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Flavonoids: An Overview

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ABSTRACT

Flavonoid constitute one of the largest class of naturally occurring plant products mostly phenols either in the Free State or as their respective glycosides. The main constituent of flavonoid drugs are 2-phenyl- γ -benzopyrones. The family includes monomeric flavanols, flavanones, anthocyanidins, flavones, isoflavonoid and flavonols. It is very probable that a number of herbal remedies, whose constituents are yet unknown, will be shown to contain active flavonoids, quercetin and rutin in plant trigonella foenum seed, they are known for its anti-inflammatory properties, The flavonones hesperidin and neohesperidin were shown to protect against the toxicity of hydrogen peroxide, as well as protecting against DNA damage. Most flavonoids have anti-tumor properties. A diet rich in flavonol such as onions, apples and berries may cut the risk of developing pancreatic cancer. Flavonoids are powerful antioxidant. Extract from onion contain quercetin which induce the cellular antioxidant system. Diets rich in either flavonoids including red wine, quercetin or catechin induce endothelium dependent vasorelaxation. More than 2000 of these compounds are known, with nearly 500 occurring in the free state. Flavonoid research began in an attempt to isolate the various individual flavonoids and to study the mechanism by which flavonoids act. There are many herbal drugs containing flavonoid which have different morphological characters and are not yet fully exhausted for their pharmacological activities.

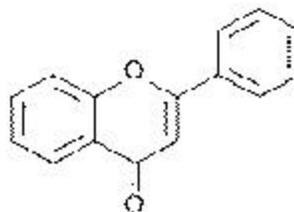
Keywords: Flavonoids, phytochemistry, pharmacological activities.

INTRODUCTION

Flavonoids are a group of polyphenolic compound ubiquitously found in fruits and vegetables. Flavonoids occur in both in the free state and a glycosides. These compounds occur as yellow and white plant pigments (Latin *flavus* = yellow). Till yet more than 4000 flavonoids has been discovered, out of which 500 are found in free state. They are abundant in Polygonaceae, Rutaceae, Leguminosae, Umbelliferae and Compositae.

Flavonoid is known for its anti-inflammatory and anti-allergic effects, antioxidant, antithrombotic and vasoprotective properties. Many flavonoid containing plants are diuretic or antispasmodic (e.g. liquorice & parsley). Some flavonoids have antibacterial and antifungal properties. They also play a role in protecting the plants from microbe and insect attacks. More importantly, the consumption of foods containing flavonoids has been linked to numerous health benefits. They are secondary metabolites, meaning they are organic compounds that have no direct involvement with the growth or development of plants. Flavonoids are widely disbursed throughout plants and gives vibrant colors to the flowers and fruits of many plants.¹⁻⁴

The current focus on the potential and beneficial role of these bioactive compounds in several chronic diseases, there is intense disclose interest in studying the health effects of bioactive compounds and sorting out the mechanisms that mediate their effects. The primary challenge in this area is to identify bioactive compounds and their associated health effects as well as their underlying biological mechanism of action. An impressive and growing number of bioactive compounds have been identified that have potentially important health benefits via distinguished mechanism actions and can act as antioxidants, enzyme inhibitors and inducers, inhibitors of receptor activities, and inducers and inhibitors of gene expression, photo protective among other actions. Flavonoids are nearly ubiquitous in plants and are recognized as the pigments responsible for the colors of leaves, especially in autumn. They are rich in seeds, citrus fruits, olive oil, tea, and red wine. They are low molecular weight compounds composed of a three-ring structure with various substitutions. This basic structure is shared by tocopherols (vitamin E).



