Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i6.6883

PLANTS FLAVONOIDS: CHEMISTRY, METABOLISM AND BIOLOGICAL ACTIVITIES

Sabira Sultana[1*], Rizwana Dilshad[2], Saiqa Muneer[3], Rabia Nisar[4], Aqsa Afzal[5], Ayesha Nazir[6], Naheed Akhtar[7], Muhammad Akram[8], Sidra Huma[9], Muhammad Saleem Qureshi[10], Momina Iftikhar[11]

[1*,8,11]Department of Eastern Medicine, Faculty of Medical Sciences, Government College University, Faisalabad Pakistan
[2]Swedish College of Pharmacy and Allied Health Sciences, Rahim Yar Khan Pakistan
[3]Times Institute, Multan Pakistan
[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, Punjab Pakistan
[5]Faculty of Pharmacy, Department of Pharmaceutics, Bahauddin Zakaria University, Multan Pakistan
[6]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, Punjab Pakistan
[7]Faculty of Medical and Health Sciences, Pharmacy Department, University of Poonch Rawalakot, AJK Pakistan
[9]Bahawalpur Medical and Dental College, Bahawalpur College of Pharmacy, Bahawalpur Pakistan
[10]Akbar Institute of Medical Sciences, Bahawalpur Pakistan

***Corresponding Author:** Sabira Sultana
*Email: drsabirachishti12@gmail.com

Abstract
Flavonoids are plant derived secondary metabolites. They are found in many fruits, vegetables, and seeds. They give the characteristic color, odor and taste. They are bioactive polyphenolic compounds and play a variety of roles in plants, including regulation of cell growth, attract insects and pollinators, and defending the biotic and abiotic stresses. Cell growth, attract insects and pollinators, and defending the biotic and abiotic stresses. Flavonoids gain attention in recent years due to high mortality rate of cardiovascular disease and low preventing rate of chronic cardiovascular disease. These compounds have anticancer, anti-aging, anti-inflammatory, neuroprotective cardio protective, immunomodulatory, anti-microbial, antidiabetic, anthelmintic, and antiviral effects in humans. In the review we discuss the current research on flavonoid, chemistry of flavonoids, their metabolism in human body, and their biological activities.

Keywords: Flavonoids, classification, biological activities, secondary metabolites

Introduction

Flavonoids are secondary metabolites found in high concentrations in fruits, plants, and seeds and are gives for color, odor, and taste. Flavonoids play a variety of roles in plants, including regulation of cell growth, attract insects and pollinators, and defending the biotic and abiotic stresses [1]. These

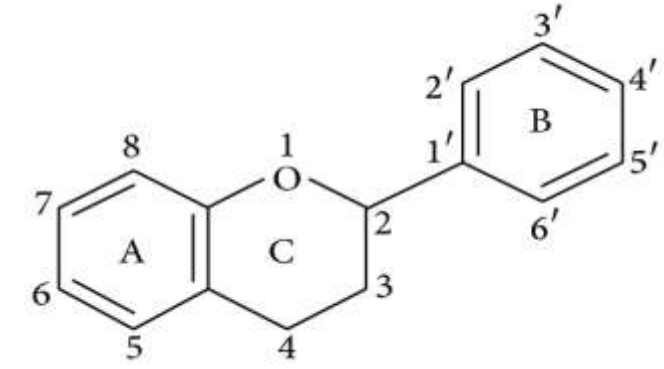
compounds have anticancer, anti-aging, anti-inflammatory, neuroprotective cardio protective, immunomodulatory, anti-microbial, antidiabetic, anthelmintic, and antiviral effects in humans [2]. Flavonoids have a C6-C3-C6 flavone skeleton with two benzene rings connected by a three-carbon pyran ring C. Catechol B-ring position on the pyran C-ring, as well as the number and position of hydroxy groups on the catechol group of the B-ring, affect the antioxidant capacity of flavonoids [3]. Flavonoids are classified into several subgroups, including flavones, chalcones, isoflavonols, and flavonols. All the subgroups have their own sources. Onions and tea, for istance, are essential nutritional sources of flavones and flavonols. Flavonoids involve in protective role in a variety of biotic and abiotic stresses, as well as acting as unique UV filters [4].

