Ind. J. Pharm. Edu. Res, 2023; 57(2):354-371.
https://www.ijper.org

Review Article

Insight into the Glycosylation Methods of the Flavonoids as an Approach to Enhance its Bioavailability and Pharmacological Activities

Aman Dahiya*, Chandana Majee*, Rupa Mazumder, Neha Priya, Salahauddin, Anmol Atriya

Department of Pharmaceutical Chemistry, Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida, Uttar Pradesh, INDIA.

ABSTRACT

Background: Flavonoids are a kind of polyphenol having a diverse set of pharmacological applications, but their bioavailability is limited due to their poor stability, poor solubility, quick clearance, and low intrinsic characteristics. According to the prior study, the flavonoids containing the sugar moiety have better solubility and stability (for example rutin) as they maintain greater plasma levels and a longer mean residence duration than the flavonoids have no glycoside moiety. **Aim:** This review focus on the enhancement of solubility and bioavailability of flavonoids by the various glycosylation techniques. **Materials and Methods:** In this review study, we discussed the various glycosylation approaches to improve solubility and bioavailability, such as microbial biotransformation, enzyme pathway manipulation, metabolic engineering, and chemical synthesis. **Results:** Glycosylation is one of the most efficient methods for increasing flavonoid solubility and stability, as a result the pharmacological activity also enhanced. The glycosylated compound such as scutellarin, quercetin 3-O-glucoside, Cyanidin 3-O-glucoside, kaempferitrin, baicalein 7-O-glucuronide and so on, shows the increased solubility and better pharmacological activities. **Conclusion:** The flavonoids need more attention to overcome their limitations, as they have plethora of applications on human health such as free radical scavenging property, anti-cancer activity, anti-diabetic, anti-inflammatory.

Keywords: Flavonoids, Classification, Limitations, Bioavailability, Glycosylation.

Correspondence:
Mr. Aman Dahiya
Department of Pharmaceutical Chemistry, Noida Institute of Engineering and Technology (Pharmacy Institute), Noida-201306, Uttar Pradesh, INDIA.
Email: amandahiya0011@gmail.com

Ms. Chandana Majee
Department of Pharmaceutical Chemistry, Noida Institute of Engineering and Technology (Pharmacy Institute), Noida-201306, Uttar Pradesh, INDIA.
Email: cmchandana1@gmail.com

Received: 04-04-2022;
Revised: 20-11-2022;
Accepted: 20-01-2023.

INTRODUCTION

Flavonoids (flavus-yellow), or bioflavonoids, are one of the most significant types of natural substances in plants, with over 9,000 identified structures.[1] They are the class of polyphenolic phytochemicals that are linked to a wide range of health-promoting impacts that are essential components in a wide range of nutraceutical, pharmaceutical, medicinal, and cosmetic applications.[2,3] Broad-spectrum pharmacological activities of flavonoids involve antibacterial, antiviral, anti-inflammatory, anti-mutagenic, anti-ulcer, anti-stress, anti-atherosclerosis, anti-diabetic, and antitumor properties, apart from these activities flavonoids are commonly considered as the most effective natural antioxidants found in plants.[4-10] They are effective against various enzymes, including COX, XO, lipoxygenase, and phosphoinositide 3-kinase.[11,12] Based on the study done on animals, it was observed that some of the flavonoids (e.g., quercetin, naringenin) can cross the blood-brain barrier as they are absorbed by the bloodstream, and acts as neuroprotectants in the brain.[13] Flavonoids are found in the fruits coat, seed, bark, and flowers of most plants, present in vegetables (kale, onion, and broccoli), and certain Chinese medicinal herbs.[14-17] They are also found inside the nucleus of mesophyll cells and in the ROS production centers.[18] Flavonoids have a lot of healthcare benefits, but their poor bioavailability has been a matter of concern.[19] Due to their limited solubility and stability, tissue and plasma concentrations of flavonoids are typically insufficient to elicit the required pharmacological effects, even when a significant daily intake of flavonoids is obtained through dietary sources. In humans, the bioavailability of flavonoids is known to be affected by phase 2 metabolism. The major portion of flavonoids are sulfated, methylated, and glucuronidated in the small intestine and liver, and conjugated metabolites are detected in plasma following flavonoid consumption. So, improving bioavailability is essential to impose health effects.[20] To overcome the limitations of the flavonoid's bioavailability, glycosylation is one of the methods by which the solubility, absorption, chemical stability, and metabolism are enhanced to some extent relative to flavonoid aglycones.[21,22]

DOI: 10.5530/ijper.57.2.45

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

This review summarizes the various glycosylation techniques of flavonoids such as microbial biotransformation, enzyme pathway manipulation, metabolic engineering, and chemical synthesis that ultimately increase the solubility and stability further improving the bioavailability of the flavonoids to give desired pharmacological effect.

Chemistry of Flavonoids

Flavonoids are a major class of polyphenolic substances with a benzo-γ-pyrone structure.[23] They refer to two benzene rings (ring A and B) with phenolic hydroxyl that is linked by three central carbon chains and forms a series of compounds with the basic carbon skeleton C6-C3-C6. Flavonoids are classified into flavanol, flavone, flavonol, flavanone, flavanones, isoflavone, and anthocyanins, based on the oxidation level of the three-carbon bonds (C3) and the substitution in the ring A and B makes difference in the individual compounds within a class (Figure 1). Therefore, the C ring is characteristic of each flavonoid subfamily. Monomers differ among subfamilies in terms of the type, location, and several substituents (e.g., hydroxyl groups), and oligomers can also be formed by some flavonoids.[24]

Classification of Flavonoids

Flavonoids are classified according to their chemical structure and source.

Flavones

Flavones have the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). They are mostly found in fruits (apple skins, oranges) and vegetables (Onions, broccoli, tomatoes) and they have a favorable impact on our health with no serious adverse effects.[25]

Flavanones or Dihydroflavones

Flavanones are also known as dihydroflavones, far from flavones the C ring is saturated, although the only variation between the flavanones and flavones is that double bond between C-2 and C-3 is also saturated. Because of their free radical scavenging characteristics, flavanones are assisted to various health benefits.

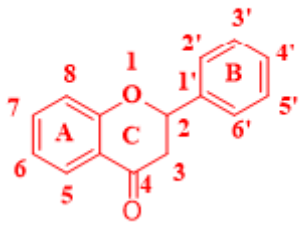


Figure 1: General structure of flavonoid.

Citrus fruit their peels and juice contain flavanones, due to which they have a bitter taste.[26,27]

Flavonols

A double bond is present between C3 and C2 in flavonols, and a hydroxyl group is linked at C3. Flavonols can be found in almost every diet. Flavonols are found in various foods (olive oil, red wine, tea) fruits (grapefruit, berries), and vegetables (onion, broccoli).[28,29]

Isoflavones

Ring B of isoflavones is attached to the C3 site of Ring C. They are mainly found in the leguminous plant family. Soy and its by-products are good sources of isoflavones.[30] Isoflavones can bind to estrogen receptors because they have phytoestrogen activity.[31]

Flavanonols

Flavanonols are usually known as catechins or dihydro flavonols that are3-hydroxy derivatives of flavanones. Flavanonols are a multi-substituted and diverse subclass of polyphenols.

Flavanols or Flavan-3-OLS

Flavanols are sometimes referred to as flavan-3-ols due to the fact that the hydroxyl group is always connected to the C ring in three locations. Far from other, flavonoids, there is no double bond between positions C-2 and C-3. The main source of flavan-3-ols is fruit, vegetables, legumes, beverages (wine and tea), and chocolate.[32]

Flavylium Salts or Anthocyanidin

Flavylium salts are the most often used water-soluble plant dye, and they protect plants against photo-oxidation, as well as serve as insect attractants and food colorants.[33,34]

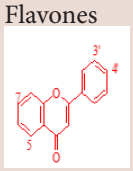
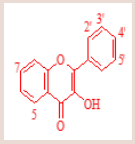
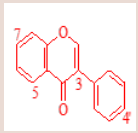
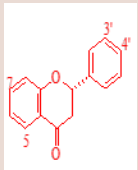
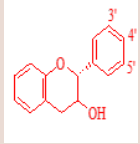
Structure-based classification of flavonoids with their examples, pharmacological activities, and sources are summarized in Table 1.

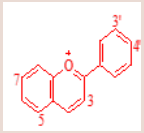
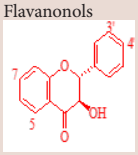
Structure-Activity Relationship of Flavonoid

The activities of flavonoids are structure dependent. Flavonoids' chemical nature is determined by their structural class, degree of hydroxylation, degree of polymerization, and other substitutions and conjugations. Flavonoids and their metabolite's metabolic activity is determined by the relative orientation and chemical structure of distinct moieties on the entity. The hydroxyl functional group in flavonoids shows their antioxidant activity by chelating metal ions or scavenging free radicals. Flavones with 2 or 3 free hydroxyl groups in rings A and B, such as chrysin, luteolin, and apigenin, show antioxidant activity at small doses.[45]

C2=C3 is beneficial for the various pharmacological activities such as antioxidant, antiviral, anti-cancer, anti-bacterial and

Table 1: Major subclasses, sources, and pharmacological activities of flavonoids.

Flavonoid's subclass	Examples	Structure							Pharmacological activities	Source	References
		3	5	7	2′	3′	4′	5′			
Flavones 									Antifungals, Antioxidants, Anti-HIV.	Fruit skin, Red wine, Buckwheat, Red pepper, Tomato	35,36
	Luteolin	-	OH	OH	-	OH	OH	-			
	Apigenin	-	OH	OH	-	-	OH	-			
	Chrysin	-	OH	OH	-	-	-	-			
Flavonols 	Quercetin	-	OH	OH	-	OH	OH		Antioxidants, Cardioprotective, Antiviral, Anti-aging.	Onion, Red wine, Kale, Olive oil, Tea, Grapes	37,38
	Kaempferol	-	OH	OH	-	-	OH				
	Galangin	-	OH	OH	-	-	-				
	Fisetin	-	-	OH	-	OH	OH				
	Myricetin	-	OH	OH	-	OH	OH	OH			
	Morin	-	OH	OH	OH	-	OH	-			
Isoflavones 	Genistein	-	OH	OH	-		OH		Antiestrogenic	Soya beans, Legumes	39,40
	Genistin	-	OH	O-glc	-		OH				
	Daidzein	-	-	OH	-		OH				
	Daidzin	-	-	O-glc	-		OH				
	Biochanin A	-	OH	OH	-		OCH_3				
	Formononetin	-	-	OH	-		OCH_3				
Flavanones 	Hesperetin		OH	OH	-	OH	OCH_3		Chemoprotective effect	Citrus fruits, Grapes, Lemons, Oranges	41
	Naringenin		OH	OH	-	-	OH				
Flavan-3-ol 	(+)-Catechin	ßOH	OH	OH	-	OH	OH		Antioxidant activity, Antiviral activity	Apple, Tea	42
	(-)-Epicatechin	aOH	OH	OH	-	OH	OH				
	(-)-Epigallocatechin	aOH	OH	OH	-	OH	OH	OH			

Flavonoid's subclass	Examples	Structure							Pharmacological activities	Source	References
		3	5	7	2′	3′	4′	5′			
Flavylium Salts 									Antioxidants, Antibacterial, Cytoprotective effect, Anti-inflammatory effect	Cherries, Strawberry, Grapes	43
	Cyanidin	OH	OH	OH	-	OH	OH	-			
	Cyanin	O-glc	OH	OH	-	OH	OH	-			
	Pelargonidin	OH	OH	OH	-	-	OH	-			
Flavanonols 	Toxifolin	-	OH	OH	-	OH	OH	-	Antioxidant, Antiradical	Oranges, Lemons	44

antiradical, antidiabetic and cardioprotective activity. C2=C3 double bond with C3-OH converts flavonols and flavones into improved antioxidant and antiradical activity than the flavanols and flavanones. Catechol moiety is mandatory for pharmacological activities such as antiviral, antibacterial, anti-inflammation, antidiabetic, antioxidant and so on. As the number of OH bond increases the affinity of flavonoids against various activities increases such as anti-cancer, antioxidant activity anti-diabetic, anti-inflammatory. Increased OH decreases the effect against antiviral and anti-bacterial. O-methylation enhances anticancer, antioxidant, and cardioprotective activity. The Carbonyl group (C=O) at C4 increases affinity against various activities such as antidiabetic, antibacterial, antiviral, anti-inflammatory, and antioxidant activities.[46-49]

Glycosylation at C6 and C8 position may increase the activities such as antioxidant, antidiabetic, and glycosylation at O3 and O7 position can improve certain activities such as tyrosinase inhibition, anti-HIV activity, anti-rotavirus activity, anti-stress activity, anticholinesterase, and anti-obesity activity[50] (Figure 2).