Flavonoid

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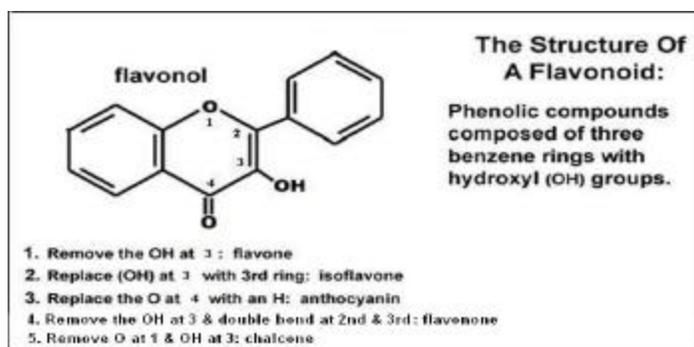
E-mail: saritakesarkar2009@gmail.com

Properties:

Flavonoids are generally soluble in water and alcohol, but insoluble in organic solvents; the genins are only sparingly soluble in water but are soluble in ether. Flavonoids dissolve in alkalis, giving yellow solutions which on the addition of acid become colorless.⁴

Chemistry:

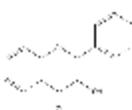
The main constituents of flavonoid drugs are 2-phenyl- γ -benzopyrones (2-phenyl-chromones) or structurally related, mostly phenolic compound. Their chemical structure are solely based upon a C6-C3-C6 carbon skeleton having a pyran or chroman ring bearing a second benzene (aromatic) ring strategically positioned at C-2, C-3 or C-4.^{2,5}



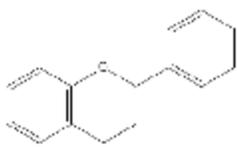
Classification with Sources:^{6,7}

Flavonoid Chemical Constituents

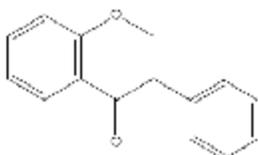
Flavonols [I]	Rutin, quercetin, kaempferol, myricetin.
Flavones [II]	Luteolin, apigenin.
Isoflavones[III]	Tephrosin, genistein, diadzein.
Flavanones [IV]	Hesperetin, naringenin,
Chalcone [V]	Carthamine
Anthocyanin	Pelargonidin, Cyanidin, Blueberry



[I]



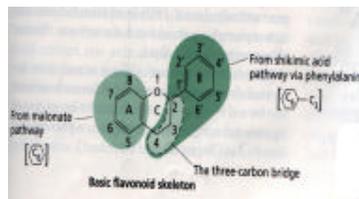
[II]



[III]

BIOSYNTHETIC PATHWAY

Flavonoids are product of both the shikimic acid and acetate pathway, being formed by the condensation of a p phenyl- γ -propanoid precursor with 3 malonyl coenzyme A units.



The biosynthesis of flavonoid compounds uses shikimic acid pathway for deriving a polyketide intermediate which produces chalcone skeleton. Chalcones works as an important intermediate for majority of flavonoid compounds in the plant kingdom. The major structural categories of flavonoids are flavones, flavanones, flavonols, isoflavones and anthocyanidins.^{2,8-9}

ISOLATION

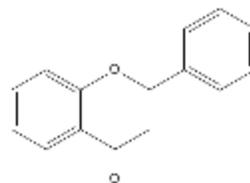
Preparation of Extracts

General Method

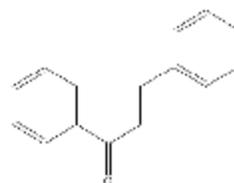
Powered drug (1 g) is extracted with 10 ml methanol for 5 min on a water bath at about 60°C and then filtered; 20-30 μ l is used for chromatography (flavonoid content, 0.5 – 1.5%). This rapid method extracts both lipophilic and hydrophilic flavonoids.

Primary Food Sources

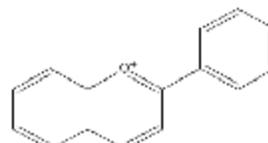
Onions, apples, teas, berries, isorhamentin	olives, bananas, lettuce, plums, & red wine.
Apples, celery, celeriac, lemons, parsley, oregano, lettuce, beets	
Soybeans, legumes.	
Oranges, grapes, lemons.	
Sufflower	
Grapes	



[IV]



[V]



[VI]

Thin-Layer Chromatography

Reference compounds

Standard compounds are prepared ad 0.05% solutions in methanol, and 10 μ is used for chromatography. The average detection limit for flavonoids is 5-10 μ g. For a general description of the flavonoid pattern of a drug, 10ul of a mixture of the compounds rutin, chlorogenic acid and hyperoside is used for TLC (test mixture T1).