Flavonoids are widely recognized as an essential compound having wide range of application in medicinal, nutraceutical, cosmetic, pharmaceutical industries. This is due to anti-oxidant, anti-mutagenic, inflammatory, anti-carcinogenic potential. They have an ability to alter the function of key enzymes. Flavonoids gain attention in recent years due to high mortality rate of cardiovascular disease and low preventing rate of chronic cardiovascular disease. The mechanisms of flavonoids are poorly known. Plant-derived derivatives have a wide range of biological activity. Emerging patterns in flavonoids research and development revolve around the extraction, isolation, characterization, identification and role of flavonoids in human health. Industries used Molecular docking and bioinformatics knowledge to determine the potential role of flavonoids. [5]. In the review we discuss the current research on flavonoid, chemistry of flavonoids, their metabolism in human body, and their biological activities.

Chemistry of Flavonoids

Flavonoids are plant derived well-known bioactive Phenolic compounds. They were discovered in 1930. First flavonoid compound was isolated from orange. They were thought that it was new class of vitamin and gave the name vitamin P. Further research and studies concluded that they were not vitamin but flavonoid. Until now four thousand flavonoids have been isolated. [6].

Flavonoids have fifteen carbon chain in which two benzene ring present which are connected by heterocyclic pyrane ring. Flavonoids are classified in to different classes i.e. Flavones, flavonols, flavanones and others. The flavonoids classes are different on the base of carbon ring pattern and their mode of oxidation [6].



.

Flavonoids present as glycosides, aglycones and methylated derivatives. Theaglycones is the basic flavonoid structure (Figure 1). Benzene ring condensed with six carbon ring either by α-pyroneor by its dihydroderivative[7]. Flavonoids mostly hydroxylated at positions 3, 5, 7, 2, 3′, 4′, and 5′. Acetyl esters and Methyl ethers of alcohol group occur in nature. When glycosides formed, the glycosidic linkage is normally present at positions 3 or 7 and carbohydrate can be L-rhamnose, glucorhamnose, arabinose D-glucose or galactose [8].

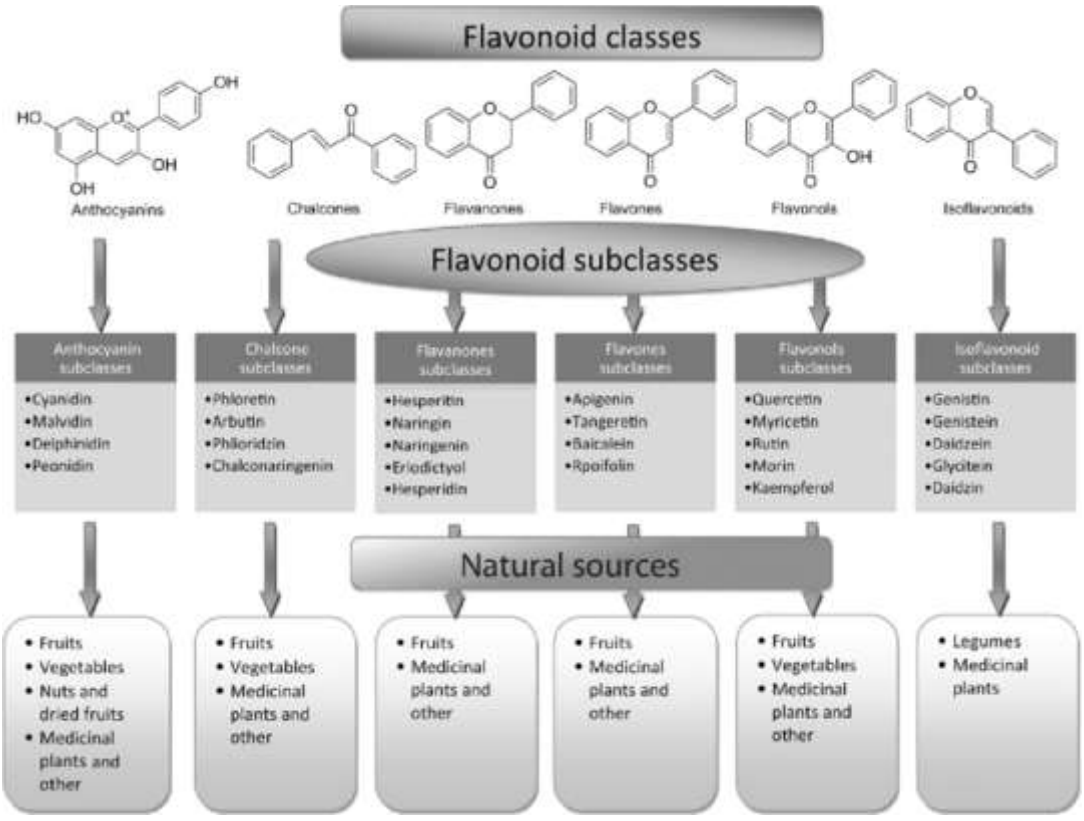
Classification

Flavonoids are classified into different subgroups. The sub groups are differing on the bases of Carbon ring on which ring B attached. The groups are also differing on the base of unsaturation and C-ring oxidation. Following are the subgroups of flavonoids