Functions of flavonoids and their biological activities

Flavonoids are plant secondary compounds that are well known for the blue, purple, and red, anthocyanin pigments found in plant tissues.[51] These chemicals help plants reproduce by attracting seed dispersers and pollinators. They are responsible for many plants species' appealing fall color, which has been proposed to shield leaf cells from photo-oxidation and hence enhance nutrient retrieval efficiency during senescence.[52] The ability of flavonoids to absorb ultraviolet (UV) light has been thought to be proof of their role in ultraviolet protection. Flavonoids are commonly seen in the epidermal cell layers of leaves, as well as in UV-sensitive tissues including apical meristem and the pollen. Flavonoids are a complex class of secondary metabolites with a wide range of biological functions, including stress reduction.[53] New evidence connecting flavonoids to control of the polar transport of the plant growth regulator auxin has been discovered after a nearly ten-year interval. This hormone may aid in the stress response by limiting stomatal opening[54] and allocating resources in low-growth conditions.[55] They have wide range of pharmacological effects, including anti-hepatotoxic, anti-inflammatory, and anti-ulcer characteristics. Some of the enzymes are also inhibited by the flavonoids which are cyclooxygenase, aldose reductase, calcium ATPase (Ca^{2+}-ATPase), xanthine oxidase, lipoxygenase, and phosphodiesterase. Flavonoids are potent antioxidants with the ability to scavenge free radicals. Many flavonoids have antiallergic and antiviral properties, and some of them can protect against cardiovascular disease (Figure 3).[56,57]

Limitation of flavonoids

The limited bioavailability of flavonoids is one of the primary issues about their usage as active ingredients and numerous health advantages. Even when flavonoids are consumed in significant amounts, tissue and plasma concentrations are typically inadequate to provide the intended pharmacological effects. Flavonoids typically have limited bioavailability due to a range of variables such as chemical structure and molecular weight, relatively poor water solubility, gastrointestinal absorption and metabolism, a lack of site-specificity in distribution, and quick elimination. Additionally, whether exposed to oxygen, temperature changes, UV light, or a pH change, this class of chemicals is very susceptible to deterioration.[58-60] As well as the presence of a double bond between C2 and C3 position of flavonols and flavones forms planar structures, resulting in a tight molecular arrangement, and making it difficult for the solvent to penetrate their molecule structures,[61,62] for example, Myricetin has a poor aqueous solubility of 16.60 g/mL and low oral bioavailability of just 9.62 percent in rats, because of a planar structure.[63,64] Furthermore, excess free hydroxyl groups in intestine cells are easily glucuronidated and sulfated, and then effluxed by some efflux transporters, gastrointestinal permeability decreased as the number of hydroxyl groups in a subclass of flavonoids increased, resulting in poor and erratic

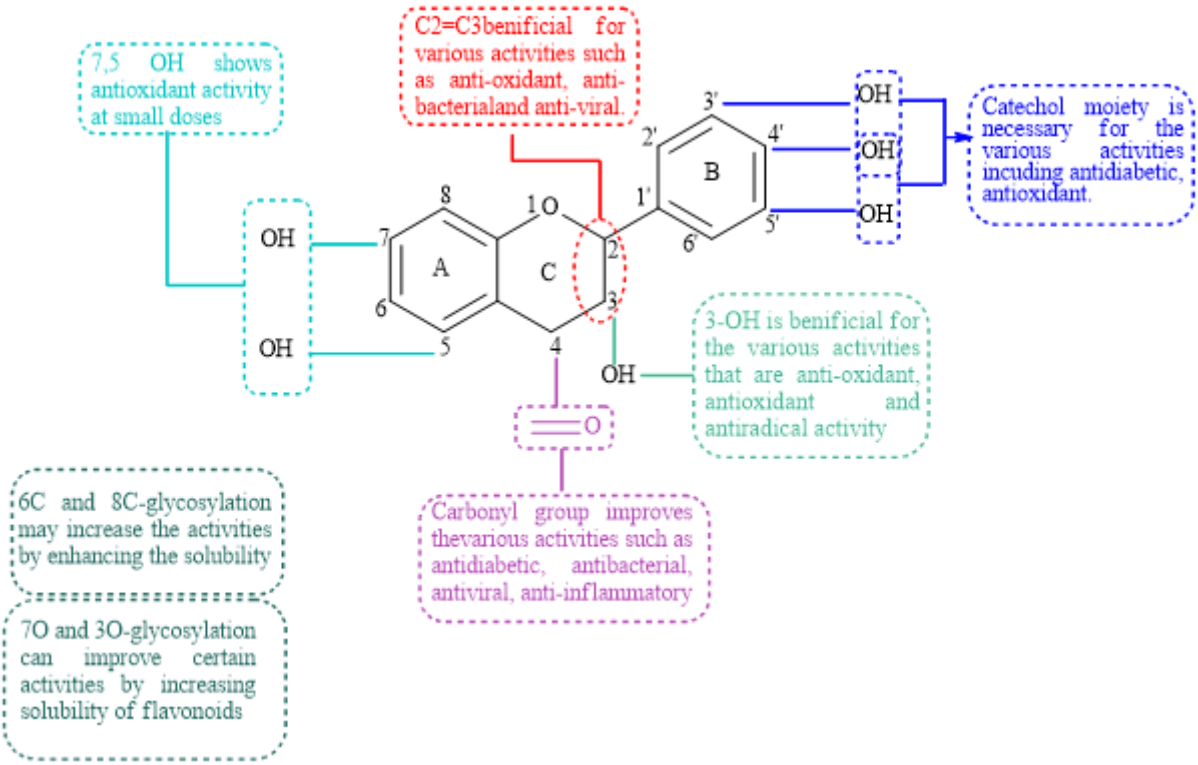


Figure 2: Summary of SAR of flavonoids.

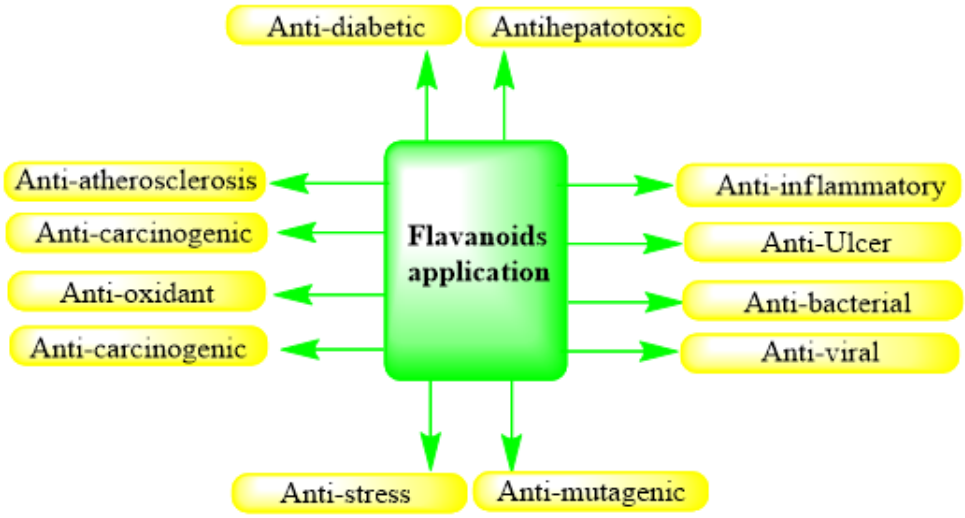


Figure 3: Application of flavonoids.

oral absorption.[65-67] As observed in catechin, a common flavonoid found in grapes, green tea, cocoa, has a poor oral bioavailability of just 5% in rats, which was ascribed to its low permeability (Apparent permeability coefficient (Papp) = 6.0 10-7 cm/s) due to its structure's 5 phenolic hydroxyl groups.[68] Flavonoids undergo extensive metabolic pathways into conjugated compounds, such as glucuronides, methylated derivatives, and sulfates, after being absorbed by the intestinal epithelium, first in the intestine and subsequently in the liver, where they are released into bile.[69,70]

Bioavailability of flavonoids in humans is known to be influenced by phase 2 metabolism.[71] In the small intestine and liver, most flavonoids are sulfated, methylated, and glucuronidated,[72] with

conjugated metabolites detected in plasma flavonoid intake.[73] In general, metabolites of flavonoids have lower bioactivity than the parent molecules.[74] As a result, increasing bioavailability is essential to achieving health benefits *in vivo*. Many attempts have been made to improve bioavailability, including using absorption enhancers,[75] novel delivery methods,[76] enhancing metabolic stability,[77] and switching the absorption location from the large to the small intestine.[78]

Glycosylation of flavonoid: A strategy to enhance its bioavailability

Glycosylation of flavonoid compounds is thought to be an effective way to make them more stable and water-soluble.[79] As well as improves their pharmacological activities by improving bioavailability, lowering acute toxicity and health risks, improving specific targeting, and minors the adverse effects.[80] Sugar chains have a role in practically every biological activity, including cell differentiation, development, immunology, ageing, carcinogenesis, and information transfer.[81] In glycosylation sugar is transferred from an activated donor to the various flavonoid acceptors, that are conjugated through the linkage between flavonoid aglycones, generally showing increased properties than their flavonoid aglycones or even distinct properties.[82] Sugar moieties, depending on the type of the sugar, improve the bioavailability of the flavonoid aglycone; for example, glucosides are absorbed faster than rhamnosides and rhamnoglucosides,[83] owing to the presence or lack of certain hydrolyzing enzymes in the human gastrointestinal tract. While intestinal lactase-phlorizin hydrolase or small intestine epithelial cell rutinosidase, and β-glucosidase may cleave glucosides, there is no human α-L-rhamnosidase or the bioavailability of rhamnose-containing flavonoids is entirely dependent on their breakdown by intestinal microbiota.[84] Furthermore, not all intestinal strains perform this function,[85] and such strains appear to be (almost) absent in other patients. Glycosylation causes considerable inter-individual variance in the bioavailability of aglycones.[86]

Effect of Glycosylation on Stability and Solubility

Few research has been demonstrated that glycosylation influences the stability of flavonoids. According to Buchner *et al.* (2006) investigated the changes in concentration at 100°C of rutin (quercetin-3-O-rutinoside or sophorin) and quercetin in an aqueous solution with air perfusion, and found that rutin was more stable than quercetin.[87,88] In another research the metal-catalyzed oxidative degradation quercetin and rutin were dissolved in phosphate buffer containing Fe^{2+} and Cu^{2+}, during the degradation process the concentration changes were measured and it was seen that rutin had a slower degradation rate.[89]

The solubility of flavonoids is another factor that influences their bioavailability. A large polar component, such as sugar, attached to a flavonoid molecule improves its water solubility, which may lead to higher absorption and bioavailability.[90] However, most published comparisons provide water solubility for distinct flavonoid compounds at a specific temperature and have seen that flavonoids glycosides are more soluble that flavonoid aglycones. For example, at 20°C aglycone naringenin has a solubility of 4.38mg/L[91] whereas naringin (naringenin+ glycoside) has a solubility of 500mg/L (more than 110-fold higher).[92] The same pattern may be seen in rutin and quercetin. Rutin has a solubility of 125 mg/L in water,[93] where as quercetin aglycone has a solubility of 0.512 mg/L (more than 240 times lower).Another example of quercetin at 25°C has a poor water solubility (0.92 g/L) and the semisynthetic glycosylated quercetin is claimed to have significantly improved water solubility.[94]

Glycosylation technique

Glycosylation is a key tailoring step for the production or modification of biologically active compounds that can modify the physico-chemical properties of hydrophobic molecules like flavonoids. For the laboratory glycosylation of flavonoid aglycones, a number of molecular biology and biochemical approaches have been employed involving microbial biotransformation, enzyme

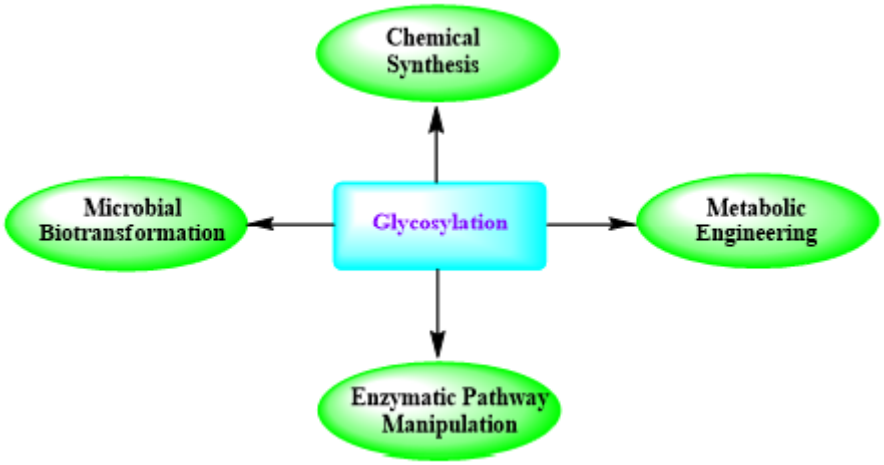


Figure 4: Approaches for Glycosylation.

pathway manipulation, metabolic engineering, and chemical synthesis[95] as shown in Figure 4.