Adsorbent

Silica gel 60 F₂₅₄-precoated TLC plates (Merck, Germany).

Chromatography solvents

Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26)

Ethyl acetate-formic acid-glacial acetic acid-ethylmethyl ketone-water (50:7:3:30:10)

Chloroform-acetone-formic acid (75:16:5:8.5)

Choloroform-ethyl acetate (60:40)

Choloform (100)

Benzene-pyridine-formic acid (72:15:10)

Toluene-ethyl formate-formic acid (50:40:10)

Toluene-dioxan-glacial acetic acid (90:25:4)

Detection

The solvent (acids) must be thoroughly removed from the silica gel layer before detection.

UV-254 nm. All flavonoids cause fluorescence quenching.

UV-365 nm. Depending of the structural type, flavonoids show dark yellow, green or blue fluoroscence, which is intensified and changed by the use of various spray reagents.

Spray regents

Natural products reagent (NP/PEG No. 28)

Typical intense fluorescence in UV-365 nm is produced immediately on spraying. Addition of polyethylene glycol solution lowers the detection limit and intensifies the fluorescence behaviour, which is structure dependent.

Flavonols: quercetin, myricetin and their glycosides-orange-yellow kaempferol, isorhamenetin and their glycosides -yellow green

Flavones : luteolin and their glycosides -orange,Apigenin and their glycosides-yellow-green

Fast blue salt B (FBS No. 15)

Blue or blue-violet (Vis) azo-dyes are formed. The color can be intensified by further spraying with 10% sodium hydroxide or potassium hydroxide solution.

TESTS

1) FLUORESCENCE QUENCHING TEST

A novel fluorescence quenching test for the detection of flavonoid degradation by microorganisms was developed. The test is based on the ability of the flavonoids to quench the fluorescence of 1,6-diphenyl-1,3,5-hexatriene (DPH). Several members of the anthocyanidins, flavones, isoflavones, flavonols, flavanones, dihydroflavanones, chalcones, dihydrochalcones and catechins were tested with regard to their quenching properties. The anthocyanidins were the most potent quenchers of DPH fluorescence, while the flavanones, dihydroflavanones and dihydrochalcones, quenched the

fluorescence only weakly. The catechins had no visible impact on DPH fluorescence. The developed test allows a quick and easy differentiation between flavonoid-degrading and flavonoid-non-degrading bacteria. The investigation of individual reactions of flavonoid transformation with the developed test system is also possible.¹⁰

2) SHINODA TEST

To dry power or extract, add 5ml. 95% ethanol. Few drops conc. HCL and 0.5 g magnesium turnings. Pink color observed. To small quantity of residue, add lead acetate solution. Yellow colored precipitate is formed. Addition of increasing amount of sodium hydroxide to the residue shows yellow coloration which decolorises after addition of acid.¹¹

MECHANISM^{5-6, 12}

Antiatherosclerotic effects

Because of their antioxidative properties, flavonoids are likely to have a major influence on the vascular system. Oxygen radicals can oxidize LDL, which injures the endothelial wall and thereby promotes atherosclerotic changes. A few clinical studies have pointed out that flavonoid intakes protect against coronary heart disease. Hertog et al stated that the flavonoids in regularly consumed foods might reduce the risk of death from coronary heart disease in elderly men. Furthermore, a Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations. Oxidative stress and vascular damage are postulated to play a key role in dementia, and the intake of red wine is reported to prevent the development of dementia. The intake of flavonoids was reported to be inversely related to the risk of incident dementia.

Antiinflammatory effects

Cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Neutrophils containing lipoxygenase create chemotactic compounds from arachidonic acid. They also provoke the release of cytokines. Selected phenolic compounds were shown to inhibit both the cyclooxygenase and 5-lipoxygenase pathways. This inhibition reduces the release of arachidonic acid. The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxygenase activities, thus diminishing the formation of these inflammatory metabolites.