1. Flavones
2. Flavonols
3. Flavanones
4. Flavanonols
5. Flavanols or catechins
6. Anthocyanins
7. Chalcones

Flavones

Flavones are the subgroups of flavonoids. These are present generally in different parts of plants and richly present in parsley, Celery, peppers, mint, ginko biloba and chamomile. Apigenin, Luteolin, and tangeritin are flavones. Highest amount of polymethoxylated flavones, nobilatin, sinensetin and tageretin present in citrus fruit. [9]. The hydroxyl group of flavones present in position 5 of ring A in vegetables and natural products. The hydroxylation in different conditions, generally in place seven of A ring or 3′ and 4′ of the B ring, may shift as indicated by ordered characterization of specific vegetable or organic product.



[6]

Flavonols

Keto group is present in these flavonoids. Flavonols are building blocks of proanthocyanins. Many fruits and vegetables contains flavonoids. Extensive research has been done on some flavonoids such as quercetin, liquiritin, kaempferol and myricetin (Fig. 2). Tomato, berries apples, onions, lettuce and grapes are rich source of flavonoids. Leafy food such as tea, lemmon grass and green tea are also rich source of flavonoids. They have many therapeutic activities. Flavonols involves in curing vascular infections. They are strong antioxidant. Flavonol are the largest class of flavonoids and are present in

most of fruits and vegetables. Quercetin is one such example which of largely distributed flavonol [10].

Flavanones

Flavanones are mostly present in citrus fruits like lemon, grapes and oranges. Naringenin, and Hesperitin, are flavanones. Flavanone have antioxidant potential that's why they have number of therapeutic effects. Citrus flavonoids have many pharmacologic
al potentials. They are also considering as bitter principle that's why citrus fruit peel is bitter in taste. They are antioxidant, antihyperlipidimic and anti-inflammatory. Flavanones, are also known as dihydroflavones. Saturation is present between double bond present at position two and three. (10).

Isoflavonoids

Isoflavonoids are an enormous and extremely particular subclass of flavonoids. Iso flavonoids present in limited number of plants. These are commonly found in leguminous plants such as soyabeans. Some isoflavonoids are also present in microbes(11). They act as precursors forphytoalexins(12,13). Isoflavonoids have many therapeutic efficacies. Isoflavones, for example, daidzein and genistein are generally considered as phyto-oestrogens. Their oestrogenic effect is also determined (Fig. 2) (14).

Neoflavonoids

Neoflavonoids are polyphenolic compounds. The neoflavone isolated from the first time from seeds of *Calophyllum inophyllum*. This neoflavonoids was callophylloides. It is also found in the bark of *Mesua thwaitesii* [15,16,17].

Flavanols

Flavanols also known as flavan-3-ols or catechinsare 3-hydroxy derivatives of flavanones. These are multisubstituted and diverse class. In this class hydroxyl group is present on position 3 of carbon ring. Therefore, these are also known as flavon-3-ols. In this class no double bond is present between 2 and 3 positions. Flavones are found richly in apples, bananas, blueberries, pears and peaches.

Anthocyanins

Anthocyanins are coloring agents in plants, fruits and flowers. Cyanidin, malvidin, delphinidin, peonidin and pelargonidin are common anthrocyanins (Fig. 2). They are present certain fruits like cranberries, blue berries, red grapes,dark currants, strawberries and raspberries. These compounds are utilizedin food industries in several applications [18]. Anthocyaninscolor depends upon the pH and methylationat the hydroxyl group on the A and B rings [10].

Chalcones

Chalcones are open chain flavonoids. In these flavonoids C ring is absent in basic structure. Some examples of isolated chalcones are arbutin, phloretin, chalconaringenin and Phloridzin. Chalcones abundantly found in in pears, tomatoes, bear berries and wheat. Chalcones and their drivatives have many nutritional and medicinal potential [19-20]. The intake of naturally occurring chalcones have significant role in combating diseases.

