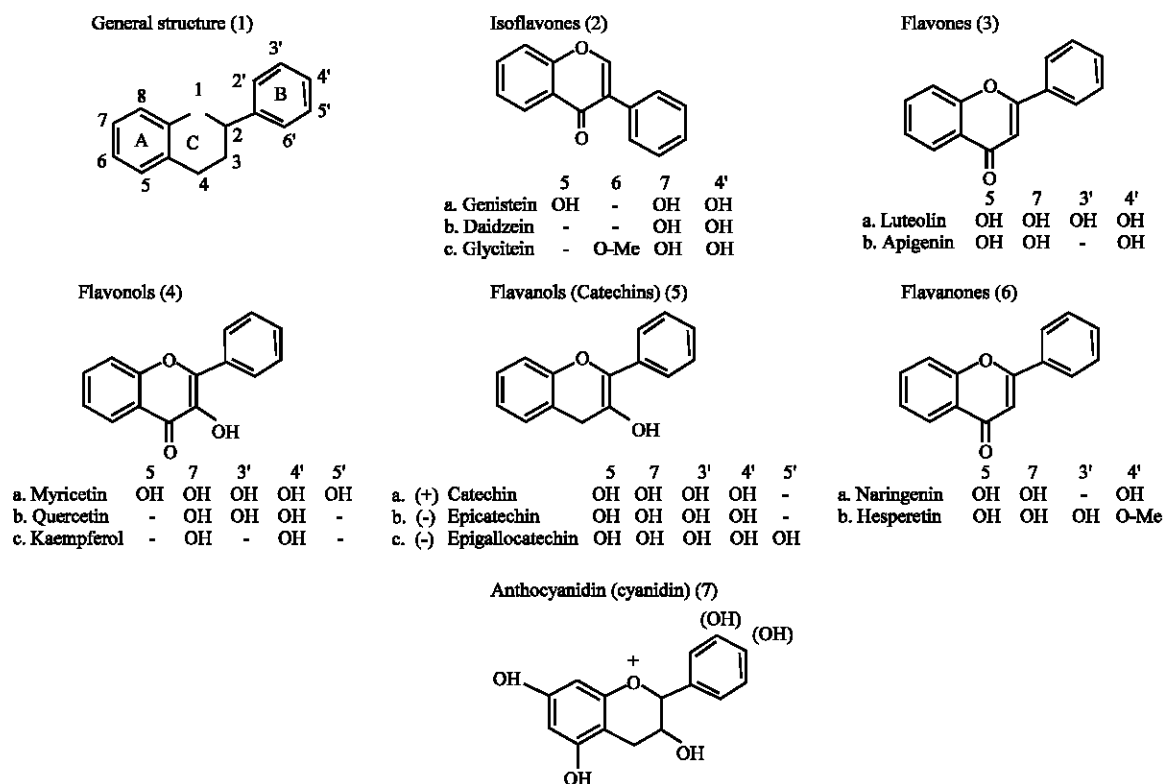


Fig. 1: Chemical structures of the major classes of flavonoids

Table 1: Major food sources of flavonoids

Sr. No.	Flavonoid subclass	Major food sources
1.	Flavonols	Onions, broccoli, apples, cherries, berries, tea, red wine
2.	Flavones	Parsley, thymus
3.	Flavanones	Citrus
4.	Catechins	Apples, tea
5.	Anthocyanidins	Cherries, grapes
6.	Isoflavones	Soya beans, legumes

(anthocyanins) are abundant in berries and red grape (Haslam, 1989). Some major food sources (Hollman and Katan, 1999) are given in Table 1.