Another antiinflammatory feature is the ability of flavonoids to inhibit eicosanoid biosynthesis, Eicosanoids, such as prostaglandins, are involved in various immunologic responses and are the end products of the cyclooxygenase and lipoxygenase pathways. Flavonoids also inhibit both monooxygenase kinase, are involved in a variety of functions, such as enzyme catalysis, transport across membranes, transduction of signals that function as receptors of hormones and growth factors, and energy transfer in ATP synthesis. Inhibition of these proteins results in inhibition of uncontrolled cell growth and

proliferation. Tyrosine kinase substrates seem to play key roles in the signal transduction pathway that regulates cell proliferation. Another antiinflammatory property of flavonoids is their suggested ability to inhibit neutrophil degranulation. This is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells.

Antitumor effects

The antitumor activity of flavonoids is still a point of discussion. Antioxidant systems are frequently inadequate, and damage from reactive oxygen species is proposed to be involved in carcinogenesis. Reactive oxygen species can damage DNA, and division of cells with unrepaired or misrepaired damage leads to mutations. If these changes appear in critical genes, such as oncogenes or tumor suppressor genes, initiation or progression may result. Reactive oxygen species can interfere directly with cell signaling and growth. The cellular damage caused by reactive oxygen species can induce mitosis, increasing the risk that damaged DNA will lead to mutations, and can increase the exposure of DNA to mutagens. It has been stated that flavonoids, as antioxidants, can inhibit carcinogenesis. Some flavonoids such as fisetin, apigenin, and luteolin are stated to be potent inhibitors of cell proliferation. A large clinical study suggested the presence of an inverse association between flavonoid intake and the subsequent incidence of lung cancer. This effect was mainly ascribed to quercetin, which provided > 95% of the total flavonoid intake in that particular study. Quercetin and apigenin inhibited melanoma growth and influenced the invasive and metastatic potential in mice. This finding may offer new insights about possible therapies for metastatic disease. Furthermore, it has been speculated that flavonoids can inhibit angiogenesis. Angiogenesis is normally a strictly controlled process in the human body. The process of angiogenesis is regulated by a variety of endogenous angiogenic and angiostatic factors. It is switched on, for example, during wound healing. Pathologic, unregulated angiogenesis occurs in cancer. Angiogenesis inhibitors can interfere with various steps in angiogenesis, such as the proliferation and migration of endothelial cells and lumen formation. Among the known angiogenesis inhibitors, flavonoids seem to play an important role. However, the mechanism behind the antiangiogenic effect of flavonoids is unclear. A possible mechanism could be inhibition of protein kinases. These enzymes are implicated to play an important role in signal transduction and are known for their effects on angiogenesis.

Antithrombogenic effects

Platelet aggregation contributes to both the development of atherosclerosis and acute platelet thrombus formation, followed by embolization of stenosed arteries. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals, which inhibit the endothelial formation of prostacyclin and nitrous oxide. It was shown in the 1960s that tea pigment can reduce blood coagulability, increase fibrinolysis, and prevent platelet adhesion and aggregation. Selected flavonoids, such as quercetin, kaempferol, and

myricetin were shown to be effective inhibitors of platelet aggregation in dogs and monkeys. Flavonols are particularly antithrombotic because they directly scavenge free radicals, thereby maintaining proper concentrations of endothelial prostacyclin and nitric oxide. One study showed that flavonoids are powerful antithrombotic agents in vitro and in vivo because of their inhibition of the activity of cyclooxygenase and lipoxygenase pathways. It is well known that arachidonic acid, which is released in inflammatory conditions, is metabolized by platelets to form prostaglandin, endoperoxides, and thromboxane A₂, leading to platelet activation and aggregation. The main antiaggregatory effect of flavonoids is thought to be by inhibition of thromboxane A₂ formation. Flavonoids affect arachidonic acid metabolism in different ways. Some flavonoids specifically block cyclooxygenase or lipoxygenase, whereas others block both enzymes. In vitro studies showed that flavonoids bind to platelet membranes and may therefore have an accumulative effect over time.