In vitro, direct enzymatic catalysis maybe even more concise and fasterthan metabolic engineering *in vivo*, but it requires equimolar volumes of expensive uridine diphosphate (UDP)-sugar. Furthermore, elucidating the mechanism of enzymatic glycosylation regioselectivity and stereoselectivity remains a challenge, preventing their future use. We expect that a regeneration strategy to prepare various sugar donors *in situ*,[96] as well as an immobilized enzyme technique and more in-depth structure analysis to reveal the mechanism of enzymatic reactions, will bedeveloped that would lower the cost, and facilitate the rational design of more novel flavonoid glycosides. *In vivo*, metabolic engineering might be an alternative way to produce glycosides as cell metabolism can offer a constant supply of UDP-glucose. However, low product yields might occur because of redox imbalance and excessive metabolic load, necessitating pathway compartmentalization for optimal function. Fortunately, progress has been constant in this field over the last five years, with improved enzyme expression, better enzymes arising from protein engineering, and better-engineered microbes utilized as carriers. Co-cultivation of multiple engineered microbial strains, for example, significantly improved product yields by distributing metabolic burden between co-cultivation partners. Several challenges exist in this area; for example, large-scale preparation is challenging, and just a few studies have reached milligram size. Biotechnology, on the other hand, has considerable potential in terms of providing an alternate method to meet industrial demands.[97]

Chemical Glycosylation of 3,7 Dihydroxyflavone

Neves *et al.* (2018) promoted the synthesis of 3-hydroxy-7-(2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl) flavone and 3,7-(2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl) flavone by glycosylation of 3,7-dihydroxyflavone with K_2CO_3 in dry acetone by using the modified Michael method. The glucose donor used was 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide for the glycosylation (Figure 5).

It was seen that glycosylation enhances the stability which alters the *in vitro* cell growth inhibitory activity of the 3 hydroxy-7-(2,3,4,6-tetra-O-acetyl-ß-glucopyranosyl) flavone and 3,7-(2,3,4,6-tetra-O-acetyl-ß-glucopyranosyl) flavone and was investigated in six human tumor cell lines that are MCF-7 (Breast adenocarcinoma), A375-C5 (malignant melanoma IL-1 insensitive), U373 (glioblastoma astrocytoma), NCI-H460 (non-small cell lung cancer), U251 (glioblastoma astrocytoma), and U87MG (glioblastoma astrocytoma). It was tested that 3-hydroxy-7-(2,3,4,6-tetra-O-acetyl-ß-glucopyranosyl) flavone has shown potent against all tumor cell lines, with growth inhibition value < 8 μM and a notable degree of selectivity for cancer cells.[98]

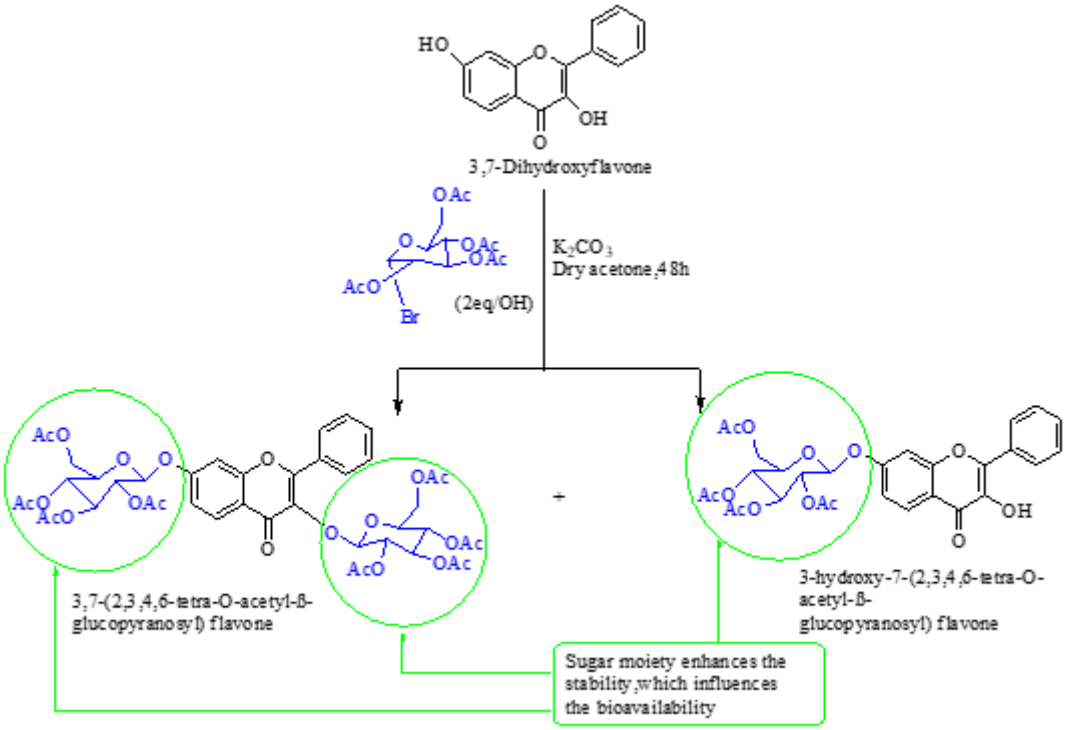


Figure 5: Synthesis of synthesis of 3,7-(2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl) flavone and 3-hydroxy-7-(2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl) flavone from 3,7-dihydroxyflavone.

Glycosylation of apigenin by microbial biotransformation and metabolic engineering

The microbial biosynthesis of scutellarin in *E. coli* from the apigenin, by glucosyltransferases. The recombinant *E. coli* strain produces the UDP-glucosyltransferase UGT71G1 from *M. truncatula* after heterologous overexpression to synthesize Apigenin-7-O-G and the enzyme flavone 6-hydroxylase converted the apigenin-7-O-G into scutellarein. Where asapigetrin was also synthesized from apigenin (derived from p-coumaric acid (PCA) using culture system) by glycotransferase and UDP-glucose was used as a donor (Figure 6).

The glycosylated derivatives of apigenin show increased stability and solubility which shows altered bioavailability i.e., Scutellarin can be used to treat metabolic syndrome caused by high-fructose and high-fat diet by decreasing leptin level and boosting adiponectin levelon-the-other-hand apigetrin have been proven to be a well-known anti-aging, anti-fungal, anti-tumor, and anti-inflammatory agents along with anti-proliferative and antioxidant activity against reactive oxygen species (ROS).[99,100]

Enzymatic glycosylation (+)-catechin

Synthesis of (+)-Catechin-3'-O-a-D-maltoside and (+)-Catechin-3'-O-a-D-glucopyranoside by trans glycosylation reaction of (+)-Catechin catalyzed by amylosucrase from *Deinococcus geothermalis* and sucrose was used as a donor (Figure 7).

The catechin shows low solubility in aqueous media due to which it has low bioavailability. After glycosylation, it was seen that the solubility of catechins was enhanced thus increasing the bioavailability of catechins that enhances the biological activities of catechins such as antioxidants.[101]

Glycosylation of Quercetin by microbial and enzymatic biosynthesis

Strugała *et al.* (2017) proposed the synthesis of Quercetin 7-O-ß-D(4"-O-methyl)-glucopyranoside from quercetin by regioselective glycosylation at position 7, that is catalyzed by *Beauveria bassiana* AM278 strain (Figure 8).

It was seen that orally administered quercetin has poor bioavailability due to low absorbance, the introduction of large polar moiety i.e., sugar to quercetin increases the solubility that enhances the absorbance by which the bioactivity towards antioxidant and anti-inflammatory activity also increased (i.e., 7-O-ß-D(4"-O-methyl)-glucopyranoside).

The microbial biotransformation of quercetin into quercetin 3-O-ß-D-glucopyranoside by *Cunninghamella elegans* ATCC 9245[102] (Figure 8). The intestinal absorption of quercetin

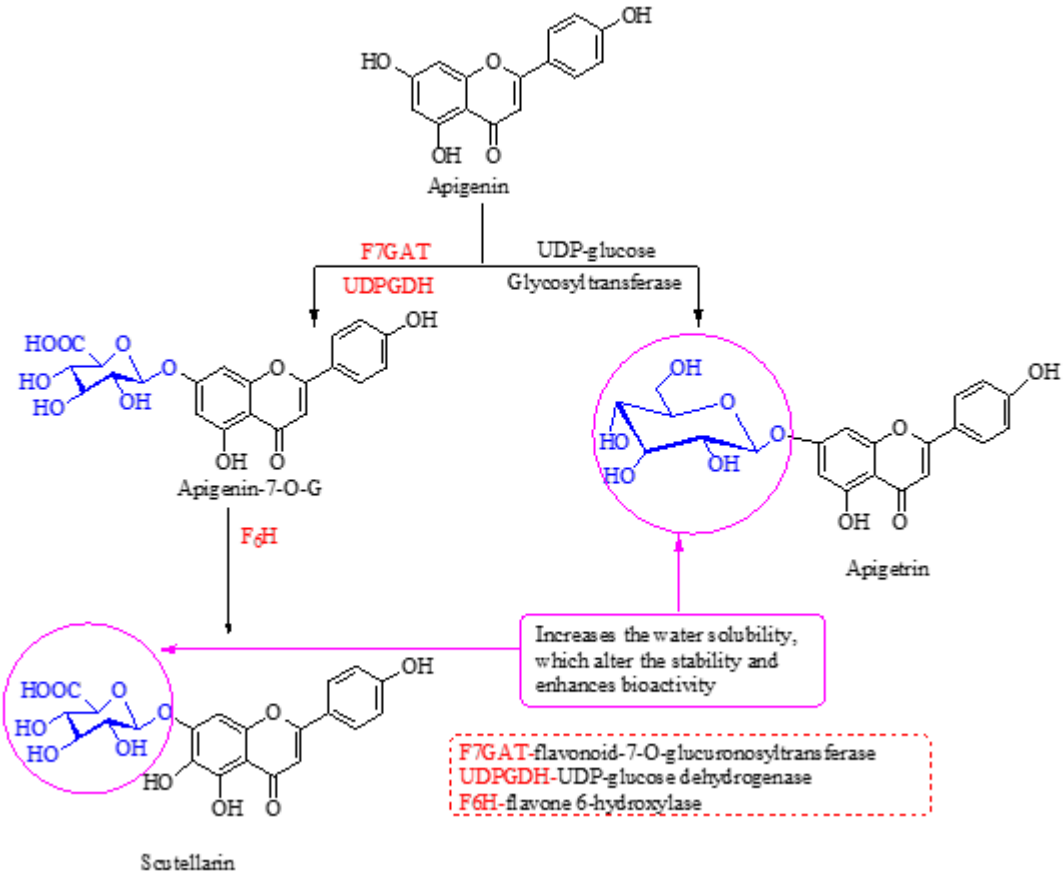


Figure 6: Microbial biosynthesis of scutellarin and metabolic engineering of apigetrin from apigenin.

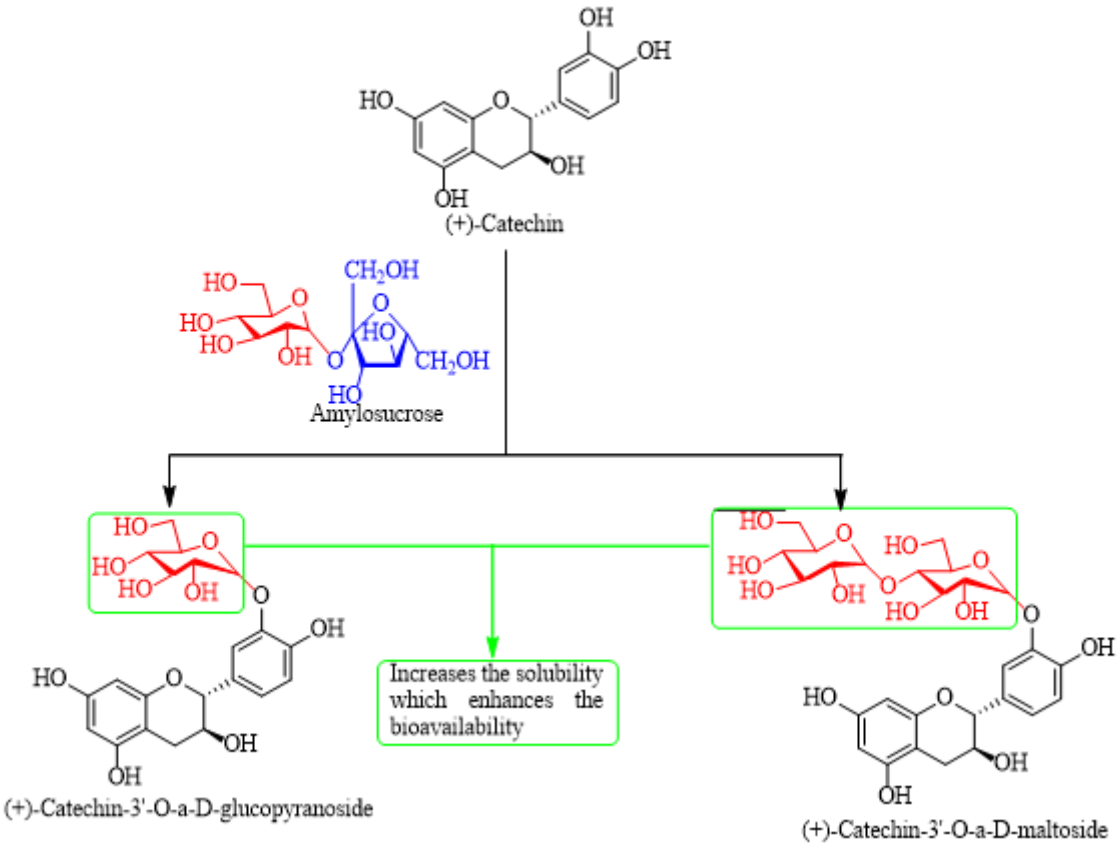


Figure 7: Synthesis of (+)-Catechin-3'-O-a-D-maltoside and (+)-Catechin-3'-O-a-D-glucopyranoside from (+)-Catechin.

