A REVIEW ON THE PHARMACOLOGICAL IMPORTANCE OF PHLORIDZIN AND ITS CONJUGATED ANALOGUES

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Abstract

Bioactive compounds from fruits play a protective role for human body against various diseases. These biologically active constituents can be isolated and employed in alternative medicine. Phloridzin is phenolic phyto-constituent, it was firstly isolated from the bark of apple trees. This secondary metabolite belongs to the chemical class of dihydrochalcones, which represent more than 90% of the soluble phenolics. Phloridzin itself is a highly potent phytochemical, however, structural modifications have made its spectrum of potential pharmacological efficacies more diverse. The current review mainly highlights the biosynthesis and pharmacological activities like anti-cancer, anti-obesity, anti-diabetic, anti-oxidant, anti-ageing, anti-microbial and melanogenic activities, exhibited by phloridzin and its conjugates.

Keywords: *Apple, Bioactive Compounds, Phloridzin, Pharmaceuticals, Biosynthesis*

Introduction

In millennia, functional foods have gained attention owing to their therapeutic potential against many chronic diseases. Most of these protective effects are considered due to the presence of a broad spectrum of phytochemicals such as phenolics, flavonoids, isoflavonoids and carotenoids. Important functional foods are fruits, vegetables, herbs, cereals, egg and fish [1,2]. Daily intake of fruits and vegetables decreases the risk of chronic diseases. These protective effects of fruits and vegetables have been attributed to phytochemicals, such as phenolics and flavonoids which are biologically active and extremely beneficial for health [3, 4, 5]. Phenolic compounds in fruit bearing plants play an important role in the plant defense mechanism against different fungal diseases and different stresses [6, 7]. Phenolic compounds act as natural antioxidants, scavenge free radicals and stimulate the synthesis of antioxidant enzymes, and thus prevent generation of oxidative species [8, 9]. The antioxidant activity of phenolic compounds is linked to other pharmacological properties of these compounds, such as anti-inflammatory, antimicrobial, anticancer, and various other activities [10, 11].

The domestic apple is one of the most worldwide cultivated fruit tree. Phloridzin (PZ or phloretin-2'-O-glucoside) is a major precursor of flavonoid glucoside largely found in apple peels [12]. Few studies on the chemical composition of leaves have been published, where phloretin glycosides, phenolic acids, catechins, and some quercetin glycosides were identified as the main phenolic compounds [13]. Phloridzin not only exists in *Malus* species, but also existed in the leaves of *Smilax glyciphylla* [14], *Lithocarpus polystachyus* [15], and in *Fragaria ananassa* [16]. In apple trees, phloridzin is found primarily in all parts while it is most abundant in fruits. Phloridzin has a bitter taste, and the color of the apple juices is due to its dimerized oxidation products [17, 18, 19]. Phloridzin exhibited a wide spectrum of interesting pharmacological bioactivities [20].

Biosynthesis of phloridzin

The biosynthetic steps leading to production of phloridzin have been recently investigated with recombinant enzymes and plant protein extracts [21, 22]. (Avadhani & Towers, 1961) identified hydroxycinnamic acid as a precursor for the phloridzin biosynthesis and they showed that 4-hydroxydihydrocinnamoyl-CoA, is an intermediate in phloridzin formation. Previous studies indicated that dihydrochalcone formation is based on a similar mechanism as chalcone formation [23]. Substrate specificity studies show that recombinant chalcone synthases (CHSs) from *Sinapis alba* [24], and *Psilotum nudum* [25], accept a broad range of hydroxycinnamoyl-CoA precursors and are thereby able to catalyze the formation of phloretin from 4-hydroxydihydrocinnamoyl-CoA and malonyl-CoA. Phloridzin formation in apple is based on three basic steps: (i) Formation of 4-hydroxydihydrocinnamoyl-CoA from p-coumaroyl CoA (ii) Phloretin formation by CHS (iii) Glucosylation of phloretin in position 2 to form phloridzin as shown on Figure 1 [22].

Pharmacological importance

It is known that phloridzin and its derivatives are commonly utilized in human physiological studies on biological membranes. Such studies are mainly conducted on obesity, diabetes, stress hyperglycemia, anti-oxidative activity, food additives, beverages, membrane permeability and longevity extending agents in the food, cosmetic, and pharmaceutical industries related to phloridzin and its derivatives [26]. Phloridzin possess anti-cancer, anti-obesity, anti-diabetic, anti-oxidant, anti-ageing, anti-microbial and melanogenic activity, the brief mechanism of actions are tabulated in Table 1.