Flavonoids Metabolism

The dietary flavonoids absorption depends on their size of molecules, lipophilicity, configuration, solubility and pKa. Absorption of dietary flavonoids takes place in small intestine. Sometime before absorption the enter in the colon. The absorption may also depend on the flavonoids structure, whether it is aglycone or glycosides. Small intestine easily absorbed aglycan, whereas, glycoside for absorption first convert in to aglycon form. Flavonoids mostly bound with sugar *b*-glycosides in plants except sub class catechin [21].The quercetin is hydrophilic glucosides. Quercetin transports across

the intestine by Na^+-dependent glucose cotransporter (SGLT1) [21]. lactase phloridzin hydrolase is an enzyme present in the intestinal brush border. This enzyme hydrolyzed flavonoids glucosides that result in the liberation of aglycone which then absorbed by small intestine [22]. Lactase phloridzin hydrolase enzyme substrate specificity is not same for every flavonoid [23]. Therefore, the glycosides which are not hydrolyzed by these enzymes are transported toward colon where intestinal bacteria have the ability to hydrolyze flavonoid glycosides, but they will also degrade the liberated aglycones [24]. Since in the colon absorption capacity as compared to small intestine. After absorption from small intestine, the flavonoids are conjugated in liver by sulfation, or methylation and convertedinto smaller phenolic compounds [25]. Because of conjugation reaction, the plasma and urine are free of flavonoids aglycones. [26]. Flavonoids from different food source are differ.They have different rate of absorption. For instance, quercetin from onion absorb more quickly than quercetin from tea and apple [27]. The flavonoids which are not absorbed by the small intestine are move towards the colon where they are degraded by microflora of small intestine. The microflora breakdown the ring structure of flavonoids. In stomach acidic environment large oligomer hydrolyzed into monomers. Among all subclasses of flavonoids, isoflavones exhibithighest bioavailability [28]. The flavonoids in the green tea after ingestion rapidly absorbed and enter the systemic circulation and cause significant increase in plasma antioxidant level [29].

Extraction, Isolation, and Characterization of Flavonoids

Flavonoids solubility varies in different solvent. The solvent is selected on the base of polarity of flavonoids. For example, ethyl acetate is used for, aglycones extraction. The solvents that are toxic such as chloroform and benzene are not used in extraction. The polar aglycones are extracted with alcohol, acetone and water [30,31].The characterization of flavonoids is done by mass spectroscopy and nuclear magnetic resonance. However, some other techniques are also used for characterization such as ultra violet absorption spectroscopy and infrared spectroscopy [32].

Several research has been published on the importance of analysis with MS fragmentation [33,34] They highlight some main fragments. Most of which cleave the heterocyclic ring and able to identify the presence of substituent in flavonoids nucleus [35, 36,37].

The evaluation of structure is most commonly determined by nuclear magnetic resonance. Different groups can be determined due to presence of heterocyclic ring [38]. For instance, isoflavones are differ from flavoneson the basis of H-2 and C-2 chemical shifts [32].

Biological Potential of Flavonoids

Antibacterial Activity

Flavonoids inhibit the biofilm formation of *Aeromonas shydrophila*, *Streptococcus mutans*, and *Escherichia coli*. [37] Quercetin and kampferol inhibit the formation of biofilm in E. coli [38]. Presence of hydroxyl phenolic group in flavonoids responsible for antibacterial effects. This group inhibit enzymes of bacteria and also interfere in their synthesis [39].

Antifungal activity

Many flavonoids have antifungal activity [40]. Flavonoids inhibit the growth of pathogenic fungal spores in plants and thus preventing fungal diseases in humans [41]. Flavonoids forms the complexes with protein in fungal cell walls.Prenylated flavones are from Moraceae family and have antifungal activity against *Saccharomyces cerevisiae* and*Candida albicans* [40]. Baicalein is flavone is isolated from *Scutellaria baicalensis* exhibit anticandidal activity against *Candida albicans*, *Candida parapsilosis, Candida tropicalis* [42].

Antiviral Activity

Flavonoids exhibit anti-viral activity. They inhibit the enzymes involves in the virus life cycle. Flavon-3-ol inhibit HIV-1 and HIV-2 and other immunodeficiency virus [43]. Anti-dengue activity of certain flavonoids such as naringin, hespertin and quercetin have been seen [44].