Antiosteoporotic effects

In an English study, bone mineral density was compared between older women who consumed tea and those who did not. Women in the study who drank tea had higher bone mineral density measurements than did those who did not drink tea. The flavonoids in tea might be responsible for the prevention of osteoporosis.

Antiviral effects

The antiviral activity of flavonoids was shown in a study by Wang et al. Some of the viruses reported to be affected by flavonoids are herpes simplex virus, respiratory syncytial virus, parainfluenza virus, and adenovirus. Quercetin was reported to exhibit both antiinfective and antireplicative abilities. The interaction of flavonoids with the different stages in the replication cycle of viruses was previously described. For example, some flavonoids work on the intracellular replication of viruses, whereas others inhibit the infectious properties of the viruses. By far, most studies of the effects on viruses were performed in vitro and little is known about the antiviral effect of flavonoids in vivo.

There is some evidence that flavonoids in their glycone form seem to be more inhibitory on rotavirus infectivity than are flavonoids in their aglycone form. Because of the worldwide spread of HIV since the 1980s, investigations of the antiviral activity of flavonoids have mainly focused on HIV. Many natural products can inhibit various stages of the replication cycle of the virus. The discovery and development of flavonoids as anti-HIV agents has expanded in the past 2 decades. Most of these studies focused on the inhibitory activity of reverse transcriptase, or RNA-directed DNA polymerase, but antiintegrase and antiprotease activities were also described. Again, flavonoids have mainly been studied in vitro experiments; therefore, no clear contribution of flavonoids to the treatment of HIV-infected patients has yet been shown.

USES^{7, 13-16}

Anti-inflammatory

Most flavonoids have anti-inflammatory properties. Hence the use of flavonoids may be appropriate in some medical conditions involved in inflammation. Flavonoid containing is quercetin & rutin in plant trigonella foenum seed.

Flavonoids and Cancer

Most flavonoids have anti-tumor properties. Flavonol rich diet may slash pancreatic cancer, flavonoids containing in onions, apples.

Flavonoids as Anti-oxidants

Flavonoids are powerful antioxidants. Extracts from onion and various flavonoids induce the cellular antioxidant system. Onion extract and quercetin were able to increase the intracellular concentration of glutathione by approximately 50%.

Vasodilatation and nitric oxide production

Diets rich in either flavonoids including red wine, quercetin or catechin induce endothelium-dependent vasorelaxation. This means that blood vessels are more dilated and this may be beneficial in vascular diseases such as claudication or coronary or heart disease.

Role in human body:^{12, 17}

Flavonoids are plant nutrients that when consumed in the form of fruits and vegetables are non-toxic as well as potentially beneficial to the human body. Flavonoids alone provide minimal antioxidant benefit due to slow absorption by the body, there is indication that they biologically trigger the production of natural enzymes that fight disease.

Flavonoids can be nutritionally helpful by triggering enzymes that reduce the risk of certain cancers, heart disease, and age-related degenerative diseases. Flavonoids may help prevent tooth decay and reduce the occurrence of common ailments such as the flu. These potential health benefits, many of which have been proven, have become of particular interest to consumers and food manufacturers.

The higher the amount of flavonoids in the diet, lower the risk for heart disease. The biological activities of flavonoids are partly related to their antioxidative effects, low risk of degenerative diseases, cardiovascular disease, hypertension, cataract, and stroke and in particular, cancers in people with a high intake of fruit and vegetables. This protective effect is assumed to be associated mainly with the antioxidant activities of either individual or interacting bioactive components present in the fruits and vegetables, and with other biochemical and physical characteristics of the identified and unknown bioactive components.

Flavonoids may play a role in the prevention and treatment of Allergy, Asthma, Atopic dermatitis, Candida infection, Cataracts, Diabetes, Gout, Hemorrhoids, Macular degeneration, Migraine, Periodontal disease, Stomach ulcer, Varicose veins.