Anti-Cancer activity

Cancer is a complex pathophysiological process involving DNA damage, multiple mutations, excessive cell proliferation, tumor expansion and metastasis. The potential of dietary phenolic compounds to decrease cancer risk has been established by *in vitro* and *in vivo* [27]. These phytochemicals are also capable of minimizing drug resistance and adverse side effects [28]. A recent study also showed that polyphenols exhibit synergism to increase the cytotoxicity of doxorubicin and etoposide in lymphoid leukemia cells [29].

Initially, phloridzin as a cytotoxic agent was not considered valuable due to concerns related to poor bioavailability [30]. To solve this problem, fatty acid

esters of phloridzin have been synthesized in laboratory by regioselective enzymatic acylation of phloridzin with six different long chain saturated, mono- and polyunsaturated fatty acids. Fatty acid esters of phloridzin, (PZ-stearic acid), (PZ-oleic acid), (PZ-linoleic acid), (PZ-alinolenic acid), (PZ-docosahexanoic acid) and (PZ-ecosapentanoic acid) have been distinctly more potent inhibitors of the growth of tumors such as hepatocellular carcinoma, breast adenocarcinoma, and leukemia than their corresponding parent molecules, phloridzin and the fatty acids [31].

A novel fatty acid ester of PZ called phloridzin-docosahexaenoate (PZ-DHA) was synthesized by conjugating PZ with docosahexaenoic acid through a lipase B enzyme-catalyzed acylation. Enzymatic acylation of phloridzin with long chain polyunsaturated fatty acids (PUFAs) has been used to enhance penetrability of flavonoids into cells [32]. Besides esterification, PUFAs exhibit significant health benefits [33]. Docosahexanoic acid (DHA), was found to be the primary tumor-suppressing fatty acid in athymic mice bearing human colon carcinoma [34]. DHA was found to be an effective adjuvant agent, as it synergistically enhances the efficacy of numerous chemotherapeutic agents both *in vitro* and *in vivo* [35].

Enzymatic conjugation of PZ with DHA is symbiotic, as the modification not only improves phloridzin bioavailability, but also increases the stability of the unsaturated fatty acid. The individual capability of PZ and DHA to induce cytotoxic effects in malignant cells suggests that the single chemical entity, PZ-DHA, could be a more potent and favorable cancer therapeutic agent. Previous studies have demostrated the anti-oxidant, anti-tyrosinase, and anti-inflammatory effects of PZ-DHA conjugate [36]. Pz-DHA conjugate had the greatest potential and efficacy to be employed as a chemotherapeutic agent. Selective targeting of receptors with inhibitors and its downstream signaling pathways has been proven to be an effective approach in anticancer therapy [37]. The anti-proliferative effect suggests that the esterification of 6´- OH of glucose moiety of phloridzin with acyl side chains enhanced the bioactivity of phloridzin as shown in Figure 2. This conformational change in sturcture could change the lipophilicity of the phloridzin molecule that improves the interactions with membrane bound receptors, cellular permeability and uptake [38].

Anti-Obesity activity

Obesity is a widely occurring but manageable disease of clinical importance. It is often considered a risk factor for the development of several disability issues and diseases. Obesity is prevalent in all age groups and in almost all regions of the world. The increasing prevalence of obesity places a huge burden on health care system. Weight loss is associated with significant health benefits during obesity. Effective weight loss approaches include diet therapy, physical activity and lifestyle modification [39].

High-fat diet is an environmental factor associated with obesity. In rodents, the high fat diet induce obesity and metabolic problems similar to the human body [40, 41]. Diet-induced obesity in C57BL/6J mice lead to leptin resistance [42]. Development of diabetes in mice is linked with an increase in plasma glucose level, hepatic triglyceride levels and adipokine levels. In visceral obesity, adipokines are released by white adipose tissue which are responsible for chronic inflammatory response and insulin resistance [43].