The basic structural unit of the flavonoid family comprises two benzene rings (A and B) (1) as shown in Fig. 1 linked through a heterocyclic pyran or pyrone ring (C), variation in the C ring and hydroxylation pattern on the A and B rings define the major classes (Cook and Samman, 1996) including Isoflavones (genistein) (2a), Flavones (luteolin) (3a), Flavonols (quercetin) (4b), Flavan-3-ols (epicatechin) (5b), Flavanones (naringenin) (6a), Anthocyanidins (cyanidin) (7). The *in vitro* anticancer assay with synthetic compounds of structurally related subcategories of flavonoids (*viz.*, flavones, isoflavones, xanthenes) indicated the maximum activity with xanthenes and least with isoflavones, however, flavones exhibited more significant activity than

isoflavones and less significant when compared with xanthenes (Wang *et al.*, 2005).

DIETARY AND INDUSTRIAL SIGNIFICANCE OF FLAVONOIDS

The flavonoids exert potential beneficial effects on health (Table 2), extensively employed in the various formulations in the industry and may also be obtained from the diet (dietary flavonoids). In addition to outstanding anti-oxidant activity, flavonoids possess a profound inhibitor action on the formation of lipid peroxides both *in vitro* (Carini *et al.*, 1992; Villa *et al.*, 1992) as well as *in vivo* (Chen *et al.*, 1990; Cholbi *et al.*, 1991; Uchida *et al.*, 1988). However, the range of dietary flavonoids varies from low content (<1 mg/100 g) to high content (5-35 mg/100 g) depending upon the biological source (Table 3). Quercetin, kaempferol, myricetin, luteolin, apigenin are some important examples of flavonoids, present in the dietary sources (Hertog *et al.*, 1992, 1993). In addition to these dietary sources, there are a number of plants, which are not the part of diet, used in therapeutics, but having appreciable flavonoidal content like beverages such as wine (red wine), tea, beer etc. (Larson, 1988). Flavonoids are present

Table 2: Flavonoids showing activities other than antioxidant activity

Activity	References
Cardioprotective activity	Huesken <i>et al.</i> (1995)
Lipid lowering activity	Igarashi and Ohmura (1995)
Antiulcer activity	Alarcon <i>et al.</i> (1994), Parmar and Parmar (1998)
Hepatoprotective activity	Lorenz <i>et al.</i> (1973)
Anti-inflammatory activity	Phyllis and James (2000)
Antineoplastic activity	Spedding <i>et al.</i> (1989)
Treatment of capillary fragility and phlebosclerosis	Chaumontet <i>et al.</i> (1996), Felicia <i>et al.</i> (1996), Boege <i>et al.</i> (1996), Paul <i>et al.</i> (1997)
Antibacterial	Wild and Fasel (1969)
Antifungal	Tencate <i>et al.</i> (1973)
Antiviral	Loewenstein (1979)
Antiallergy	Verma and Kinoshita (1976)

Table 3: Dietary sources of flavonoids

High (5-35 mg/100 g)	Medium (1-4 mg/100 g)	Low (<1 mg/100 g)
Onion, kale, celery, broccoli, French bean, broad bean	Apple, black tea, leek, apricot, lettuce, cherry, redcurrant, red capsicum, plum, tomato	Cabbage, white cabbage, cauliflower, mushroom, pea, spinach, beetroot, cucumber, peach, carrot, citrus juices, coffee, white wine