Role in plant physiology:²

Flavonoids are universal within the plant kingdom; they are the most common plant pigment next to chlorophyll and carotenoids. They are recognized as the pigment responsible for autumnal leaf colors as well as for the many shades of yellow, orange and red in flowers. Their functions include protection of plant tissues from damaging UV radiation, acting as antioxidant, enzyme inhibitors, pigments and light screens. The compounds are involved in photo-sensitization and energy transfer, action of plant growth hormones and growth regulators, as well as defense against infection the plant response to injury result in increased synthesis of flavonoid aglycone at the site of injury or infection. Flavonoids aglycone that reach the large bowel are subject to ring fission by intestinal bacteria, a process in which the middle carbon ring is split apart into smaller fission metabolites. These metabolites are readily absorbed and some are known to possess therapeutic benefits in their own right due to the process of enterohepatic circulation, the therapeutic benefits of flavonoid metabolites are sustained over a relatively long duration. As antioxidants, some flavonoids, such as quercetin, protect LDL ("bad") cholesterol from oxidative damage. Others, such as the anthocyanidins from bilberry, purple cabbage, and grapes, may help protect the lens of the eye from cataracts. Animal research suggests that naringenin, found in grapefruit, may have anticancer activity.² Soy isoflavones are also currently being studied to see if they help fight cancer.

While they are not considered essential nutrients, some flavonoids support health by strengthening capillaries and other connective tissue, and some function as anti-inflammatory, antihistaminic, and antiviral agents. Quercetin has been reported to block the "sorbitol pathway" that is linked to many problems associated with diabetes. Rutin and several other flavonoids may also protect blood vessels.

Pharmacokinetics:⁶

Their beneficial effects will be dependent upon their uptake and disposition in tissues and cells. Small intestinal absorption ranges from 0 to 60% of the dose and elimination half-life (T_{1/2}) range from 2 to 28h. Absorbed flavonoids undergo extensive first-pass Phase II metabolism in the small intestine epithelial cells and in the liver. Metabolites conjugated with methyl, glucuronate and sulfate groups are the predominant forms present in plasma.

Absorption

Absorption, metabolism, and excretion of flavonoids in humans are contradictory and scarce. Some dietary flavonoid, quercetin, is absorbed in significant amounts naturally occurring flavones exist predominantly in a glycosylated form rather than in their aglycone form. The form of the flavonoid seems to influence the rate of absorption.

tion, the glycosylated forms of quercetin are absorbed more readily than are the aglycone forms.

Conjugation

It is generally accepted that the conjugation pathway for flavonoids (catechins) begins with the conjugation of a glucuronide moiety in intestinal cells. The flavonoid is then bound to albumin and transported to the liver. The liver can extend the conjugation of the flavonoid by adding a sulfate group, a methyl group, or both. The addition of these groups increases the circulatory elimination time and probably also decreases toxicity.

There are several possible locations for the conjugates on the flavonoid skeleton. The type of conjugate and its location on the flavonoid skeleton probably determine the enzyme-inhibiting capacity, the antioxidant activity, or both of the flavonoid.

Toxicity

High doses of quercetin over several years might result in the formation of tumors in mice. However, in other long-term studies, no carcinogenicity was found. In contrast with the potential mutagenic effects of flavonoids, quercetin, seem to be antimutagenic.

Flavonoids are toxic to cancer cells or to immortalized cells, but are not toxic or are less toxic to normal cells. If this is true, flavonoids might play a role in the prevention of cancer that is worthy of further investigation.

ENZYME MODULATOR ACTIVITY OF FLAVONOIDS ⁶

SR NO	ENZYMES	FLAVONOIDS	MECHANISM
1	Protein Kinase	Quercetin	Inhibited the phosphorylated activity of the rous sarcoma virus-transforming gene.
2	Phospholipase A 2 (pla2)	Kaempferol	Inhibition of phospholipase kinase
3	Phospholipase C ATPases		and also of protein tyrosine kinase. Catalyzes the Myosin light chain kinase (MLICK)
4	Lipoxygenases and Cyclooxygenases	Quercetin. Kaempferol-3-O-glycoside and scutellarein. Genistein Quercetin Quercetin	Inhibition of PLA3 from human & rabbit leukocytes. Blocking the PLC activation & formation of inositol trisphosphate (IP3) & diacylglycerol (DGA). Mg21-ectolATPase of human leukocytes. Increase in ATPase activity by conformational changes in the structure of myosin. Effect on arachidonic acid metabolism via the LO and CO (TxB2, PGE2, 6-keto-PGFIA) pathways.