Phloridzin causes the suppression of visceral and subcutaneous fat accumulation and prevent adipocyte hypertrophy in high fat diet induced obese mice. Both visceral fat and subcutaneous fat are responsible for metabolic disorders [44]. Furthermore, the phloridzin reduced total white adipose tissue weight. Obesity related metabolic disturbances include impaired insulin resistance and secretion, diabetes, hypertension, inflammation and dyslipidemia. Supplementation of phloridzin to high fat diet-induced obesity decreased the levels of lipids and free fatty acids in liver. The phloridzin also increased plasma HDL-cholesterol and apo A-I, lipoprotein. Low levels of HDL-cholesterol and apo A-I can be responsible for metabolic syndrome that includes abdominal obesity, obesity-associated hepatic steatosis, insulin resistance, elevated fasting glucose, and pro-inflammatory states [45, 46].

Another mechanism by which phloridzin affected the activities of hepatic enzymes, as well as the decrease in white adipose tissue weight is the decrease in the activities of hepatic fatty acid

synthase and phosphatidate phosphatase, which are basic enzymes in the regulation of *de novo* fatty acid and triglyceride synthesis. This decrease in hepatic lipids level by phloridzin may be attributed to control of hepatic enzymes involved in lipogenesis and cholesterol synthesis [46].

Increases in the expression of pro-inflammatory adipokines (TNF-α, MCP-1, IFN-γ, and IL-6), and reduction in adiponectin expression is noticebale in adipose tissue of obese individuals [47]. Circulating leptin levels regulates the production of cytokines [43]. Phloridzin decreased not only leptin levels, but also pro-inflammatory adipokines level in plasma. It also lowered plasma level of adipsin, responsible for adiposity, insulin resistance and dyslipidemia. It plays additional role in homeostasis and lipid metabolism [48].

The veterinary literature has proposed that chronic administration of phlorizin in lactating cows induces lipolysis. A phlorizin analogue, dapagliflozin shown in Figure 2, induces reduced adiposity, and ultimately weight loss [49].

Anti-Diabetic activity

Diabetes mellitus is one of the most prevalent chronic diseases that is associated with abnormalities in insulin secretion, mode of action or both. These defects in insulin secretion and activities cause disturbance in nutrient metabolism that leads to hyperglycemia. These nutrients mainly include carbohydrates, proteins and fats [50, 51].

In recent era, fruits and vegetables have captured larger importance due to the presence of many important bioactive compounds such as fiber which are associated with various beneficial effects such as antioxidant, anti-inflammation, anti-metabolic syndrome, anti-obesity, cardio-protective, anti-cancer and improved endothelial function [52]. Among bioactive moieties of fruits, phloridzin from apple pomace has strong therapeutic potential against both types of diabetes mellitus. It acts as a strong inhibitor against sodium or glucose transportation in the intestine (SGLT1) and kidney (SGLT2). This in turn decreases the chances of risk factors and complications associated with both types of diabetes [53, 54, 55]. In some researches, when phloridzin was injected in rodents models, it was found that phloridzin improved the hyperglycemia level in both types of diabetes and the main mode of

action behind this positive potential was the mitigation of renal glucose reabsorption and increased excretion of glucose into urine [5, 56].

In a study, Oku et al. (1999) claimed the antidiabetic effect of phloridzin in diabetic mice by lowering postprandial blood glucose levels [57, 58]. Park et al. (2004) converted the phoridzin in two components i.e. phloretin and glucose and found that phloretin lowered the digestion and absorption of carbohydrates, fats and proteins in the small intestine. Along with the reduced metabolism of carbohydrates and fats, their transport in the gastrointestinal tract was also reduced. Phloridzin intake was resulted in notable decrease in hyperglycemia and increase in the lipids and carbohydrates metabolism defects. The reduction in blood glucose level led to decrease in insulin level in blood and it was found that chalcone, an important derivative from phloridzin had ability of insulin derivation [59, 60].

A number of researches were conducted to probe the effect of phloridzin against both types of diabetes and related complications. In a study of Malatiali et al. (2008), it was noticed that phloridzin was considered effective against proteinuria, hyperfiltration and kidney hypertrophy. These consequences increased the early kidney activity and mitigate few structural changes in diabetes. An important derivative of phloridzin, T-1095, was found to decreased the albuminuria occurrence and increase in glomerular mesangial area in db/db mice. This in turn combat the diabetic nephropathy [61]. In few previous studies, it was observed that phloridzin reduced the high glucose induced structural and functional modifications and defects in cultured bovine retinal pericytes [63, 63]. At the end, it was proved that phloridzin has strong therapeutic potential against complications and risk factors related to diabetes.