Table 4: Description of some important flavonoidal drugs

Common name	Botanical name and family	Useful part	Flavonoid	Uses
Maidenhair Tree	<i>Ginkgo biloba</i> Linn. (Ginkgoaceae)	Leaf	Quercetin, kaempferol-3-rhamnosides and 3-rutinoides	In chronic arterial diseases, Raynaud's disease, in senile cerebral insufficiency, vertigo, tinnitus, hearing loss, piles, etc.
Passion Flower	<i>Passiflora incarnata</i> Linn. (Passifloraceae)	Aerial parts	Flavone di-C-glucoside, shaftoside, isoshaftoside, vitexin, isovitexin, orientin, iso-orientin, saponarin	Sedative, antispasmodic, tranquilizers and in cardiac rhythm abnormalities
Thyme	<i>Thymus vulgaris</i> Linn. and <i>Thymus zygis</i> (Lamiaceae)	Leaf and flower	5,4'-Dihydroxy-6,7,3'-trimethoxyflavone, 5,6,4'-trihydroxy-7,8,3'-trime-, toxyflavone, 5,4'-Dihydroxy-6,7-dimethoxyflavone	Antibacterial, antifungal, gastrointestinal disturbances and in cough
Roman camomile	<i>Chamaemelum nobile</i> (Linn.) All. (Asteraceae)	Leaf	Apigenin and luteolin glucosides	In gastrointestinal disorders, spasmodic colitis pain and skin disorders
Yarrow	<i>Achillea millefolium</i> Linn. (Asteraceae)	Flowering tops	Flavones and 6-methoxylated or di and trimethylated flavonols	In gastrointestinal disorders, skin disorders, anti inflammatory and antispasmodic
Field horsetail	<i>Equisetum arvense</i> Linn. (Equisetaceae)	Aerial parts	Flavone-5-glucosides and their malonic esters	Diuretic, in cosmetology, to enhance renal excretion of water, in bone fragility and cramps

in a number of drugs/plants, they may occur in any part of the plant but, generally found in more concentration in leaves or flowers (Table 4). Rutin, a flavonoid, bearing pronounced therapeutic activity, widely used in the industry. It is well documented the plants *viz.* *Saphora japonica* L. (Fabaceae), *Fagopyrum esculentum* Moench. (Polygonaceae), *Eucalyptus macrorrhynca* F. Muell. (Myrtaceae) are been used at large in industry for extraction of rutin (Bruneton, 1995). Flavonoids, tannins and/or polyphenolic compounds found in some *Ficus* spp. also showed antioxidant or free radicals scavenging activity (Sharma and Gupta, 2007a, b, 2008).

PHARMACOKINETIC/PHARMACODYNAMIC STUDIES OF FLAVONOIDS

It has been proved that flavonoids from dietary sources exert significant antioxidant effect. It is believed that flavonol, flavone and isoflavone glycosides are initially hydrolyzed to their respective aglycones

Table 5: Flavonoidal content of tea

Flavonoid	Green tea (Fresh leaf tea) (%)	Black tea (%)
Epi-gallocatechin gallate	9-13	4.6
Epi-catechin gallate	3-6	3.9
Epi-gallocatechin	3-6	1.1
Epi-catechin	1-3	1.2
Flavonols	3-4	Trace

(Manach *et al.*, 1996; Nielsen *et al.*, 1997). The glycoside quercetin-3-rutinoside was detected in the blood (after consumption of tomato puree) (Mauri *et al.*, 1999), naringin (4', 5, 7-trihydroxyflavanone-7-rhamnoglucoside) in urine (after taking naringin orally) (Ishii *et al.*, 2000), epigallocatechin gallate and epicatechin gallate detected in human blood after intake of green tea (green tea has more flavonoidal content as compare to black tea (Anonymous, 1991) (Table 5), decaffeinated green tea extracts and dark chocolates (Michelle *et al.*, 1999; Nakagawa and Miyazawa, 1997; Unno *et al.*, 1996). So, all these facts support that glycosides are absorbable.

Absorption of flavonoids (flavonols, flavones, isoflavones and catechins) in the human body takes place

in two ways; first, a small portion of it transformed into their glucouronides and sulfates (King and Brusill, 1998). This small fraction of the absorbed flavonoids is metabolized by the liver enzymes, resulting in more polar conjugates being excreted in the urine or returned to the duodenum via gall bladder. However, the major part of the ingested flavonoids is not absorbed and is largely degraded by the intestinal microflora. The bacterial enzymes catalyze several reactions including hydrolysis, dehydrogenation, cleavage of the heterocyclic oxygen containing ring, decarboxylation etc. In this way several phenolic acids are produced (Pietta *et al.*, 1997). These phenolic acids can be reabsorbed and account a large fraction of the ingested flavonoids (30-60%). Phenolic acids bearing catechol structure possess a radical scavenging ability comparable to that of their intact precursors (Merfort *et al.*, 1996). Further, TEAC values of these metabolites confirm their antioxidant potential (Pietta *et al.*, 2000).