3-O-glucoside increases +184% compared to quercetin in a rat model.[103] Quercetin 3-O-glucoside shows a greater anti-obesity effect than quercetin aglycon and its anti-obesity impact is mediated by inhibiting adipocyte differentiation and lipogenesis, lowering serum lipid levels through changing hepatic lipid metabolism, and decreasing body weight gain. It was also seen that quercetin 3-O-glucoside shows stronger activity against the gram-positive bacteria.[104]

Synthesis of isorhamnetin 3-O-glucoside from Quercetin by converting it into isorhamnetin by O-methyltransferase from rice (ROMT9) which was eventually converted into isorhamnetin 3-O-glucoside by PGT Glu82Leu (Figure 8).

The glycosylation of quercetin has a profound effect on its solubility, stability, and bioactivity.

Isorhamnetin 3-O-glucoside shows the effect on diabetic complications.[105]

Glycosylation of 6-methylflavone by microbial biotransformation

Synthesis of 6-methylflavone 4'-*O*-β-D-(4º-*O*-methyl)-glucopyranoside and (6-methylflavone 8-*O*-β-D-(4º-*O*-methyl)-glucopyranoside from 6-methylflavone in the culture of *I. fumosorosea.* They reported being the first to introduce a sugar moiety to a flavonoid molecule that lacked a hydroxyl group (Figure 9). It was seen that 6-methylflavone is the most potent inhibitor of GABAA/benzodiazepine receptors, but its application is limited because of its low water solubility that has low bioavailability. The addition of the sugar moiety to 6-methylflavone improves its stability, water solubility, and bioavailability. UV absorption of 6-methylflavone and its biotransformation derivatives were compared, and it was seen that 6-methylflavone 4'-*O*-β-D-(4º-*O*-methyl)-glucopyranoside) shows the highest absorbance and is more active in the inhibition of the GABAA/benzodiazepine receptors.

Enzymatic Glycosylation of Cyanidin

The synthesis of Cyanidin 3-O-glucoside from Cyanidin in the presence of catalyst *Vitis vinifera* UDP-Glucose. The addition of glucose to the 3-O site of cyanidin by 3-O-glycosyltransferase comes from a UDP-glucose donor (Figure 10).

Cyanidin is an unstable flavonoid to enhance its stability, it was glycosylated to yield the cyanidin 3-O-glucoside, as the stability enhances, the desired the pharmacological activities were obtained against anti-inflammatory, and antioxidant.[106]

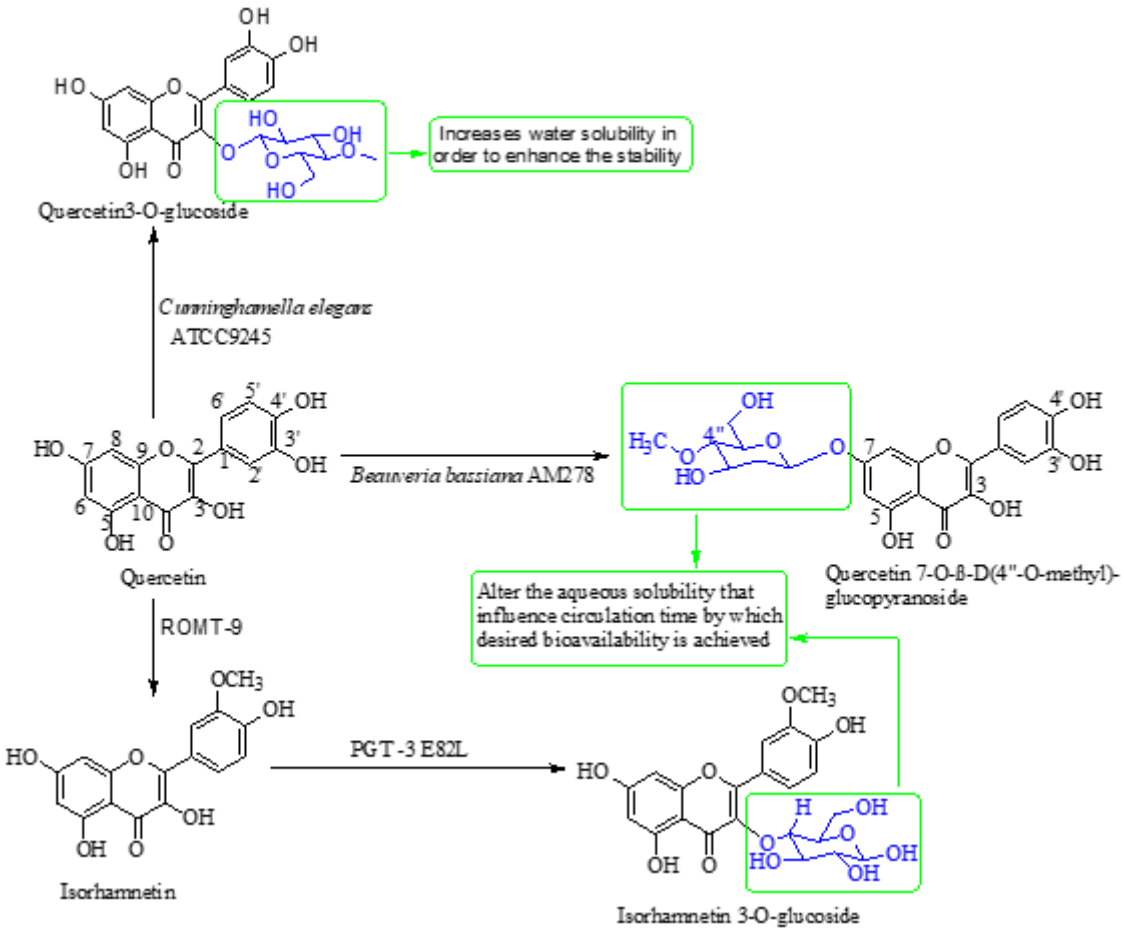


Figure 8: Synthesis of Quercetin 7-O-ß-D(4"-O-methyl)-glucopyranoside, quercetin 3-O-glucoside, and isorhamnetin 3-O-glucoside from quercetin.

Glycosylation of Baicalein by metabolic engineering

The synthesis of baicalein 7-O-glucuronide from the baicalein by glycosylation, UDP (uridine diphosphosphate) glucuronic acid was used as the glycosyl donor (Figure 11).

The glycosylation of baicalein increases the solubility that shows the increased antiallergic, antioxidative, and anti-inflammatory activities. Various activities such as tyrosinase inhibition, anti-HIV, anti-rotavirus, anti-stress, anticholinesterase, anti-obesity potential, antiadipogenic, antiallergic, and treatment for chronic kidney disease can be improved from O-glycosylation.[107]

Glycosylation of Xanthohumol by microbial biotransformation

The synthesis of α, ß-Dihydroxanthohumol 4'-O-ß-D-glucopyranoside by converting xanthohumol into α, ß- xanthohumol by chemical method, in this method regioselective hydrogenation of xanthohumol takes place in the presence of palladium on the charcoal catalyst and hydrogen gas was used as a donor of hydrogen atoms, after that microbial(fungal) glycosylation of α, ß-dihydroxanthohumol into α, ß -dihydroxanthohumol 4'-O-ß-D-glucopyranoside by *Absidiacoeruela* AM93 and *Absidia glauca* AM177 was done (Figure 12).

The introduction of sugar moiety enhances the absorbance of α, ß -Dihydroxanthohumol 4'-O-ß-D-glucopyranoside and seen thar a, ß -Dihydroxanthohumol 4'-O-ß-D-glucopyranoside shows strong anti-inflammatory activity by inhibiting the cyclooxygenase enzyme.

Enzymatic Glycosylation of Puerarin

The glycosylation of puerarin into monofructosylpuerarin and difructosylpuerarin was attained in a 25% DMSO solvent system, β -fructosidase (solvent stable) and sucrose as a donor (Figure 13). It was seen that the glycosylation promoted the solubility of puerarin by which the bioavailability towards the treatment of heart disease, ocular disease, diabetes, and cancer increased.[108]

Chemical Glycosylation of Kaempferol

Kaempferitrin was synthesized by using kaempferol through Koenigs-Knorr glycosylation of the 3, 7-diol to afford 3, 7-O-bisglycoside as an intermediate (Figure 14). It was seen that the solubility of kaempferol was enhanced which increases

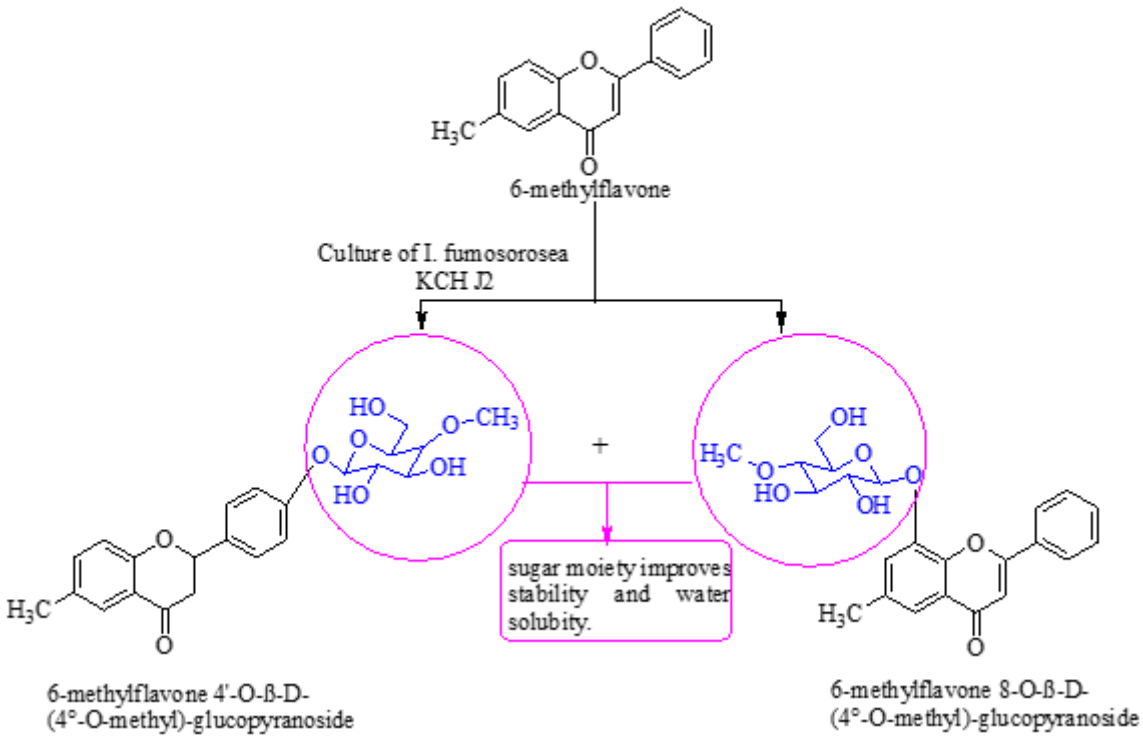


Figure 9: Synthesis the 6-methylflavone 4'-*O*-β-D-(4º-*O*-methyl)-glucopyranoside and (6-methylflavone 8-*O*-β-D-(4º-*O*-methyl)-glucopyranoside from 6-methylflavone.