Steroid-genesis modulators

Abyssinones flavonoids are steroid-genesis modulators against the enzymes aromatase, 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase of the steroid-genesis pathway [45].

Xanthine oxidase modulators

Xanthine oxidase involves in gouty arthritis [46, 47, 48]. Flavonoids such as Licoisoflavone-A extracted from root of Glycyrrhiza glabra showed inhibitory effect against xanthine oxidase [49]. The flavonoidstricin,fisetin, butein, diosmetin, genistein,tricetin,vitexycarpin, rhamnetin, herbacetin,isorhamnetin,robinetin, biochanin, peonidin and okanin showed inhibitory activity. Benzopyran ring is present in flavonoids basic structure and involves in XO-inhibitory activity. [50]. Lin *et al*. studied many flavonoids having xanthine oxidase inhibitory activity [51]. Luteolinand Apigenin 5-hydroxy-6, 7, 3′, 4′-tetramethoxyflavone showed inhibitory potential of *gnaphalium affine* against xanthine oxidase activity [52].

Anti-cholinesterase activity

Acetylcholinesterase enzyme present in central nervous system and inhibit neural acetylcholine levels which is involve in the relives of Alzheimer disease [53]. Several number of flavonoids such as macluraxanthone and quercetin showed inhibitory activity against AChE and cholinesterase [54]. macluraxanthone exhibit strong inhibitory activity [55].

Anti-oxidant Activity

Studies showed anti-oxidant activity of flavonoids [56]. Antioxidant are chemical compounds and protect the human from damage to reactive oxygen species. Flavonoids are the phytochemicals exhibit antioxidant activity and thus inhibits the disease causing agents [57-58].

Antiaging property

Since many factors involve in aging process, but the most common cause is the oxidative damage of the cells[59] [60]. Study showedpolyphenol present in açaí pulp have antiaging properties beause they have the ability to remove superoxide and peroxyl radicals [61]. Quercetin and resveratrol flavonoids causes antiaging effects [62].

Anticancer Activity

Many flavonoids isolated from *Achyrocline satureioides* have been shown to possess anticancer activity. The flavonoids isolated from this plant are luteolin, quercetin, 3-O-methyl-quercetin and achyrobichalcone [63].

Future research and development programs

Flavonoids gained more attention in research since past ten years. They have many beneficial effects. Number of *in vitro* and *in silico* studies have been done to determine the therapeutic role of flavonoids. There is a need of further studies on to evaluate the health related benefits of flavonoids. Flavonoids study is complex as heterogeneity present in different molecular structures and less data available on the bioavailability. Moreover, deficientmethods are available to determinethe invivo oxidative damage. There is need of techniques to determine the absorption and excretion of flavonoids. No data is available on the consequences of the chronic ingestion of flavonoids. In silico and molecular docking studies are needed to evaluate the molecules of flavonoid and their therapeutic efficacy in human beings. More studies are needed to search the more and more flavonoids so that the harmful synthetic medicine are replace by new molecules.

Conclusion

Flavonoids are naturally occurring bioactive compounds and have number of beneficial effects. They are plants secondary metabolites. Studies reported that flavonoids have antioxidant, hypoglycemic,

anticancer, cardio protective, ant parasitic, antiviral, antiaging and antimicrobial activities. Flavonoids now recognized as an essential component in a wide range of nutraceutical, pharmaceutical, medicinal, and cosmetic applications. The mechanisms of flavonoids are poorly known. Emerging patterns in flavonoids research and development revolve around the isolation, identification, characterization, and role of flavonoids to cure disease. Molecular docking and bioinformatics knowledge are also being used by industry to estimate potential applications and manufacturing.