Flavonoids/polyphenolic rich substances of natural origin will always exert beneficial therapeutic effects, is not true all times. The most suitable example is tree nuts, a rich source of tocopherols, total phenols, containing wide variety of flavonoids and proanthocyanidins, has not been reported significant antioxidant *in vivo* (Bolling *et al.*, 2010). Absorption is the other important aspect, which could not be neglected as there are many flavonoids which are poorly absorbed and could not justify their therapeutic potential. So, this area needs to be explored further. However, The clinical applicabilities of polyphenols and other poorly absorbed plant medicines can be improved by phytosome technology which creates intermolecular bonding between individual polyphenol molecules and one or more molecules of the phospholipids, phosphatidylcholine (Kidd, 2009). Research based on strategies to determine phenolic acids and flavonoids in biological fluids, beverages, plant and food exudates may explore the applications in a better way, which is need of the day.

MECHANISM OF ACTION

The free radical scavenging activity of flavonols, flavones and anthocyanins have been reported through various *in vitro* models (Afanasev *et al.*, 1989; Cui *et al.*, 2002; Dobask *et al.*, 1999; Duthie and Doboson, 1999; Formica and Regelson, 1995; Kerr *et al.*, 1999; Mahesh and Menon, 2004; Pataki *et al.*, 2002; Pietri *et al.*, 1997; Yamashiro *et al.*, 2003). Flavonoids act as antioxidant due to having more number of target sites for free radicals in the oligomeric compounds produced from their

semiquinone radicals (Rohdewald, 2002; Bors and Michel, 1999; Bors *et al.*, 2000; Robak and Gryglewski, 1988). Chemically, flavonoids are single electron donors. In *in vitro* cell culture, flavonoids have good antioxidant potential as they serve as derivative of conjugated ring structures and hydroxyl groups. They act as antioxidant by scavenging superoxide anion (Husain *et al.*, 1987), singlet oxygen (Wang and Goodman, 1999) and lipid peroxy radicals (Fuchs *et al.*, 1989; Lotito and Frei, 2004).

In addition to their free radical scavenging activity, flavonoids enhance intracellular antioxidant defense against free radicals by increasing production of antioxidative enzymes (Bayeta and Lau, 2000; Kandaswami and Middleton, 1994; Lewis, 1993; Wei *et al.*, 1997). Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase (Hanasaki *et al.*, 1994) and protein kinase C (Ursini *et al.*, 1994). Flavonoids have also been shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, all involved in reactive oxygen species generation (Brown *et al.*, 1998; Korkina and Afanasev, 1997).

Due to their lower redox potentials (Jovanoic *et al.*, 1994) flavonoids (FI-OH) are thermodynamically able to reduce highly oxidizing free radicals with redox potentials in the range 2.13-1.0 V (Buettner, 1993), such as superoxide, peroxy, alkoxy and hydroxyl radicals by hydrogen atom donation:



where, R[•] represents superoxide anion, peroxy, alkoxy and hydroxyl radicals (Husain *et al.*, 1987; Robak and Gryglewski, 1988; Terol *et al.*, 1986). The aroxy radical (FI-O[•]) may react with second radical, acquiring a stable quinone structure (Fig. 2).

The aroxy radicals could interact with oxygen, generating quinines and superoxide anion, rather than terminating chain reactions. The last reaction may take place in the presence of high levels of transient metal ions and is responsible for the undesired prooxidant effect of flavonoids (McCord, 1995). So, it shows the flavonoids to act as antioxidants depends not only on the redox potential of the couple FI-O[•]/FI-OH but also on possible side reactions of the aroxy radical. Scavenging of superoxide is particularly important, because the radical is ubiquitous in aerobic cells and, despite its mild activity, is a potential precursor of the hydroxyl radical in the Fenton and Haber-Weiss reactions (Cao *et al.*, 1997).