Moreover, when the composition and structure of phloridzin is overview, it is observed that phloridzin has large content of flavonoids and it acts as precursor for flavonoids. Furthermore, owing to the presence of these flavonoids in phloridzin, it shows strong antioxidant effect in peroxynitrite scavenging and inhibiting lipid peroxidation [64, 65, 66]. In the study of Thornalley et al. (1999), it was noticed that advanced glycation end products (AGEs) levels were

decreased due to the intake of phloridzin by db/db mice whereas, without phloridzin, AGEs levels were high. The main reason behind this mechanism was the increase in glycation reactions and non-enzymatic glycation due to hyperglycemia, which was resulted in reactions with amino acids of proteins, and nucleic acid that in turn resulted in formation of AGEs. This AGEs level is associated with oxidative stress and is inversely associated with the anti-oxidant capacity of phloridzin. In a study, when phloridzin was given to db/db mice, a significant decrease in blood glucose level and body weight was observed. So it was proved that phloridzin has ability to reduce body weight due to glucose loss in urine. This was due to the sodium-glucose cotransport inhibition nature of the phloridzin [53, 67].

Moreover, γ-PGA-phloridzin, an important conjugate shown in Figure 2, also acts as an oral anti-diabetic medicine. It can reduce the glucose reabsorption in the blood and this can be improved by maintaining the phloridzin content in the conjugate. In the study of Ikumi et al. (2008), it was observed that PGA-PRZ was very effective against glucose transport from mucosal to serosal sides of rat's jejunum and glucose level in plasma was significantly decreased. Whilst, when the phloridzin was used in free form in such case, it rarely affected the glucose level during hyperglycemia [68].

Anti-Oxidant activity

Major hallmark of number of heath related problems is oxidative stress. Oxidative stress is responsible for pathogenesis of diabetes related cardiovascular disease, which is associated with an abnormal lipid profile and insulin resistance [69]. Oxidative stress results from an imbalance between antioxidant defense mechanism and the levels of reactive oxygen species [70, 71].

Dietary supplementation with antioxidants, including vitamins and phenolic compounds obtained from plants, may help to maintain an appropriate anti-oxidative balance [72]. Epidemiological studies indicate that the consumption of fruits and vegetables reduces the risk of various diseases related to oxidative damage, such as cancer, cardiovascular diseases, diabetes and Alzheimer's disease. The effectiveness and therapeutic potential of these fruits is owing to the presence of bioactive moieties in them [73, 74, 75].

Among different fruits, apple is capturing greater importance which is associated with the higher polyphenolic content. These polyphenols showed stronger antioxidant activity against various diseases, complications and risk factors. Along with this antioxidant activity, these phenolics have two to three times more DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging and 10±30 times superoxide-scavenging activities of vitamins C or E. Due to the strong antioxidant potential of polyphenols, phloridzin is considered as potent antioxidant agent against many diseases such as diabetes, cardiovascular disease, cancer, obesity and many others [76].

Moreover, when the apple extract was administrated in the blood, it was resulted in notable maintenance of endogenous rate of α-tocopherol, and lipids from oxidation. In a study, Vieira et al. (2012) claimed that consumption of apple and its juice would result in improvement in the antioxidant capacity of blood. In a study, Gutmann et al. (2014) evaluated that the most prominent bioactivity is the antioxidant activity of phloridzin. Furthermore, phloridzin was proved to be the predominant polyphenol in the *Golden Delicious* apple pomace extract with higher antioxidant capacity. When different antioxidant assay techniques were applied to probe the change in antioxidant capacity through intake of phloridzin, it was observed that phloridzin showed highest scavenging activity against peroxyl radicals. Phloridzin have the potential to be used as natural antioxidants for used in the food industry, especially for removing the peroxyl radicals formed in food [77].

Furthermore, when the phloridzin was attached with Hydroxylpropyl-β-cyclodextrin and resulted in phloridzin & Hydroxylpropyl-β-cyclodextrin complex. This complex increased the solubility of phloridzin in water. Along with increase in water solubility, antioxidant capacity of phloridzin was also increased which in turn result in increase in DPPH and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activities. This improvement in water solubility and antioxidant property of phloridzin improved the applications and utilization of phloridzin in food, pharmaceutical and nutraceutical industries as supplement and other forms [78].