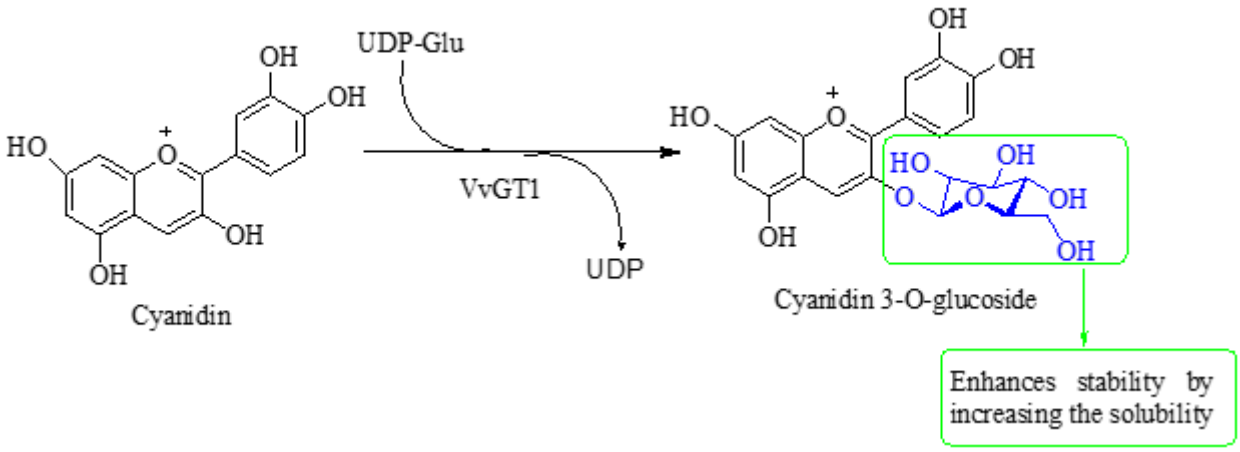


Figure 10: Synthesis of Cyanidin 3-*O*-glucoside from Cyanidin.

the bioactivity. As a result, antimicrobial activity, inhibition of bacterial cell division, and inhibitory effect against DNA topoisomerase also increase.[109]

Enzymatic Glycosylation of phloretin

The glycosylation of phloridzin from phloretin by glycosyltransferase clones isolated from pyrus (UGGT88F2) as shown in Figure 15.

As the solubility of phloretin increases by glycosylation which makes it possible to get the desired bioavailability as a result anti-diabetic anticarcinogenic activity and anti-HIV activity increase. It was seen that O-glycosylation promotes different types of bioactivities including anti-HIV activity, tyrosinase inhibition, anti-rotavirus activity, and anti-cholinesterase potential.[110]

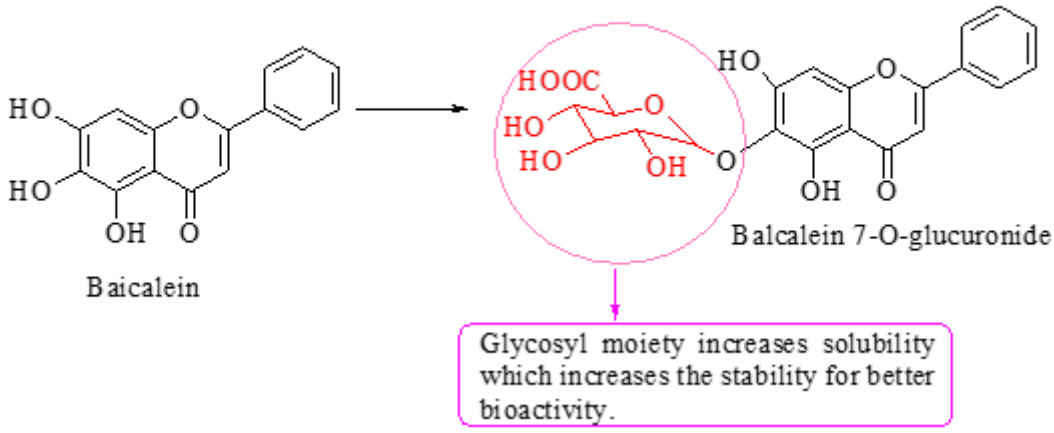


Figure 11: Synthesis of baicalein 7-O-glucuronide from baicalein.

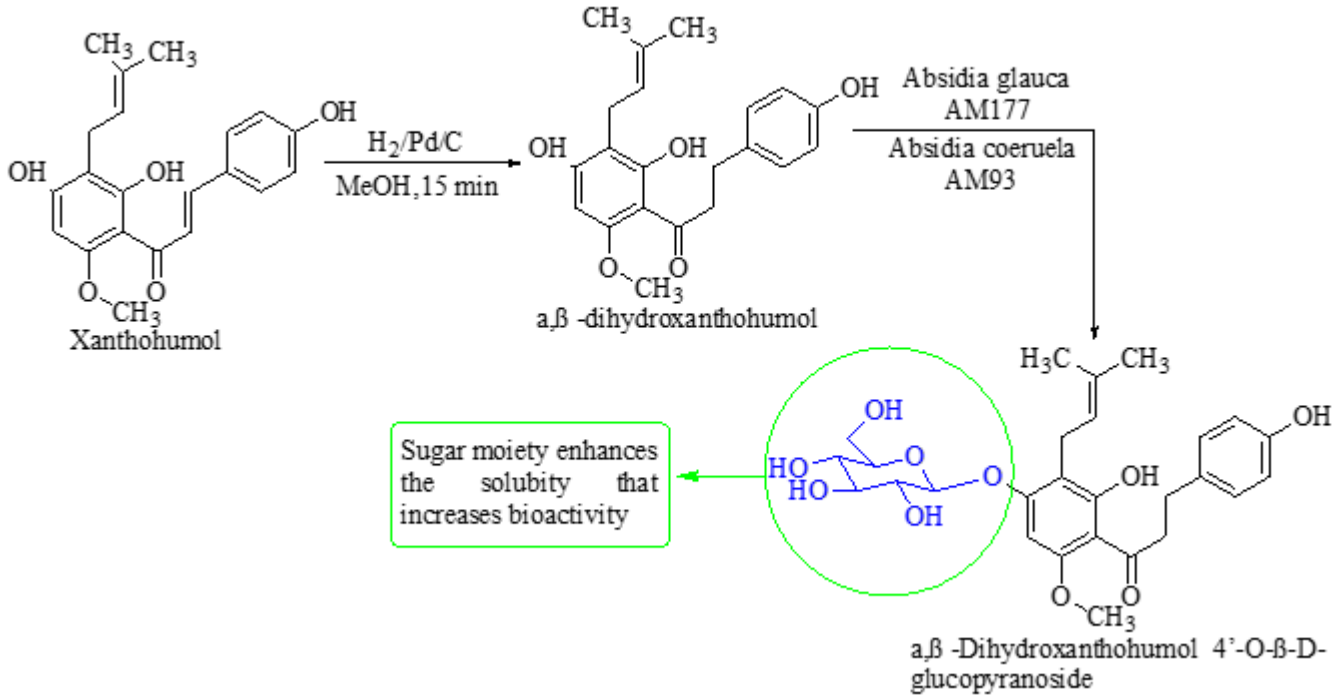


Figure 12: Synthesis of α, ß-Dihydroxanthohumol 4'-O-ß-D-glucopyranoside from xanthohumol.

Microbial Glycosylation of isoangustone A

Isoangustone A 7-O-β-D-glucopyranoside is derived from the biotransformation of Isoangustone A in the culture of *Mucor hiemalis* CGMCC 3.14114 at 30°C for 48 hr (Figure 16).

Isoangustone A 7-O-β-D-glucopyranoside shows enhanced bioavailability than isoangustone A against antimicrobial, antioxidative, anti-inflammatory, and antitumor activities due to enhanced water solubility.[111]

Microbial Glycosylation of Kuarinone

Kurarinone glycosylation is performed by Strain *C. echinulata*AS 3.3400 to form kurarinone 7-O-β-D-glucopyranoside. It was seen that kurarinone 7-O-β-D-glucopyranoside shows better pharmacological properties such as tyrosinase inhibitors and cytotoxicity than Kurarinone due to enhanced water solubility (Figure 17).[111]

Table 2 summarizes the patents on glycosylate flavonoids.

Table 2: Patents offlavonoids.

Sl. No.	Patent No.	Patent Date	Inventor	Description	References
1	EP 1 891 967	Feb. 27, 2008	Williamson, Jankovic, Rein	This invention provides a method for sustaining and improving the bioavailability of flavonoids (hesperidin).	112
2	US 20100055081	Mar. 4, 2010	Myriam Richelle, Gary Williamson, Ivana Jankovic, Maarit Rein.	This invention provides a method for sustaining and improving the bioavailability of rhamnose-containing flavonoids (hesperidin).	113
3	US008449927	May 28, 2013	Thomas Eidenberger	This invention describes a unique composition of anthocyanin and stabilizing agent which does not readily undergo degradation such as oxidation, pH instability.	114
4	WO 2014/191524	Dec. 4, 2014	Rabausch, Streit, Jurgensen, Julia	This invention comprises enzymatic glycosylation of the flavonoids that enhance the solubility, which impacts the flavonoid's functions.	115
5	US 20150224158	Aug. 13, 2015	Chantale, Serge, Christian	The inventors promised that glycosylated flavonoids show higher solubility and are more active than aglycone against the activities such as anti-cancer.	116
6	WO 2017/121863	July 20, 2017	Rabausch, Rosenfeld, Ilmberger, Plambeck, Ruprecht, Bonisch	This invention promotes the production of rhamnosylatedflavonoids which leads to higher solubility and increases stability against radiation and temperature, furthermore, the flavonoid glycosides show modulated bioavailability and pharmacological activities.	117
7	USOO894.6407	Feb. 3, 2015	Bingfang He, Xueming Wu, Jianlin Chu, Bin Wu, Sen Zhang, Pingkai Ouyang	In the invention, the inventors proposed the preparation method for fructosylatedmangiferin with improved dissolvability and bioavailability to resist inflammation, to relieve cough, and reduce phlegm.	118
8	US 20160090578	Mar. 31, 2016	Ulrich Rabausch, Wolfgang Streit, Julia Juergensen,	This invention comprises enzymatic glycosylation of the flavonoids that enhance the solubility, which impacts the flavonoid's functions.	119
9	US009828407	Nov. 28, 2017	Daniel Auriol, Renaud Nalin, Patrick Robe, Fabrice Lefevre	This invention proposed the production of the polyphenols (flavonoid) glycoside by enzymatic condensation, the derivatives have the higher water solubility than that of their parent compound which have useful applications in cosmetic and pharmacological such as antioxidant, anti-inflammatory, antiviral, antibacterial, etc.	120
10	AU 2003270537	April 30, 2004	Deavours, Bettina, Liu, Chang-Jun, Dixon, Richard A	The inventors produced flavonoids and iso-flavonoids nutraceuticals by converting them into their glycosides.	121

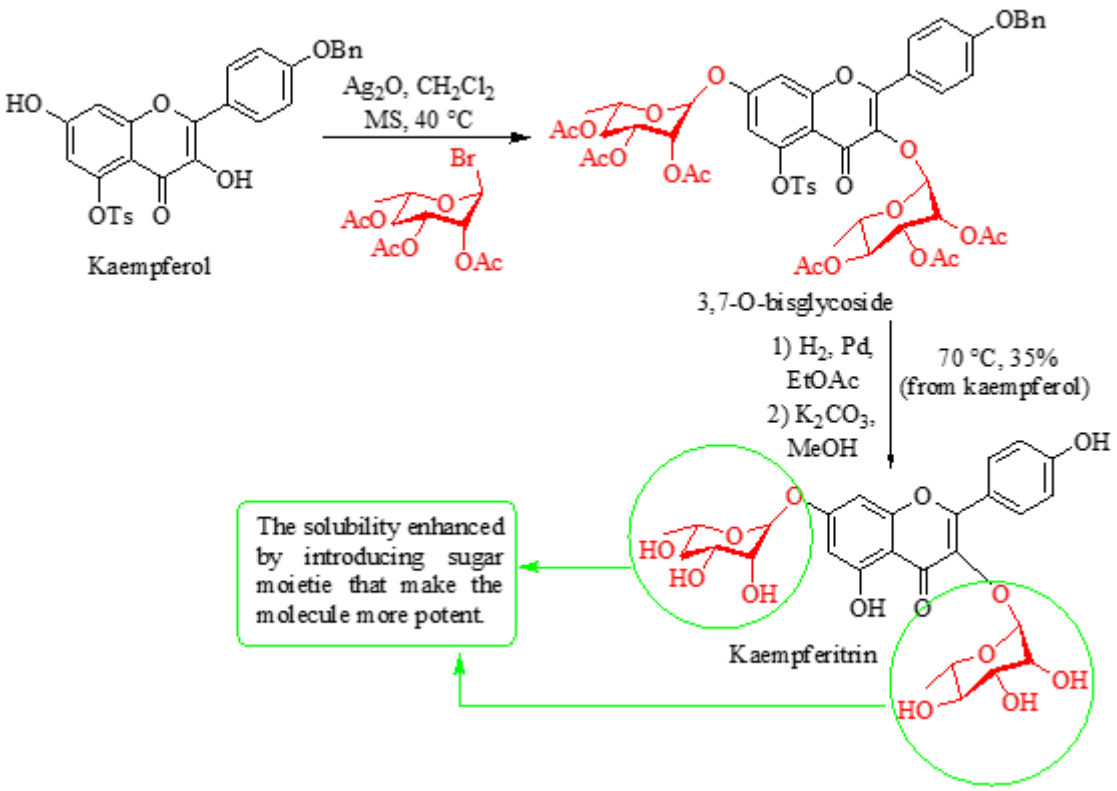


Figure 14: Synthesis of kaempferitrin from kaempferol.