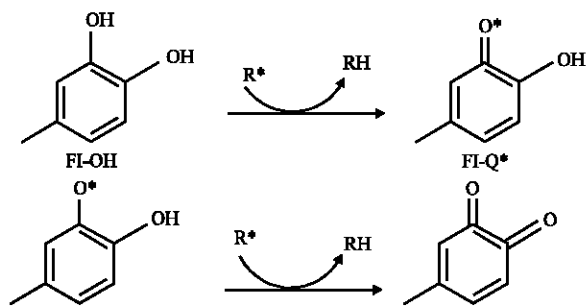


Fig. 2: Scavenging of ROS (R*) by flavonoids

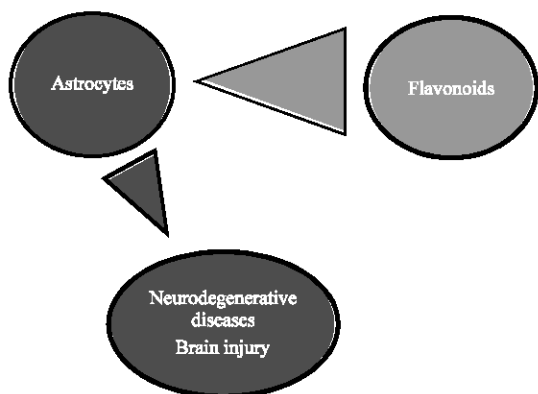


Fig. 3: Flavonoids lead to brain development by targeting astrocytes

Flavonoids present in diet are natural antioxidants and possess the potential to stabilize various radicals (oxygen-centered, carbon-centered, alkoxy peroxy, or phenoxy radicals) and ROS involved in oxidative processes through hydrogenation or complexing with oxidizing species (Nones *et al.*, 2010; Shahidi and Wanasundara, 1992).

Some scientists strongly believe that the physiological benefits of flavonoids is not due to their antioxidant and free radical scavenging effects rather to their capability to target to astrocytes especially in brain development, as astrocytes are pivotal characters in neurodegenerative diseases and brain injury (Fig. 3) (Hackl *et al.*, 2002).

ANALYSIS OF FLAVONOIDS

There are many reports that plant-derived phenolic compounds such as flavonoids have antioxidant properties capable of reducing the risk of developing age related diseases such as atherosclerosis, Alzheimer and osteoarthritis. Many herbal formulation have been prepared and therapeutic effects and flavonoidal content was successfully analyzed through thin-layer

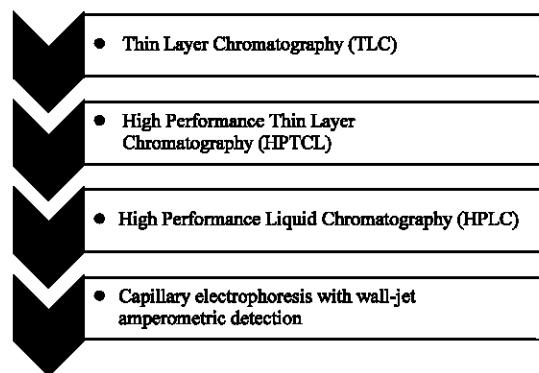


Fig. 4: Various techniques involved in analysis of flavonoids

chromatography and high performance thin layer chromatography (Pendry *et al.*, 2005). A study was conducted in Taiwan on harvested soybeans to determine major and minors of isoflavones, after subjected to methanol-H₂O extraction and HPLC analysis with the acetic acid-acetonitrile mobile phase. Among the detected soybeans, daidzin, genistin, malonyldaidzin and malonylgenistin were the majors and glycitin, malonylglycitin, daidzein and genistein were the minors of isoflavones (Tsai *et al.*, 2007). Flavonoids can also be satisfactorily determined by capillary electrophoresis with wall-jet amperometric detection by monitoring the effects of several important factors, such as the running buffer and its corresponding pH and concentration, separation voltage, injection time to acquire the optimum conditions for separation of the flavonoids (Fig. 4) (Xu *et al.*, 2006).