Anti-Ageing activity

Ageing is a process of consistent physiological integrity loss and impairment in functions of organs, which is leading cause of death. This change is the basic risk factor for the rise of many chronic diseases such as cancer, cardiovascular diseases, diabetes, neuro-degeneration and many other aberrations. In recent era, lot of research has been done on aging process (genetic and biochemical pathways) and its control [79]. To study aging process, yeast model has been considered one of the most important bioassay systems [80].

Mainly *Saccharomyces cerevisiae* K6001 yeast was used to study process of aging. Many fruits and vegetables have captured importance in this context owing to the presence of various bioactive compounds in them. Among fruits, apple has an active ingredient named phlroidzin. Various researches were conducted to investigate the effect of phloridzin on aging process. In a study, the effect of phloridzin on *Saccharomyces cerevisiae* K6001 yeast strain lifespan was checked at 3, 10, and 30 mM and it was found that phloridzin significantly increase the aging process owing to the antioxidant capacity and anti-aging effects to notably improve the survivability of the yeast under oxidative stress. This suggests that anti-oxidative stress due to phloridzin plays an important role in extending the replicative lifespan of the yeast [81]. In many other studies, anti-aging effect of phloridzin was tested at different doses. The main mechanism behind the antiaging effect of phloridzin was its antioxidant capacity [82, 83, 84].

The sirtuin family is very crucial regulator of life duration. Its optimum level is necessary to maintain because when it is more or less than optimum level, it significantly affect the organism's life span [85]. When this protein is treated with phloridzin, it proved useful in enhancing the lifespan of organisms. In a study, Xiang et al. (2011) treated the SOD gene used for protein coding and SIRT1 (Sirutin 1) protein of human with 5 and 10 mM phloridzin, and it was observed that after the intake of such phloridzin treated sirtuin, organism lifespan was increased significantly [81].

Anti-Microbial activity

Phloridzin, an important bioactive compound of apple peel, is gaining greater attention owing to its larger effectiveness as strong anti-microbial agent. This bioactive moiety shows greater anti-microbial activity against Gram-positive *S. aureus* and comparatively less activity against Gram-negative *E. coli*. As far as the anti-bacterial activity of phloridzin is concerned, it is effective against both *S. aureus* and *E. coli*. In a study, Zhang et al. (2016) described the comparative anti-microbial activity of two important bioactive compounds including phloretin and phloridzin and it was found that highest anti-microbial activity against both *S. aureus* and *E. coli* was shown by phloridzin. The position of phloridzin was considered stronger as compared to phloretin because phloridzin was more soluble in water, and possessed better stability. These results have proved the presence of higher antimicrobial agent with higher anti-oxidant activity in apple pomace and these results are leading and increasing the greater trend of apple pomace utilization in pharmaceutical and nutraceutical industries as food supplements with strong potential to cure diseases [77].

As far as the anti-fungal activity of phloridzin and its related derivatives mainly phlorizin tetraproprionate against nine different fungal species including *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton violaceum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum gypseum*, *Nannizzia cajetani*, *Microsporum canis*, *Nannizzia gypsea*, *Microsporum gypseum* is concerned, it was found that phloridzin and its related derivative gave desirable results so both can be used as an anti-fungal agent against different fungal species [38].

Phloridzin tetraproprionate, a new semi-synthetic derivative of phloridzin sown in Figure 2, have been found to be more stable in formulations as antifungal agent. It possesses a potential to be used in finished formulations for topical use. The modification in structure of phloridzin leads to change in its activities. Change in sugar hydroxyl groups improves the lipophilicity but doesn't bind the active phenol constituents. This structural modification also obtained an active but more stable molecule in dermo-cosmetic formulae. Whereas, without structural modification and with the use of whole phloridzin compound, a quick appearance of brown

color take place which is not desirables for various applications and utilization [38].

Melanogenic activity

Melanin is the major pigment of skin, which act as a shield against UV injury under normal physiological conditions. Melanin synthesis is controlled by a cascade of enzymatic reactions. Tyrosinase, which is the rate-limiting enzyme in the melanin synthesis, converts tyrosine to dopaquinone which is subsequently converted to dopachrome through auto-oxidation, and finally to dihydroxyindole to form eumelanin pigment. The latter reaction occurs in the presence of dopachrome tautomerase (tyrosinase-related protein-2; TRP-2) and DHICA oxidase (tyrosinase-related protein-2; TRP-1). These melanocyte specific enzymes, tyrosinase, TRP-1 and TRP-2 are involved in melanin synthesis [86, 87].