References

1. De Luna S.L., Ramírez-Garza R.E., Saldívar S.O.S. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. *Sci. World J.* 2020;2020:6792069. doi: 10.1155/2020/6792069.
2. Saini N., Gahlawat S.K., Lather V. Flavonoids: A nutraceutical and its role as anti-inflammatory and anticancer agent. In: Gahlawat S., Salar R., Siwach P., Duhan J., Kumar S., Kaur P., editors. *Plant Biotechnology: Recent Advancements and Developments.* Springer; Singapore: 2017.
3. D'Amelia V., Aversano R., Chiaiese P., Carputo D. The antioxidant properties of plant flavonoids: Their exploitation by molecular plant breeding. *Phytochem. Rev.* 2018;17:611–625. doi: 10.1007/s11101-018-9568-y10.
4. Takahashi A & Ohnishi T (2004) The significance of the study about the biological effects of solar ultraviolet radiation using the exposed facility on the international space station. *Biol Sci Space* 18, 255–260.
5. Panche, A.N., Diwan, A.D. and Chandra, S.R., 2016. Flavonoids: an overview. *Journal of nutritional science*, *5*.
6. E. J. Middleton, "Effect of plant flavonoids on immune and inflammatory cell function," *Advances in Experimental Medicine and Biology*, vol. 439, pp. 175–182, 1998.
7. K. R. Narayana, M. S. Reddy, M. R. Chaluvadi, and D. R. Krishna, "Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential," *Indian Journal of Pharmacology*, vol. 33, no. 1, pp. 2–16, 2001.
8. E. Middleton, "The flavonoids," *Trends in Pharmacological Sciences*, vol. 5, pp. 335–338, 1984.
9. Manach C, Scalbert A, Morand C, et al. (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr* 79, 727–747.
10. Iwashina T (2013) Flavonoid properties of five families newly incorporated into the order Caryophyllales (Review). *Bull Natl Mus Nat Sci* 39, 25–51.
11. Matthies A, Clavel T, Gütschow M, et al. (2008) Conversion of daidzein and genistein by an anaerobic bacterium newly isolated from the mouse intestine. *Appl Envrion Microbiol* 74, 4847–4852.
12. Aoki T, Akashi T & Ayabe S (2000) Flavonoids of leguminous plants: structure, biological activity, and biosynthesis. *J Plant Res* 113, 475–488.
13. Dixon R & Ferreira D (2002) Molecules of interest: genistein. *Phytochemistry* 60, 205–211.
14. Szkudelska K & Nogowski L (2007) Genistein – a dietary compound inducing hormonal and metabolic changes. J Steroid Biochem Mol Biol 105, 37–45. [PubMed] [Google Scholar]
15. Linuma M, Tanaka T, Hamada K, et al. (1987) Revised structure of neoflavone in Coutarea hexandra. Phytochemistry 26, 3096–3097. [Google Scholar]
16. 23. Nishimura S, Taki M, Takaishi S, et al. (2000) Structures of 4-aryl-coumarin (neoflavone) dimers isolated from Pistacia chinensis BUNGE and their estrogen-like activity. Chem Pharm Bull (Tokyo) 48, 505–508. [PubMed] [Google Scholar]
17. Garazd M, Garazd Y & Khilya V (2003) Neoflavones. 1. Natural distribution and spectral and biological properties. Chem Nat Comp 39, 54–121.
18. Giusti M & Wrolstad R (2003) Acylated anthocyanins from edible sources and their applications in food systems. *Biochem Eng J* 14, 217–225.
19. Hertog MG, Hollman PC & Van De PB (1993) Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J Agric Food Chem* 41, 1242–1246.