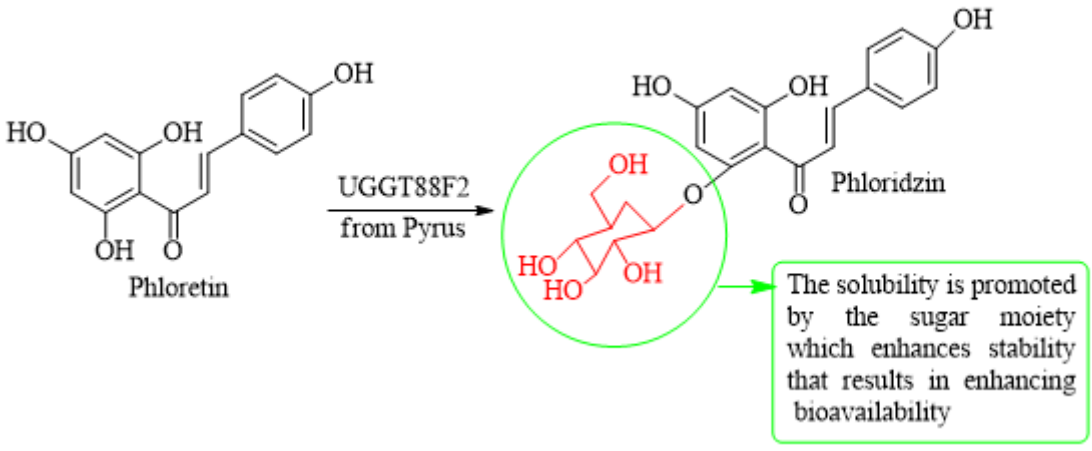


Figure 15: Synthesis of phloridzin from phloretin.

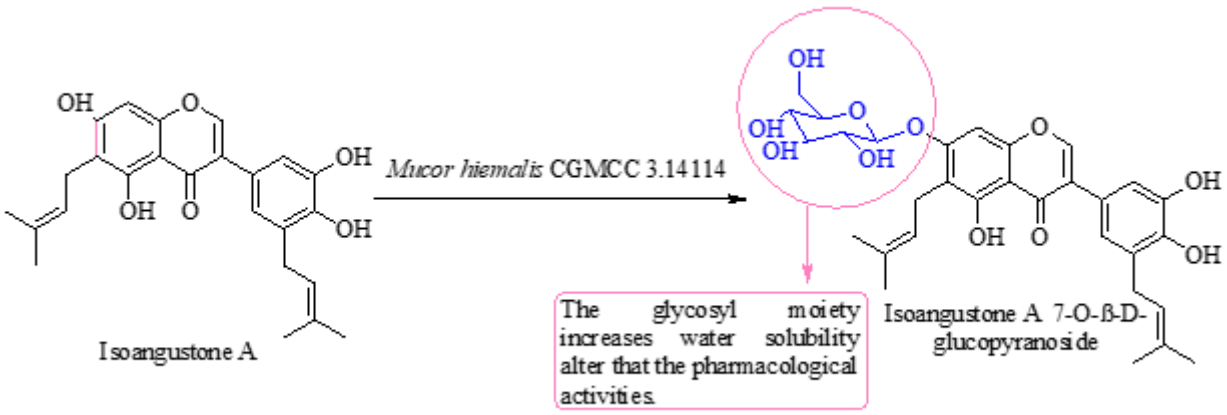


Figure 16: Biotransformation of Isoangustone A into Isoangustone A 7-O-β-D-glucopyranoside.

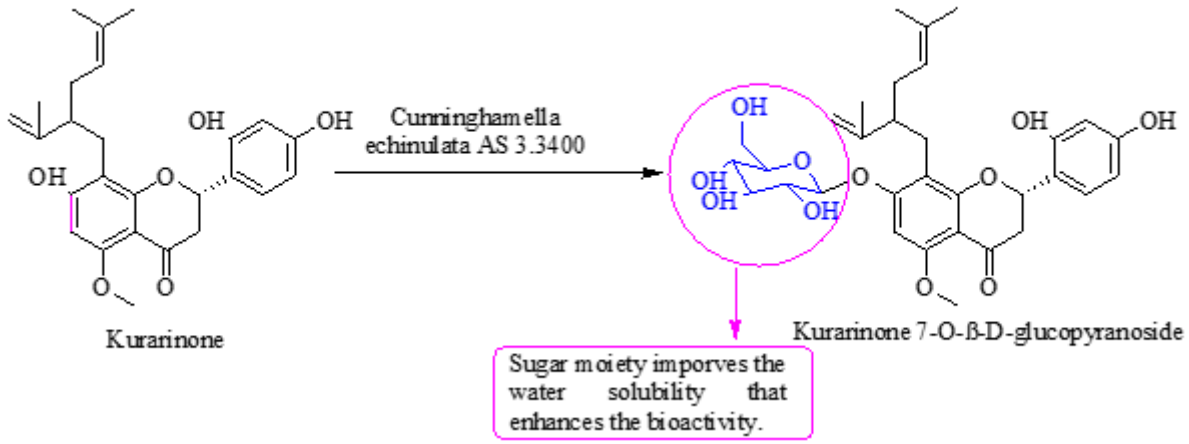


Figure 17: Synthesis of kurarinone 7-O-β-D-glucopyranoside from kurarinone.

CONCLUSION

The flavonoids are the natural polyphenols with various applications on human health such as free radical scavenging property, anti-cancer activity, anti-diabetic, anti-inflammatory. Despite their use is limited due to poor solubility and bioavailability. Glycosylation of flavonoids is one of the potent methods to enhance the water solubility and thus increases the bioavailability of the bioactive compound. The glycosylation of flavonoids by various biochemical and molecular biology methods, including metabolic engineering, enzymatic glycosylation, chemical glycosylation, and microbial glycosylation. The glycosylated compound such as scutellarin, quercetin 3-O-glucoside, Cyanidin 3-O-glucoside, kaempferitrin, baicalein 7-O-glucuronide and so on, shows the increased solubility and better pharmacological activities than their aglycone. More research should be done on flavonoid glycosylation so that it can be employed effectively, as it is a more accessible method than medication development.

ACKNOWLEDGEMENT

I would like to thank our managing director Dr. O.P. Agrawal of Noida Institute of Engineering and Technology (Pharmacy Institute) for providing the facilities and their support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

COX: Cyclooxygenase; **XO:** Xanthineoxidase; **ROS:** Reactive Oxygen Species; **UV:** Ultraviolet; **ATPase:** Adenosine Triphosphatase; **UDP Glucose:** Uridine Diphosphate Glucose; ***E-coli*:** *Escherichia coli;* **GABA A:** Gamma-Aminobutyric acid Type A; **HIV:** Human Immunodeficiency Virus; **DMSO:** Dimethyl Sulfoxide; **DNA:** Deoxyribonucleic Acid.

SUMMARY

The naturally occurring polyphenol, flavonoids have various health benefits with plenty of pharmacological activities including anti-diabetic, anti-tumor, antioxidant, anti-ulcer, anti-bacterial, anti-viral.

Even after they have various pharmacological effects, but their use is limited due to less solubility and that have an impact on the bioavailability that makes them less potent.

There are several approaches to overcome the solubility and bioavailability, but the glycosylation is the most efficient methods for increasing flavonoid solubility, stability and the bioavailability and make them potential towards the pharmacological activities.

In this review, we have focused on the glycosylation techniques to overcome the bioavailability that are metabolic engineering, enzymatic glycosylation, chemical glycosylation, and microbial glycosylation. These techniques of glycosylation effectively increase the bioavailability.

REFERENCES

1. Pilon AC, Gu H, Raftery D, Bolzani VDS, Lopes NP, Castro-Gamboa I, *et al*. Mass spectral similarity networking and gas-phase fragmentation reactions in the structural analysis of flavonoid glycoconjugates. Anal Chem. 2019;91(16):10413-23. doi: 10.1021/acs.analchem.8b05479, PMID 31313915.
2. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-56. doi: 10.1016/j.ijantimicag.2005.09.002, PMID 16323269.
3. Badshah SL, Faisal S, Muhammad A, Poulson BG, Emwas AH, Jaremko M. Antiviral activities of flavonoids. Biomed Pharmacother. 2021;140:111596. doi: 10.1016/j.biopha.2021.111596, PMID 34126315.
4. Aquila S, Giner RM, Recio MC, Spegazzini ED, Ríos JL. Anti-inflammatory activity of flavonoids from *Cayaponia tayuya* roots. J Ethnopharmacol. 2009;121(2):333-7. doi: 10.1016/j.jep.2008.11.002, PMID 19041703.
5. Miyazawa M, Hisama M. Antimutagenic activity of flavonoids from *Chrysanthemum morifolium*. Biosci Biotechnol Biochem. 2003;67(10):2091-9. doi: 10.1271/bbb.67.2091, PMID 14586095.
6. Borrelli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies. Phytother Res. Int J Respir To devoted Pharmacol Toxicol Evaluation of Nat. Prod. Derivatives. 2000;14(8):581-91.
7. Watanabe M, Ayugase J. Anti-stress effects of flavonoids from buckwheat sprouts in mice subjected to restraint stress. Food Sci Technol Res. 2008;14(3):253-60. doi: 10.3136/fstr.14.253.
8. Wu Y, Wang Y, Liu X, Jiang L, Guli A, Sailike J, *et al. Ziziphora clinopodioides* flavonoids based on network pharmacology attenuates atherosclerosis in rats induced by high-fat emulsion combined with vitamin D3 by down-regulating VEGF/AKT/NF-κB signaling pathway. Biomed Pharmacother. 2020;129:110399. doi: 10.1016/j.biopha.2020.110399, PMID 32768933.
9. Babu PV, Liu D, Gilbert ER. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. J Nutr Biochem. 2013;24(11):1777-89. doi: 10.1016/j.jnutbio.2013.06.003, PMID 24029069.
10. Jan NU, Kifayatullah M, Amin F, Rahim H, Abbas S, Mohani SNUH, *et al*. Antioxidant and cytotoxic activity of Steroidal alkaloids Isolated from *Sarcococca saligna* against DPPH and HeLa Cell Lines. Indian J Pharm Educ Res. 2022;56(2):489-96. doi: 10.5530/ijper.56.2.70.
11. Sebastian N, Yu WC, Balram D. Synthesis of amine-functionalized multi-walled carbon nanotube/3D rose flower-like zinc oxide nanocomposite for sensitive electrochemical detection of flavonoid morin. Anal Chim Acta. 2020;1095:71-81. doi: 10.1016/j.aca.2019.10.026, PMID 31864632.
12. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci. 2016;5:e47. doi: 10.1017/jns.2016.41, PMID 28620474.
13. Panhwar QK, Memon S, Bhanger MI. Synthesis, characterization, spectroscopic and antioxidation studies of Cu(II)–morin complex. J Mol Struct. 2010;967(1-3):47-53. doi: 10.1016/j.molstruc.2009.12.037.
14. Cook NC, Samman S. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem. 1996;7(2):66-76. doi: 10.1016/0955-2863(95)00168-9.
15. Tiwari SC, Husain NI. Biological activities and role of flavonoids in human health–A. Indian J Sci Res. 2017;12(2):193-6.
16. Dymarska M, Grzeszczuk J, Urbaniak M, Janeczko T, Pląskowska E, Stępien Ł, *et al*. Glycosylation of 6-methylflavone by the strain *Isaria fumosorosea* KCH J2. PLoS One. 2017;12(10):e0184885. doi: 10.1371/journal.pone.0184885, PMID 28981527.
17. Yan S, Xie M, Wang Y, Xiao Q, Ding N, Li Y. Semi-synthesis of a series natural flavonoids and flavonoid glycosides from scutellarin. Tetrahedron. 2020;76(8). doi: 10.1016/j.tet.2020.130950, PMID 130950.
18. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Scientific World Journal. 2013;2013:162750. doi: 10.1155/2013/162750, PMID 24470791.
19. Thilakarathna SH, Rupasinghe HP. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients. 2013;5(9):3367-87. doi: 10.3390/nu5093367, PMID 23989753.
20. Leonarduzzi G, Testa G, Sottero B, Gamba P, Poli G. Design and development of nanovehicle-based delivery systems for preventive or therapeutic supplementation with flavonoids. Curr Med Chem. 2010;17(1):74-95. doi: 10.2174/092986710789957760, PMID 19941477.
21. Alseekh S, Perez de Souza LP, Benina M, Fernie AR. The style and substance of plant flavonoid decoration; towards defining both structure and function. Phytochemistry. 2020;174:112347. doi: 10.1016/j.phytochem.2020.112347, PMID 32203741.
22. Xiao J, Chen T, Cao H. Withdrawn: Flavonoid glycosylation and biological benefits. Biotechnol Adv. 2014. doi: 10.1016/j.biotechadv.2014.05.004, PMID 24858477.
23. Scheidt HA, Pampel A, Nissler L, Gebhardt R, Huster D. Investigation of the membrane localization and distribution of flavonoids by high-resolution magic angle spinning NMR spectroscopy. Biochim Biophys Acta Biomembr.;1663;1-2:97-107.
24. Singla RK, Dubey AK, Garg A, Sharma RK, Fiorino M, Ameen SM, *et al*. Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. J AOAC Int. 2019;102(5):1397-400. doi: 10.5740/jaoacint.19-0133, PMID 31200785.
25. Singh M, Kaur M, Silakari O. Flavones: An important scaffold for medicinal chemistry. Eur J Med Chem. 2014;84:206-39. doi: 10.1016/j.ejmech.2014.07.013, PMID 25019478.
26. Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A, *et al*. Flavanones: Citrus phytochemical with health-promoting properties. BioFactors. 2017;43(4):495-506. doi: 10.1002/biof.1363, PMID 28497905.
27. Bellocco E, Barreca D, Laganà G, Leuzzi U, Tellone E, Ficarra S, *et al*. Influence of L-rhamnosyl-D-glucosyl derivatives on properties and biological interaction of flavonoids. Mol Cell Biochem. 2009;321(1-2):165-71. doi: 10.1007/s11010-008-9930-2, PMID 18987944.
28. Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, *et al*. Natural polyphenols: An overview. Int J Food Prop. 2017;20(8):1689-99. doi: 10.1080/10942912.2016.1220393.
29. Zhang Y, Li Y, Cao C, Cao J, Chen W, Zhang Y, *et al*. Dietary flavonol and flavone intakes and their major food sources in Chinese adults. Nutr Cancer. 2010;62(8):1120-7. doi: 10.1080/01635581.2010.513800, PMID 21058200.
30. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727-47. doi: 10.1093/ajcn/79.5.727, PMID 15113710.
31. Garazd MM, Garazd YL, Khilya VP. Neoflavones. 1. Natural distribution and spectral and biological properties. Chem Nat Compd. 2003;39(1):54-121. doi: 10.1023/A:1024140915526.
32. Aron PM, Kennedy JA. Flavan-3-ols: Nature, occurrence and biological activity. Mol Nutr Food Res. 2008;52(1):79-104. doi: 10.1002/mnfr.200700137, PMID 18081206.
33. Francis FJ, Markakis PC. Food colorants: Anthocyanins. Crit Rev Food Sci Nutr. 1989;28(4):273-314. doi: 10.1080/10408398909527503, PMID 2690857.
34. Forschner R, Knelles J, Bader K, Müller C, Frey W, Köhn A, *et al*. Flavylium salts: A blooming core for bioinspired ionic liquid crystals. Chemistry. 2019;25(56):12966-80. doi: 10.1002/chem.201901975, PMID 31418972.
35. Souza J, Santos RH, Ferreira MM, Molfetta FA, Camargo AJ, Honório KM, *et al*. A quantum chemical and statistical study of flavonoid compounds (flavones) with anti-HIV activity. Eur J Med Chem. 2003;38(11-12):929-38. doi: 10.1016/j.ejmech.2003.06.001, PMID 14642325.
36. Middleton E. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol. 1998;439:175-82. doi: 10.1007/978-1-4615-5335-9_13, PMID 9781303.