CONCLUSION

There are many herbal drugs containing flavonoid which have different pharmacological activities and are not yet totally exploited. It is important to note that the difference aspect of flavonoid focused in review as Trigonella foenum¹⁴⁻¹⁶(fenugreek), Centella asiatica¹⁷⁻¹⁹,

Bacopa monnieri²⁰⁻²¹, Eclipta alba²², Cartaeva nurvala²³⁻²⁵ is exploited for anti-inflammatory^{14, 15, 17, 21}, hepatoprotective^{15, 20}, immunomodulator, neuroprotective, wound healing¹⁸ nootropic¹⁹, antioxidant²⁰, anticancer, anticomplement²⁴ activities.

In future, such study claims an open area of research, for sound consideration for development of flavonoid.

FUTURE IMPLICATIONS

Flavonoids have received much attention in the literature over the past 10 years and a variety of potential beneficial effects have been elucidated. However, most of the research involved in vitro studies; therefore, it is difficult to draw definite conclusions about the usefulness of flavonoids in the diet.

The study of flavonoids is complex because of the heterogeneity of the different molecular structures and the scarcity of data on bioavailability. Furthermore, insufficient methods are available to measure oxidative damage in vivo and the measurement of objective endpoints remains difficult.

There is a need to improve analytic techniques to allow collection of more data on absorption, excretion and on the long-term consequences of chronic flavonoid ingestion which is especially scarce.

REFERENCES

- 1) Ronald R Watson, Vegetables fruits and herbs in health promotion, 1st edition, CRC Press, London, 2001, 7, 140-141.
- 2) Andrew pengelly, the constituents of medical plant, 2nd edition, 2006, 33-36.
- 3) Ashutosh kar, Pharmacognosy and pharmacobiotechnology, 2nd edition, new age international publication, 2007, 157-167.
- 4) William charles Evans, Trease, Pharmacognosy, 13th edition, ELBS, 1989, 419-421.
- 5) H. Wagner, S Bladt, Plant drug analysis, 2nd edition, spinger publications, 1996, 195-197.
- 6) Swarnlata Saraf, Mahendra Sing Ashawat and Shailendra Saraf Pharmacognosy review:: Review Article :Flavonoids A Nutritional protection againgt oxidative and UV induced cellular damages, Pharmacog-

nosy Reviews Vol 1, Issue 1, Jan-MAY, 2007. Page no:30-36.

7) <http://www.raysahelian.com/flavonoids.html>

8) Vinod. D. Rangari, Pharmacognosy & phytochemistry, 1st edition, part-1, carrier publication, Nashik, 2008, 171,242.

9) Paul M. Dewick, Medicinal natural product and Biosynthetic approach, 2nd edition, John Wiley and sons, 2001, 149-151.

10) L.Schoefer, A Braune, M Blaut, A fluorescence quenching test for the detection of flavonoid transformation, FEMS microbiology letters, Vol. 204, No. 2, 2001, 277-280.

11) Khandelwal K. R., Practical pharmacognosy, Techniques & Experiments, 18th edition, Nirali prakashan, 2007, 153.

12) Robert J Nijveldt, Els van Nood, Danny EC van Hoorn, Petra G Boelens, Flavonoids: a review of probable mechanisms of action and potential applications, American Journal of Clinical Nutrition, Vol. 74, No. 4, 2001, 418-425.