Phloridzin induces an increase in the protein levels of tyrosinase, TRP-1 and TRP-2. cAMP signaling pathway may play prominent roles in phloridzin-induced melanogenesis. Phloridzin stimulated cAMP production and CREB phosphorylation. Protein kinase A inhibitor, H89, attenuated the phloridzin-induced increase in tyrosinase activity, tyrosinase expression and melanin synthesis. Phloridzin acts through a cAMP pathway to increase tyrosinase transcriptional activities, thereby leading to the stimulation of melanogenesis. In addition, utilization of the pigmenting effect of phloridzin might be useful as an adjunctive therapy for treatment of hypopigmentation-related disorders [88].

Shoji et al. reported that phloridzin induces melanogenesis through the inhibition of Protein kinase C activity in B16 melanoma cells. Polyphenols absorb ultraviolet rays, and phloridzin was identified as the one of the important compounds stimulating to produce melanin Phloridzin at the concentration of 500 pg/ml increased the melanin content in B16 mouse melanoma cells [89]. Biosynthesis of melanin in our skin is one of the major protective responses against ultraviolet irradiation. Therefore, the compounds that increase melanogenesis are speculated to protect human skin from damage by ultraviolet irradiation [90].

Conclusion

Phloridzin is a potent phytochemical derived from apple. Pharmacological actions of its conjugates as well as parent compound emphasize its used in daily diet. Isolation of this phytochemical would lead to highly efficacious in food and pharmaceutical industry. Besides health effects, structural modifications and conjugations make it an exceptional phytochemical to use in healthcare. However, chemical natures of its derivatives need to be explored more.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table 1. Mechanism of action exhibited by phloridzin in different pharmacological activities.

| Pharmacological activity	Mechanism of action	References
Anti-Cancer	Enzymatic acylation with PUFAs	[32]
Anti-obesity	↑ Plasma HDL and Apo A-1 lipoprotein ↓ Activity of FAS and PAP ↓ Leptin levels and cytokine expression	[46, 48]
Anti-diabetic	↓ Posprandial glucose levels and AGEs Inhibition of SGLT1 and SGT2	[57]
Anti-oxidant	DPPH and ABTS scavenging	[78]
Anti- ageing	SOD and SIRT1 gene expression	[81]
Anti-microbial	Gram positive bacterial and fungal inhibition ↑ Lipophilicity	[38, 77]
Melanogenesis	↑Tyrosine kinase activity through cAMP signaling pathway	[88]