37. López M, Martínez F, Del Valle C, Orte C, Miró M. Analysis of phenolic constituents of biological interest in red wines by high-performance liquid chromatography. J Chromatogr A. 2001;922(1-2):359-63. doi: 10.1016/S0021-9673(01)00913-X, PMID 11486883.
38. Kreft S, Knapp M, Kreft I. Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. J Agric Food Chem. 1999;47(11):4649-52. doi: 10.1021/jf990186p, PMID 10552865.
39. Stewart AJ, Bozonnet S, Mullen W, Jenkins GI, Lean ME, Crozier A. Occurrence of flavonols in tomatoes and tomato-based products. J Agric Food Chem. 2000;48(7):2663-9. doi: 10.1021/jf000070p, PMID 10898604.
40. Kaufman PB, Duke JA, Brielmann H, Boik J, Hoyt JE. A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: Implications for human nutrition and health. J Altern Complement Med. 1997;3(1):7-12. doi: 10.1089/acm.1997.3.7, PMID 9395689.
41. Reinli K, Block G. Phytoestrogen content of foods—a compendium of literature values. Nutr Cancer. 1996;26(2):123-48. doi: 10.1080/01635589609514470, PMID 8875551.
42. Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000;63(7):1035-42. doi: 10.1021/np9904509, PMID 10924197.
43. Topal F, Nar M, Gocer H, Kalin P, Kocyigit UM, Gulçin İ, *et al*. Antioxidant activity of taxifolin: An activity–structure relationship. J Enzyme Inhib Med Chem. 2016;31(4):674-83. doi: 10.3109/14756366.2015.1057723, PMID 26147349.
44. Rana AC. R, Gulliya B. Chemistry and pharmacology of flavonoids-a review. Indian JPharmEducRes. 2019;53(1):8-20. doi: 10.5530/ijper.53.1.3.
45. Musialik M, Kuzmicz R, Pawłowski TS, Litwinienko G. Acidity of hydroxyl groups: An overlooked influence on antiradical properties of flavonoids. J Org Chem. 2009;74(7):2699-709. doi: 10.1021/jo802716v, PMID 19275193.
46. Chen L, Teng H, Xie Z, Cao H, Cheang WS, Skalicka-Woniak K, *et al*. Modifications of dietary flavonoids towards improved bioactivity: An update on structure–activity relationship. Crit Rev Food Sci Nutr. 2018;58(4):513-27. doi: 10.1080/10408398.2016.1196334, PMID 27438892.
47. Fan M, Ding H, Zhang G, Hu X, Gong D. Relationships of dietary flavonoid structure with its tyrosinase inhibitory activity and affinity. LWT. 2019;107:25-34. doi: 10.1016/j.lwt.2019.02.076.
48. Sarian MN, Ahmed QU, Mat So'ad SZ, Alhassan AM, Murugesu S, Perumal V, *et al*. Antioxidant and antidiabetic effects of flavonoids: A structure–activity relationship-based study. BioMed Res Int. 2017;2017:8386065. doi: 10.1155/2017/8386065, PMID 29318154.
49. Wang TY, Li Q, Bi KS. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J Pharm Sci. 2018;13(1):12-23. doi: 10.1016/j.ajps.2017.08.004, PMID 32104374.
50. Brazier-Hicks M, Evans KM, Gershater MC, Puschmann H, Steel PG, Edwards R. The C-glycosylation of flavonoids in cereals. J Biol Chem. 2009;284(27):17926-34. doi: 10.1074/jbc.M109.009258, PMID 19411659.
51. Winkel-Shirley B. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol. 2001;126(2):485-93. doi: 10.1104/pp.126.2.485, PMID 11402179.
52. Feild TS, Lee DW, Holbrook NM. Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol. 2001;127(2):566-74. doi: 10.1104/pp.010063, PMID 11598230.
53. Winkel-Shirley B. Biosynthesis of flavonoids and effects of stress. Curr Opin Plant Biol. 2002;5(3):218-23. doi: 10.1016/S1369-5266(02)00256-X, PMID 11960739.
54. Dietrich P, Sanders D, Hedrich R. The role of ion channels in light-dependent stomatal opening. J Exp Bot. 2001;52(363):1959-67. doi: 10.1093/jexbot/52.363.1959, PMID 11559731.
55. Palme K, Gälweiler L. PIN-pointing the molecular basis of auxin transport. Curr Opin Plant Biol. 1999;2(5):375-81. doi: 10.1016/S1369-5266(99)00008-4, PMID 10508762.
56. Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr Cancer. 1993;20(1):21-9. doi: 10.1080/01635589309514267, PMID 8415127.
57. Agrawal AD. Pharmacological activities of flavonoids: A review. PCI-Approved-IJPSN;4(2):1394-8. doi: 10.37285/ijpsn.2011.4.2.3.
58. Khan H, Ullah H, Martorell M, Valdes SE, Belwal T, Tejada S, *et al*. Flavonoids nanoparticles in cancer: Treatment, prevention and clinical prospects. Semin Cancer Biol. 2021;69:200-11. doi: 10.1016/j.semcancer.2019.07.023, PMID 31374244.
59. Palafox-Carlos H, Ayala-Zavala JF, González-Aguilar GA. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. J Food Sci. 2011;76(1):R6-R15. doi: 10.1111/j.1750-3841.2010.01957.x, PMID 21535705.
60. Ruenroengklin N, Zhong J, Duan X, Yang B, Li J, Jiang Y. Effects of various temperatures and pH values on the extraction yield of phenolics from litchi fruit pericarp tissue and the antioxidant activity of the extracted anthocyanins. Int J Mol Sci. 2008;9(7):1333-41. doi: 10.3390/ijms9071333, PMID 19325806.
61. Chuang SY, Lin YK, Lin CF, Wang PW, Chen EL, Fang JY. Elucidating the skin delivery of aglycone and glycoside flavonoids: How the structures affect cutaneous absorption. Nutrients. 2017;9(12):1304. doi: 10.3390/nu9121304, PMID 29189718.
62. Fukuhara K, Nakanishi I, Kansui H, Sugiyama E, Kimura M, Shimada T, *et al*. Enhanced radical-scavenging activity of a planar catechin analogue. J Am Chem Soc. 2002;124(21):5952-3. doi: 10.1021/ja0178259, PMID 12022823.
63. Dang Y, Lin G, Xie Y, Duan J, Ma P, Li G, *et al*. Quantitative determination of myricetin in rat plasma by ultra performance liquid chromatography tandem mass spectrometry and its absolute bioavailability. Drug Res. 2014;64(10):516-22. doi: 10.1055/s-0033-1363220, PMID 24357136.
64. Yao Y, Lin G, Xie Y, Ma P, Li G, Meng Q, *et al*. Preformulation studies of myricetin: A natural antioxidant flavonoid. Pharmazie. 2014;2;69(1):19-26. doi: 10.1691/ph.2014.3076, PMID 24601218.
65. Fang Y, Cao W, Xia M, Pan S, Xu X. Study of structure and permeability relationship of flavonoids in Caco-2 cells. Nutrients. 2017;9(12):1301. doi: 10.3390/nu9121301, PMID 29186068.
66. Otake Y, Hsieh F, Walle T. Glucuronidation versus oxidation of the flavonoid galangin by human liver microsomes and hepatocytes. Drug Metab Dispos. 2002;30(5):576-81. doi: 10.1124/dmd.30.5.576, PMID 11950790.
67. Tian XJ, Yang XW, Yang X, Wang K. Studies of intestinal permeability of 36 flavonoids using Caco-2 cell monolayer model. Int J Pharm. 2009;367(1-2):58-64. doi: 10.1016/j.ijpharm.2008.09.023, PMID 18848870.
68. Ezzat HM, Elnaggar YSR, Abdallah OY. Improved oral bioavailability of the anticancer drug catechin using chitosomes: Design, *in vitro* appraisal and *in vivo* studies. Int J Pharm. 2019;565:488-98. doi: 10.1016/j.ijpharm.2019.05.034, PMID 31100382.
69. Li C, Lee MJ, Sheng S, Meng X, Prabhu S, Winnik B, *et al*. Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. Chem Res Toxicol. 2000;13(3):177-84. doi: 10.1021/tx9901837, PMID 10725114.
70. Lambert JD, Sang S, Yang CS. Biotransformation of green tea polyphenols and the biological activities of those metabolites. Mol Pharm. 2007;4(6):819-25. doi: 10.1021/mp700075m, PMID 17963356.
71. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bio efficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81(1);Suppl:230S-42S. doi: 10.1093/ajcn/81.1.230S, PMID 15640486.
72. Mullen W, Edwards CA, Crozier A. Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. Br J Nutr Nutr 2006. 2006;96(1):107-16. doi: 10.1079/BJN20061809, PMID 16869998.
73. Rupasinghe HP, Ronalds CM, Rathgeber B, Robinson RA. Absorption and tissue distribution of dietary quercetin and quercetin glycosides of apple skin in broiler chickens. J Sci Food AgricAgric.. 2010;90(7):1172-8. doi: 10.1002/jsfa.3944, PMID 20393998.
74. Thilakarathna SH, Rupasinghe HP, Needs PW. Apple peel bioactive rich extracts effectively inhibit *in vitro* human LDL cholesterol oxidation. Food Chem. 2013;138(1):463-70. doi: 10.1016/j.foodchem.2012.09.121, PMID 23265512.
75. Shen Q, Li X, Li W, Zhao X. Enhanced intestinal absorption of daidzein by borneol/menthol eutectic mixture and microemulsion. AAPS PharmSciTech. 2011;12(4):1044-9. doi: 10.1208/s12249-011-9672-4, PMID 21842308.
76. Zhang Z, Huang Y, Gao F, Gao Z, Bu H, Gu W, *et al*. A self-assembled Nano delivery system enhances the oral bioavailability of daidzein: *in vitro* characteristics and *in vivo* performance. Nanomedicine (Lond). 2011;6(8):1365-79. doi: 10.2217/nnm.11.39, PMID 22026378.
77. Cao H, Jing X, Wu D, Shi Y. Methylation of genistein and kaempferol improves their affinities for proteins. Int J Food Sci Nutr. 2013;64(4):437-43. doi: 10.3109/09637486.2012.759186, PMID 23311465.
78. Nielsen ILF, Chee WS, Poulsen L, Offord-Cavin E, Rasmussen SE, Frederiksen H, *et al*. Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: A randomized, double-blind, crossover trial. J Nutr. 2006;136(2):404-8. doi: 10.1093/jn/136.2.404, PMID 16424119.
79. Plaza M, Pozzo T, Liu J, Gulshan Ara KZ, Turner C, Nordberg Karlsson E. Substituent effects on *in vitro* antioxidizing properties, stability, and solubility in flavonoids. J Agric Food Chem. 2014;62(15):3321-33. doi: 10.1021/jf405570u, PMID 24650232.
80. Xu L, Qi T, Xu L, Lu L, Xiao M. Recent progress in the enzymatic glycosylation of phenolic compounds. Carbohydr Chem. 2016;35(1):1-23. doi: 10.1080/07328303.2015.1137580.
81. Jiang JR, Yuan S, Ding JF, Zhu SC, Xu HD, Chen T, *et al*. Conversion of puerarin into its 7-O-glycoside derivatives by Microbacterium oxydans (CGMCC1788) to improve its water solubility and pharmacokinetic properties. Appl Microbiol Biotechnol. 2008;81(4):647-57. doi: 10.1007/s00253-008-1683-z, PMID 18795283.
82. Xiao J. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? Crit Rev Food Sci Nutr. 2017 Jun 13;57(9):1874-905. doi: 10.1080/10408398.2015.1032400, PMID 26176651.
83. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: Facts and fancies. Biochem Pharmacol. 2012;83(1):6-15. doi: 10.1016/j.bcp.2011.08.010, PMID 21856292.
84. Valentova K, Vrba J, Bancirova M, Ulrichova J, Kren V. Isoquercitrin. Isoquercitrin: pharmacology, toxicology, and metabolism. Food Chem Toxicol. 2014;68:267-82.2014:267-82. doi: 10.1016/j.fct.2014.03.018, PMID 24680690.
85. Amaretti A, Raimondi S, Leonardi A, Quartieri A, Rossi M. Hydrolysis of the rutinose-conjugates flavonoids rutin and hesperidin by the gut microbiota and bifidobacteria. Nutrients. 2015;7(4):2788-800. doi: 10.3390/nu7042788, PMID 25875120.
86. Almeida AF, Borge GIA, Piskula M, Tudose A, Tudoreanu L, Valentová K, *et al*. Bioavailability of quercetin in humans with a focus on inter individual variation.