20. López-Lázaro M (2009) Distribution and biological activities of the flavonoid luteolin. *Mini Rev Med Chem* 9, 31–59.
21. P. C. H. Hollman, M. N. C. P. Buijsman, Y. van Gameren, P. J. Cnossen, J. H. M. de Vries, and M. B. Katan, "The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man," *Free Radical Research*, vol. 31, no. 6, pp. 569–573, 1999.View at: Google Scholar
22. A. J. Day, F. J. Canada, J. C. Diaz et al., "Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase," *FEBS Letters*, vol. 468, no. 2-3, pp. 166–170, 2000.View at: Publisher Site | Google Scholar
23. T. Walle, "Serial review: flavonoids and isoflavones (phytoestrogens: absorption, metabolism, and bioactivity): absorption and metabolism of flavonoids," *Free Radical Biology and Medicine*, vol. 36, no. 7, pp. 829–837, 2004.View at: Publisher Site | Google Scholar
24. R. R. Scheline, "Metabolism of foreign compounds by gastrointestinal microorganisms," *Pharmacological Reviews*, vol. 25, no. 4, pp. 451–532, 1973.View at: Google Scholar
25. L. Bravo, "Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance," *Nutrition Reviews*, vol. 56, no. 11, pp. 317–333, 1998.View at: Google Scholar
26. P. C. H. Hollman, "Absorption, bioavailability and metabolism of flavonoids," *Pharmaceutical Biology*, vol. 42, pp. 74–83, 2004.View at: Publisher Site | Google Scholar
27. P. C. H. Hollman, J. M. P. van Trijp, M. N. C. P. Buysman et al., "Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man," *FEBS Letters*, vol. 418, no. 1-2, pp. 152–156, 1997.View at: Publisher Site | Google Scholar
28. J. E. Spencer, F. Chaudry, A. S. Pannala, S. K. Srai, E. Debnam, and E. C. Rice, "Decomposition of cocoa procyanidins in the gastric milieu," *Biochemical and Biophysical Research Communications*, vol. 272, no. 1, pp. 236–241, 2000.View at: Publisher Site | Google Scholar
29. I. F. F. Benzie, Y. T. Szeto, J. J. Strain, and B. Tomlinson, "Consumption of green tea causes rapid increase in plasma antioxidant power in humans," *Nutrition and Cancer*, vol. 34, no. 1, pp. 83–87, 1999.View at: Google Scholar
30. Stobiecki M., Kachlicki P. Isolation and identification of flavonoids. In: Grotewold E., editor. *The Science of Flavonoids.* Springer; Cincinnati, OH, USA: 2006.
31. Tzanova M., Atanasov V., Yaneva Z., Ivanova D., Dinev T. Selectivity of current extraction techniques for flavonoids from plant materials. *Processes.*
32. Awouafack M.D., Tane P., Morita H. Isolation and structure characterization of flavonoids. In: Justino G.C., editor. *Flavonoids—From Biosynthesis to Human Health.* IntechOpen; London, UK: 2017.
33. Boukhalkhal S., Gourine N., Pinto D.C.G.A., Silva A.M.S., Yousfi M. UHPLC-DAD-ESI-MS^n profiling variability of the phenolic constituents of *Artemisia campestris* L. populations growing in Algeria. *Biocatal. Agric. Biotechnol.* 2020;23:101483. doi: 10.1016/j.bcab.2019.101483.
34. Ye M., Yang W.-Z., Liu K.-D., Qiao X., Li B.-J., Cheng J., Feng J., Guo D.-A., Zhao Y.-Y. Characterization of flavonoids in *Millettia nitida* var. *hirsutissima* by HPLC/DAD/ESI-MS^n. *J. Pharmaceut. Anal.* 2012;2:35–42. doi: 10.1016/j.jpha.2011.09.009.
35. Simões M.A.M., Pinto D.C.G.A., Neves B.M.R., Silva A.M.S. Flavonoid profile of the *Genista tridentate* L., a species used traditionally to treat inflammatory processes. *Molecules.* 2020;25:812. doi: 10.3390/molecules25040812.
36. Mabry T.J., Markham K.R., Thomas M.B. Reagents and procedures for the ultraviolet spectral analysis of flavonoids. In: Mabry T.J., Markham K.R., Thomas M.B., editors. *The Systematic Identification of Flavonoids.* Springer; Berlin/Heidelberg, Germany: 1970.
37. Lee JH, Regmi SC, Kim JA, Cho MH, Yun H, Lee CS, Lee J. 2011. Apple flavonoid phloretin inhibits *Escherichia coli* O157:H7 biofilm formation and ameliorates colon inflammation in rats. Infect Immun. 79:4819–4827.