13) <http://www.whfoods.com/genpage.php?tname=nutrient&dbid=109#nutrientdescr>

14) Savita Vyas, Rajendra Agrawal, Pooja Solanki, Piyush Trivedi; Analgesic And Anti-Inflammatory Activities Of Trigonella Foenum-Graecum (Seed) Extract; Acta Poloniae Pharmaceutica AND Drug Research, Vol. 65 No. 4 pp. 473-476, 2008

15) Ahmet Cihat, Onera Ufuk, Mercana Hatice, Onturkb Nureddin, Cengizc Remzi Ertend, Hanefi Ozbeke; Anti-inflammatory and Hepatoprotective Activities of Trigonella foenum-graecum L.; Pharmacologyonline; 2: 126-132 (2008)

16) Pritee Wagh, Mahendra Rai, S K Deshmukh and Marta Cristina, Teixeira Durate3 Bio-activity of oils of Trigonella foenum-graecum and Pongamia pinnata; African Journal of Biotechnology Vol. 6 (13), pp. 1592-1596, 4 July 2007.

17) M. N. Somchit, M. R. Sulaiman, A. Zuraini, L. Samsuddin, N. Somchit, D. A. Israf, S. Moin; Antinociceptive and antiinflammatory effects of Centella asiatica; Indian J Pharmacol, December 2004, Vol 36, Issue 6, 377-380.

18) Yadav NP, Pal A, Mathur Archana, Singh SC and Gupta MM; Wound healing activity of in-vitro grown shoots of different accessions of Centella asiatica; National Interactive Meet, 2008, 82.

19) Sushma Tiwari, Shinjini Singha, Kishor Patwardhan, Sangeeta

Gehlot, I.S.Gambhir; Effect of centella asiatica on mild cognitive impairment (mci) and other common age-related clinical problems; Digest Journal of Nanomaterials and Biostructures Vol. 3, No.4, December 2008, p. 215 – 220.

20) Tirtha Ghosh, Tapan Kumar Maity, Mrinmay Das, Anindya Bose And Deepak Kumar Dash; In Vitro Antioxidant and Hepatoprotective Activity of Ethanolic Extract of Bacopa monnieri Linn. Aerial Parts; Iranian Journal of Pharmacology & Therapeutics; 6:77-85, 2007.

21) Channa S, Dar A, Anjum S, Yaqoob M, Atta-Ur-Rahman; Anti-inflammatory activity of Bacopa monniera in rodents; J Ethnopharmacol; 2006 Mar 8;104(1-2):286-289.

22) Pimolpan Pithayanukul, Sasitorn Laovachirasuwan, Rapepol Bavovada, Narumol Pakmanee, Rutt Suttisir; Anti-venom potential of butanolic extract of Eclipta prostrata against Malayan pit viper venom; Journal of Ethnopharmacology; Volume 90, Issues 2-3, February 2004, Pages 347-352.

23) M. A. Alam, M. E. Haque, J. A. Shilpi, K. A. Daulla; Antinociceptive effect of the crude ethanolic extract of crataeva nurvala. Buch. On mice; Bangl. J. Vet. Med.; (2006). 4 (1): 65–68.

24) Geetha T., Varalakshmi P.; Anticomplement activity of triterpenes from Crataeva nurvala stem bark in adjuvant arthritis in rats; General pharmacology; 1999, vol. 32, no4, 495-497.

25) Annie Shirwaikar, Manjunath Setty M & Praveen Bommu and Krishnanand B; Effect of lupeol isolated from Crataeva nurvala Buch.-Ham. Stem bark extract against free radical induced nephrotoxicity in rats; Indian Journal of Experimental Biology; Vol. 42, July 2004, pp. 686-690.

26) Williamson EM, Major herbs of Aired, 1st edition, Churchill Livingstone, 2002, 316-318.

27) Indian herbal pharmacopeia, Volume 1, Joint publication of region research laboratory and Indian drug manufacture association, Mumbai, 1998, 30-37, 47-53, 56.

28) Mukherjee PK, Quality control of herbal drugs an approach to evaluation of botanicals, 1st edition, Business horizons pharmaceutical publication, 2008,707

29) Kirtikar, Basu, Indian medicinal plants, 2nd edition, vol-3, Periodical experiment book agency, 1993,

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