Compr Rev Food Sci Food Saf. 2018;17(3):714-31. doi: 10.1111/1541-4337.12342, PMID 33350133.

87. Ji Y, Li B, Qiao M, Li J, Xu H, Zhang L, *et al*. Advances on the *in vivo* and *in vitro* glycosylations of flavonoids. Appl Microbiol Biotechnol. 2020;104(15):6587-600. doi: 10.1007/s00253-020-10667-z, PMID 32514754.
88. Buchner N, Krumbein A, Rohn S, Kroh LW. Effect of thermal processing on the flavonolsrutin and quercetin Int. Rapid Commun Mass Spectrom. 2006;20(21):3229-35. doi: 10.1002/rcm.2720, PMID 17016866.
89. Makris DP, Rossiter JT. Heat-induced, metal-catalyzed oxidative degradation of quercetin and rutin (quercetin 3-O-rhamnosylglucoside) in aqueous model systems. J Agric Food Chem. 2000;48(9):3830-8. doi: 10.1021/jf0001280, PMID 10995278.
90. Strugała P, Tronina T, Huszcza E, Gabrielska J. Bioactivity *in vitro* of quercetin glycoside obtained in *Beauveria bassiana* culture and its interaction with liposome membranes. Molecules. 2017;22(9):1520. doi: 10.3390/molecules22091520, PMID 28891998.
91. Wen J, Liu B, Yuan E, Ma Y, Zhu Y. Preparation and physicochemical properties of the complex of naringenin with hydroxypropyl-β-cyclodextrin. Molecules. 2010;15(6):4401-7. doi: 10.3390/molecules15064401, PMID 20657449.
92. Pulley GN. Solubility of naringin in water. Ind Eng Chem Anal Ed. 1936;8(5):360. doi: 10.1021/ac50103a020.
93. Saija A, Tomaino A, Trombetta D, Pellegrino ML, Tita B, Messina C, *et al*. '*In vitro*' antioxidant and photoprotective properties and interaction with model membranes of three new quercetin esters. Eur J Pharm Biopharm. 2003;56(2):167-74. doi: 10.1016/S0939-6411(03)00101-2, PMID 12957629.
94. Li J, Shi M, Ma B, Niu R, Zhang H, Kun L. Antitumor activity and safety evaluation of nanoparticle-based delivery of quercetin through intravenous administration in mice. Mater Sci Eng C Mater Biol Appl. 2017;77:803-10. doi: 10.1016/j.msec.2017.03.191, PMID 28532095.
95. Zhao J, Yang J, Xie Y. Improvement strategies for the oral bioavailability of poorly water-soluble flavonoids: An overview. Int J Pharm. 2019;570:118642. doi: 10.1016/j.ijpharm.2019.118642, PMID 31446024.
96. Feng Y, Yao M, Wang Y, Ding M, Zha J, Xiao W, *et al*. Advances in engineering UDP-sugar supply for recombinant biosynthesis of glycosides in microbes. Biotechnol Adv. 2020;41:107538. doi: 10.1016/j.biotechadv.2020.107538, PMID 32222423.
97. De Bruyn F, Maertens J, Beauprez J, Soetaert W, De Mey M. Biotechnological advances in UDP-sugar based glycosylation of small molecules. Biotechnol Adv. 2015;33(2):288-302. doi: 10.1016/j.biotechadv.2015.02.005, PMID 25698505.
98. Neves AR, Correia-da-Silva M, Silva PMA, Ribeiro D, Sousa E, Bousbaa H, *et al*. Synthesis of new glycosylated flavonoids with inhibitory activity on cell growth. Molecules. 2018;23(5):1093. doi: 10.3390/molecules23051093, PMID 29734739.
99. Sheng H, Sun X, Yan Y, Yuan Q, Wang J, Shen X. Metabolic engineering of microorganisms for the production of flavonoids. Front Bioeng Biotechnol. 2020;8:589069. doi: 10.3389/fbioe.2020.589069, PMID 33117787.
100. Thuan NH, Chaudhary AK, Van Cuong D, Cuong NX. Engineering co-culture system for production of apigetrin in *Escherichia coli*. J Ind Microbiol Biotechnol. 2018;45(3):175-85. doi: 10.1007/s10295-018-2012-x, PMID 29362971.
101. Slámová K, Kapešová J, Valentová K. 'Sweet flavonoids': Glycosidase-catalyzed modifications. Int J Mol Sci. 2018;19(7):2126. doi: 10.3390/ijms19072126, PMID 30037103.
102. Xiao J, Muzashvili TS, Georgiev MI. Advances in the biotechnological glycosylation of valuable flavonoids. Biotechnol Adv. 2014;32(6):1145-56. doi: 10.1016/j.biotechadv.2014.04.006, PMID 24780153.
103. Tronina T, Strugała P, Popłoński J, Włoch A, Sordon S, Bartmańska A, *et al*. The influence of glycosylation of natural and synthetic prenylated flavonoids on binding to human serum albumin and inhibition of cyclooxygenases COX-1 and COX-2. Molecules. 2017;22(7):1230. doi: 10.3390/molecules22071230, PMID 28754033.
104. Lee CW, Seo JY, Lee J, Choi JW, Cho S, Bae JY, *et al*. 3-O-Glucosylation of quercetin enhances inhibitory effects on the adipocyte differentiation and lipogenesis. Biomed Pharmacother. 2017;95:589-98. doi: 10.1016/j.biopha.2017.08.002, PMID 28869898.
105. Kim BG, Sung SH, Jung NR, Chong Y, Ahn JH. Biological synthesis of isorhamnetin 3-O-glucoside using engineered glucosyltransferase. Mol CatBEnzym. 2010;63(3-4):194-9. doi: 10.1016/j.molcatb.2010.01.012.
106. Offen W, Martinez-Fleites C, Yang M, Kiat-Lim E, Davis BG, Tarling CA, *et al*. Structure of a flavonoid glucosyltransferase reveals the basis for plant natural product modification. EMBO J. 2006;25(6):1396-405. doi: 10.1038/sj.emboj.7600970, PMID 16482224.
107. Zhang J, Cai W, Zhou Y, Liu Y, Wu X, Li Y, *et al*. Profiling and identification of the metabolites of baicalin and study on their tissue distribution in rats by ultra-high-performance liquid chromatography with linear ion trap-Orbitrap mass spectrometer. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;985:91-102. doi: 10.1016/j.jchromb.2015.01.018, PMID 25661005.
108. Wu X, Chu J, Wu B, Zhang S, He B. An efficient novel glycosylation of flavonoid by β-fructosidase resistant to hydrophilic organic solvents. Bioresour Technol. 2013;129:659-62. doi: 10.1016/j.biortech.2012.12.041, PMID 23298773.
109. Sun J, Laval S, Yu B. Glycosylation reactions in the synthesis of flavonoid glycosides. Synthesis. 2014;46(8):1030-45. doi: 10.1055/s-0033-1341052.
110. Nair SV, Ziaullah HV, Rupasinghe HP. Fatty acid esters of phloridzin induce apoptosis of human liver cancer cells through altered gene expression. PLoS One. 2014;9(9):e107149. doi: 10.1371/journal.pone.0107149, PMID 25229655.
111. Sordon S, Popłoński J, Huszcza EW. Microbial glycosylation of flavonoids. PolJMicrobiol. 2016;65(2):137-51. doi: 10.5604/17331331.1204473.
112. Myriam R, Gary W, Ivana J, Maarit R. SA Nestec. long-lasting absorption of flavonoids. European patent. 1891967.2006August24.
113. Myriam R, Gary W, Ivana J, Maarit R, Nestec SA. Long-lasting absorption of flavonoids. US Patent. 0055081. 2010March04.
114. Eidenberger T, OmnicaGmbH. Stabilized anthocyanin composition. US Patent. 8449927. 2013May28.
115. Ulrich R, Wolfgang S, Julia J, Universität Hamburg. Enzymes catalyzing the glycosylation of polyphenols. International patent. 191524.2014May28.
116. Chantale H, Serge R, ChristianD Kl. Fermented plant extracts, methods of production and uses. US patent. 0224158.2015August13.
117. Ulrich R, Henning R, Nele I, Tanja P, Constantin R, Friederike B, *et al*. Methods for the production of rhamnosylated flavonoids.International patent. 121863.2017 July 20.
118. He B, Wu X, Chu J, Wu B, Zhang S, Ouyang P, *et al*. Fructosylated mangiferin and preparation method therefor and use thereof.US patent. 8946407.2015 February 03.
119. Ulrich R, Wolfgang S, Julia J, Universität Hamburg. Enzymes catalyzing the glycosylation of polyphenols. US patent. 0090578. 2016 March 31.
120. Auriol D, Nalin R, Robe P, Lefevre F, Libragen. Water soluble and activable phenolic derivatives with dermocosmetic and therapeutic applications and process for preparing said derivatives. US patent. 9828407. 2017 November 28.
121. Bettina D, Chang-Jun L, Richard AD, Roberts SP, Nobel Foundation. Methods and compositions for production of flavonoid and isoflavonoid nutraceuticals. Australian Patent. 2003270537. 2004 April 30.

Cite this article: Dahiya A, Majee C, Mazumder R, Priya N, Salahauddin, Atriya A. Insight into the Glycosylation Methods of the Flavonoids as an Approach to Enhance its Bioavailability and Pharmacological Activities. Indian J of Pharmaceutical Education and Research. 2023;57(2):354-71.