38. Winter J, Moore LH, Dowell VR, Bokkenheuser VD. 1989. C-ring cleavage of flavonoids by human intestinal bacteria. Appl Environ Microbiol. 55:1203–1208.
39. Li Y, Luo Y, Hu Y, Zhu DD, Zhang S, Liu ZJ, Gong HB, Zhu HL. 2012. Design, synthesis and antimicrobial activities of nitroimidazole derivatives containing 1,3,4-oxadiazole scaffold as FabH inhibitors. Bioorganic Med Chem. 20:4316–4322.
40. Arif T, Mandal TK, Dabur R. 2011. Natural products: Anti-fungal agents derived from plants. J Asian Nat Prod Res. 11:621–638.
41. Salas MP, Céliz G, Geronazzo H, Daz M, Resnik SL. 2011. Antifungal activity of natural and enzymatically-modified flavonoids isolated from citrus species. Food Chem. 124:1411–1415.
42. Serpa R, França EJG, FurlanetoMaia L, Andrade CGTJ, Diniz A, Furlaneto MC. 2012. In vitro antifungal activity of the flavonoid baicalein against Candida species. J Med Microbiol. 61:1704–1708.
43. Gerdin B and Srensso E: Inhibitory effect of the flavonoid on increased microvascular permeability induced by various agents in rat skin. International Journal of Microcirculation, Clinical and Experimental 1983; 2(1): 39-46.
44. Zandi K, Teoh BT, Sam SS, Wong PF, Mustafa MR and Abubakar S: Antiviral activity of four types of bioflavonoid against dengue virus type-2. Vir Jour 2011; 8: 560.
45. Hatti K, Diwakar L, Rao G, et al. (2009) Abyssinones and related flavonoids as potential steroidogenesis modulators. *Bioinformation* 3, 399–402.
46. Borges F, Fernandes E & Roleira F (2002) Progress towards the discovery of xanthine oxidase inhibitors. Curr Med Chem 9, 195–217.
47. 75. Hille R (1996) The mononuclear molybdenum enzymes. Chem Rev 96, 2757–2816.
48. Alnajjar B (2008) *Computational studies of natural flavonoids towards the discovery of a potential xanthine oxidase inhibitor*. MSc Thesis, Universiti Sains, Malaysia.
49. Umamaheswari M, Madeswaran A, Kuppusamy A, et al. (2011) Discovery of potential xanthine oxidase inhibitors using *in silico* docking studies. *Der Pharma Chemica* 3, 240–247.
50. Shoba G, Hari S, Prabhavathi G, et al. (2010) Flavonoids – natural therapeutic agents for polycystic kidney disease. *Int J Pharm Bio Sci* 1, B89–B105.
51. Lin W, Xie J, Wu X, et al. (2014) Inhibition of xanthine oxidase activity by gnaphalium affine extract. *Chin Med Sci J* 29, 225–230.
52. De Souza V, De Franco E, De Araujo M, et al. (2016) Characterization of the antioxidant activity of aglycone and glycosylated derivatives of hesperetin: an *in-vitro* and *in-vitro* study. *J Mol Recognit* 29, 80–87.
53. Perry EK, Tomlinson BE, Blessed G, et al. (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br Med J* 2, 1457–1459.
54. Khan MT, Orhan I & Enol SS (2009) Cholinesterase inhibitory activities of some flavonoid derivatives and chosen xanthone and their molecular docking studies. *Chem Biol Interact* 181, 383–389.
55. Sheng R, Lin X, Zhang J, et al. (2009) Design, synthesis and evaluation of flavonoid derivatives as potent AChE inhibitors. *Bioorg Med Chem* 17, 6692–6698.
56. Zhang J, Wu Y and Zhao X: Chemopreventive effect of flavonoids from Ougan (Citrus reticulata cv. Suavissima) fruit against cancer cell proliferation and migration. J Funct Foods 2014; 10: 511-519.
57. Kelly EH, Anthony RT and Dennis JB: Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. Nutri. Biochem 2002; 13(10): 572-584.
58. Kukic J, Petrovic C and Niketic: Antioxidant activity of four endemic Stachys taxa. Biol Pharmaceut Bull 2006; 29: 725-729.
59. Vasconcelos SML, Goulart MOF, Moura JBDF, Manfredini V, Benfato MDS, Kubota LT. 200 7. Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano oxidativo em sangue humano: Principais métodos analíticos para sua determinação. Quim Nova. 30:1323–1338.

60. Mamede FEAR , Pastore GM . 2004 Wine phenolic compounds: structure and antioxidant action . B Cepp. 22: 233 - 252 .
61. Portinho JA, Zimmermann LM, Bruck MR. 2012. Efeitos Benéficos do Açaí Beneficial effects of açaí. Int J Nutrology. 5:15–20.
62. Cerqueira F, Cordeiro-Da Silva A, Araújo N, Cidade H, Kijjoa A, Nascimento MSJ. 2003. Inhibition of lymphocyte proliferation by prenylated flavones: artelastin as a potent inhibitor. Life Sci. 73:2321–2334.
63. de Souza PO, Bianchi SE, Figueiró F, Heimfarth L, Moresco KS, Gonçalves RM, Hoppe JB, Klein CP, Salbego CG, Gelain DP, Bassani VL. Anticancer activity of flavonoids isolated from Achyrocline satureioides in gliomas cell lines. Toxicology in Vitro. 2018 Sep 1; 51:23-33.