It is well documented that flavonoids (quercetin, rutin, etc.) after absorption produce good therapeutic effect in a number of other ailments also, apart from antioxidant activity (Table 6).

NUTRITIVE VALUE OF FLAVONOIDS

The flavonoids have been used over a period of time in other ailments (Table 2, 6) except as an antioxidant. Tea, the top drink in the world, has flavonoids which act as antioxidants. Apple provides the most concentrated food source of flavonoids, a group of phytochemicals, believed to protect against cancer, heart disease and other serious health problems, lending some truth to the old adage an apple a day keeps the doctor away. Blueberries are another good source of antioxidants, especially good for healthy eyesight. Recent studies have found that chocolate may actually be a healthy food because it provides plenty of flavonoids which are reported to be more effective than tea. Soybean isoflavones are structurally similar to estrogen and exhibit weak

Table 6: Diseases treated with flavonoids

Flavonoids	Target	Disease	References
Hydroxyethylrutosides, Quercetin, silymarin	PG synthesis	Pain, inflammation, cramps, tiredness, oedema	Alcaraz and Ferrandiz (1987), Morino <i>et al.</i> (1997), Shahidi <i>et al.</i> (1998)
Quercetin	Aldose reductase	Diabetes mellitus	Jager <i>et al.</i> (1998)
Quercetin	Mast cell	Allergy	Verma and Kinoshita (1976)
Quercetin	Capillary wall (PG)	Parodontosis	Morino <i>et al.</i> (1997)
Quercetin	Na ⁺ /K ⁺ ATPase	Cancer	Izzo <i>et al.</i> (1991), Kontruck <i>et al.</i> (1986), Murakami <i>et al.</i> (1992)
Quercetin	H ⁺ ATPase of lysosomal membrane	Virus infection, common cold	Kyo <i>et al.</i> (1998), Middleton (1998), Zhai <i>et al.</i> (1998a, b)
Sofalcone, Quercetin	PG synthesis	Oral surgery, stomach, duodenal ulcer	Alcaraz and Ferrandiz (1987), Morino <i>et al.</i> (1997), Shahidi <i>et al.</i> (1998)
Rutin, Quercetin, Kaempferol	PAF	Antiulcer	Izzo <i>et al.</i> (1991)
Rutin/citrin	Capillary wall (PG)	Allergy	Zhai <i>et al.</i> (1998a)
(+)-Cyanidanol-3-mecidanol, catechins	Gastric H ⁺ /K ⁺ ATPase	Antiulcer	Castillo <i>et al.</i> (2006)

estrogenic activity (Ishimi, 2009). It was claimed after experimentation on female rats that the administration of a soy extract containing isoflavones prevents oxidative changes in hepatocytes isolated from old ovariectomized female rats, without modifying uterus weight (Castillo *et al.*, 2006). Some epimeric new triterpenoid such as 3 α -hydroxy-20-oxo-3 β -norlupane and new flavanone (nubatin; 3) have not been successfully isolated from *Salvia* species rather these metabolites were found to be moderately bio-active also (Ali *et al.*, 2005).

CONCLUSION

Vitamin C, E, selenium, carotenoids are well known antioxidants however they do not come under flavonoids but constitute a vital part of our diet. The total daily intake of these dietary antioxidants is quite low, vitamin C (70 mg), vitamin E (7-10 mg) or carotenoids (2-3 mg) as compared to the flavonoids (50-800 mg), which makes a substantial contribution to the antioxidant defense system. There is adequate clinical evidence that flavonoids exert crucial therapeutic effects, many of which have been used in traditional systems of medicine for thousands of years. But, their full potential is yet to be recognized in all aspects. The utility of flavonoids in medicines should be elaborated. More pharmacokinetic and pharmacodynamic studies are required to define the protective role of flavonoids by scavenging free radicals in the mammals.

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