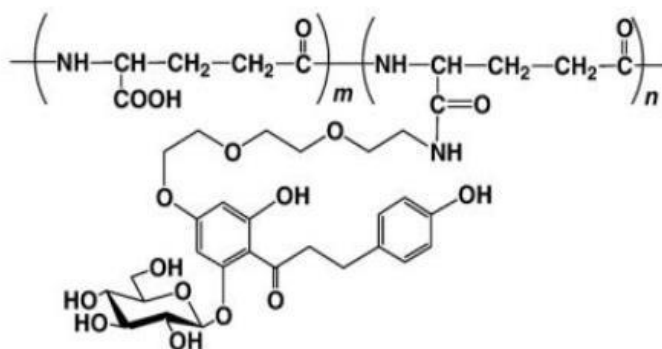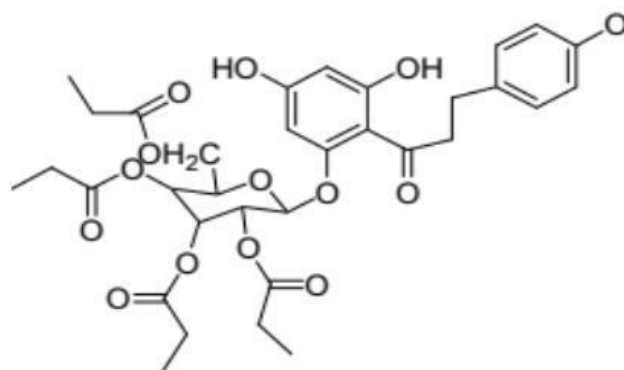**Figure 1.** Biosynthesis of Phloridzin
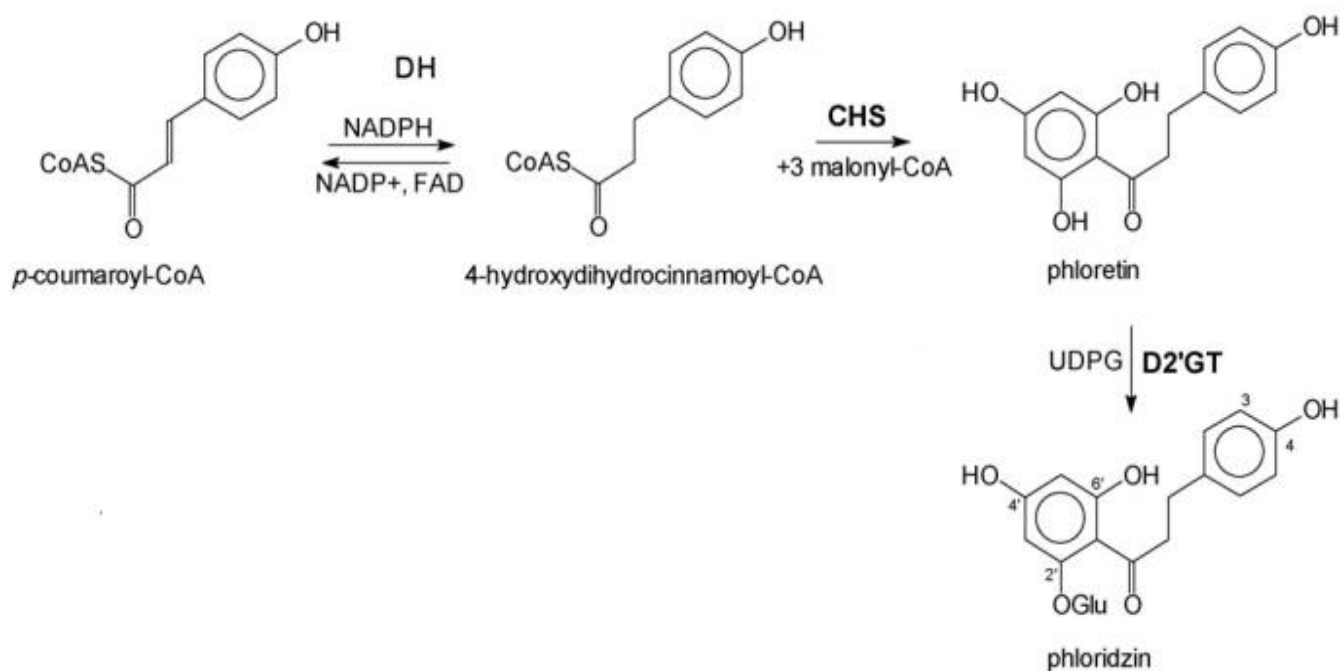
Figure 2. Structural formulas of phloridzin analogues.
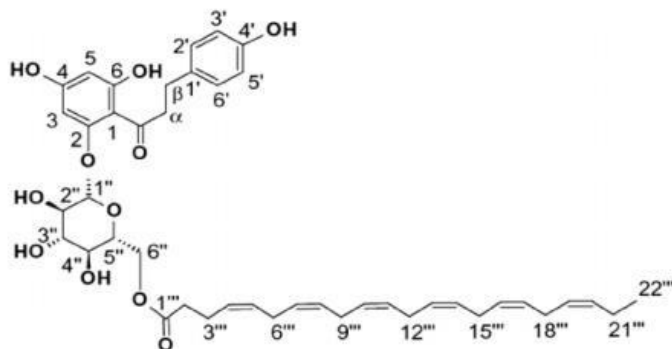
Phloridzin docosahexaenoate
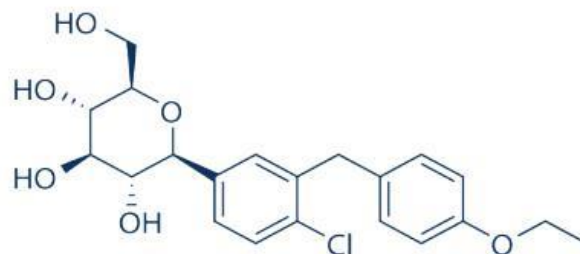
Dapagliflozin

γ-PGA-phloridzin

Phloridzin tetraproprionate