Therapeutic Potential of Phytoestrogens

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Atiya Fatima, Asrar Alam, and Ram Singh

Abstract

Phytoestrogens are naturally occurring constituents of plants present in the significant proportion of our diet. They have been extensively studied due to their potential as pharmacological targets and nutraceutical benefits. The potential pharmacological applications of these molecules include cardioprotection, antimicrobial, anticancer, anti-obesity, antiosteoporosis, antidiabetic, and neuroprotection. Phytoestrogens are polyphenolic nonsteroidal compounds of plant origin with estrogen-like biological activity. They mimic estradiol-like effects in several tissue/tissues of the mammalian body. The health benefits accredited to them are due to their ability to mimic estrogenic actions. In this chapter, we aim to provide comprehensive coverage of the pharmacological aspects of the most pronounced phytoestrogens of our daily life. We will discuss different classes of phytoestrogens under the subcategory of flavonoids and non-flavonoids. Numerous plant-derived compounds like genistein, daidzein, 8-prenylnaringenin, equol, quercetin, coumestrol, isoliquiritigenin, and resveratrol belonging to different classes of phytoestrogens will be discussed with their therapeutic values and clinical applications in a broad range of health-related problems and disorders.

Keywords

Phytoestrogens · Flavonoids · Estrogenic activity · RBA (Receptor binding affinity) · Genistein · Resveratrol

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15.1 Introduction

Phytoestrogens are naturally occurring constituents of plants, having structural similarity to mammalian female sex hormones estrogens (xenoestrogens). They exhibit estradiol-like effects in one or more target tissues in mammalian body [1]. Under the classical definition of phytoestrogens fall those compounds, which can induce estrus, exert estrogenic effects on the central nervous system (CNS), and stimulate growth of the genital tract of female animals. Broadly, the phytoestrogen can be defined as the chemicals which can display effects suggestive of estrogenicity which include binding to the estrogen receptors, thereby inducing specific estrogen-responsive gene products leading to stimulation of estrogen receptor(s)-positive breast cancer cell growth [2]. They cause both estrogenic and antiestrogenic effects [3] by binding to estrogen receptors alpha and beta with a preference estrogen receptor [1, 4]. Since estrogen receptors are present in different tissues all over the mammalian body, central nervous system (CNS), reproductive system, mammary gland, bones, lungs, and ovary, phytoestrogens have specific hormonal effects on different tissues [5]. Phytoestrogens act as natural defense against herbivorous animals by controlling their female fertility, attributed to their mimicry and action as antagonists of estrogen [3]. Phytoestrogens exert their effects mediated *via* multiple modes of actions including both genomic and non-genomic mechanisms [6]. Estrogenic and antiestrogenic effects on estrogen receptors (ERs) are some genomic mechanisms of action, while other effects' direct interaction with ERs may not be involved [7]. Phytoestrogens have stable structure and low molecular weight which gains them access through cell membranes [8]. They interact with enzymes and receptors, bind to ERs and provoke specific estrogen-responsive gene products, and stimulate ER-mediated effects in the body. Non-genomic effects exerted by phytoestrogens do not involve direct interaction with ERs. Some of the non-genomic actions include inhibition of tyrosine kinase, antioxidant effects and suppression of angiogenesis, induction of cancer cell differentiation, and DNA topoisomerase activities [9]. Among these, the most well-characterized mode of phytoestrogen action appears to be through estrogen receptor (ER) binding.

A plethora of information related to health benefits of phytoestrogens is available in the literature. The first major attributed health benefits of phytoestrogen are an alternative to hormone replacement therapy (HRT) to alleviate premenopausal symptoms and osteoporosis. Phytoestrogens have also been suggested to have health benefits in cardiovascular diseases, metabolic disorders [10], and breast cancer, but their beneficial role in breast cancer is contradicted by some adverse effects. They have been reported to possess antiviral [11] and antimicrobial properties [12]. In this chapter, we have presented the classification and a detailed description of the role of phytoestrogens in human health along with their positive and negative effects.

15.2 Clinical Importance of Different Classes of Phytoestrogens

Phytoestrogens classification is based on their chemical structures, which resemble estradiol (E2). There are several classes of phytoestrogens found in plants. Broadly, phytoestrogens are classified into two major classes such as flavonoids and non-flavonoids. Flavonoids are further divided into isoflavones, flavones, isoflavans, flavonols, and coumestans. Non-flavonoid consists mainly of lignans, stilbenes, and chalcones. The detailed classification of phytoestrogens along with their clinical significance is presented below.

15.2.1 Flavonoids

15.2.1.1 Isoflavones

The isoflavonoids are 3-phenyl chromones having C6-C3-C6 skeleton in which each C6 moiety is a benzene ring linked *via* a heterocyclic pyrone ring (Fig. 15.1). They can exist as glucosides or as aglycones. The glucosides are further hydrolyzed in the gut to their respective aglycones.

Isoflavones are substituted derivatives of isoflavonoids where two or three hydrogen atoms are replaced by hydroxyl groups. Their structures encompass a planar ring system with a substituted *p*-hydroxy aromatic ring and second in-plane hydroxyl group which are approximately 12Å away from each other. This distance is almost identical to that between the C-3 and C-17 hydroxyls of estradiol [13] (Fig. 15.2). This is why they can bind to receptors. Two ring structures separated with two carbon atoms as well as spacing between two hydroxyls play an important role in binding affinity of isoflavones to ERs [14].

Hydrogen bond interactions with amino acids of the ligand-binding site of the ER are determined by these distances [15]. Isoflavones have gained considerable importance in the areas of clinical nutrition and disease prevention during last few decades. They show beneficial effects on cancer, lipid metabolism, cardiovascular diseases, osteoporosis, and blood coagulation and act as antioxidants [16]. They proved their efficacy as insecticidal, piscicidal, antifungal, and antimicrobial agents. Two of the most extensively studied isoflavones are genistein and daidzein.

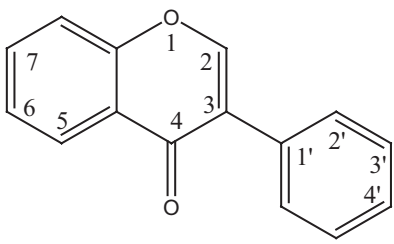


Fig. 15.1 Basic isoflavone skeleton

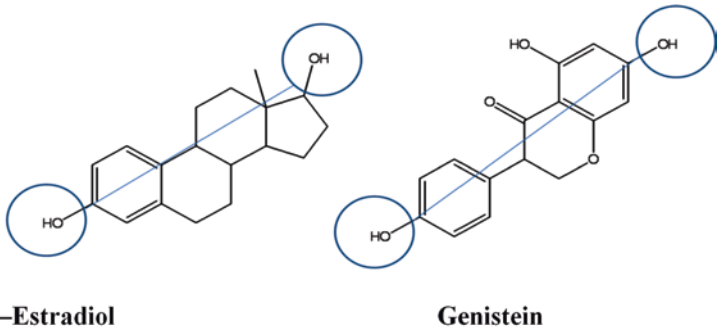
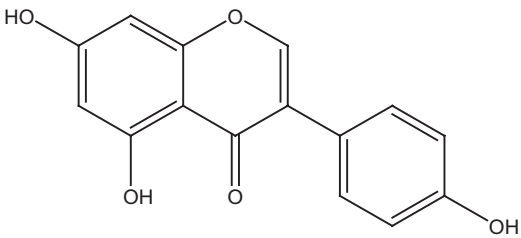


Fig. 15.2 Relative comparison of distances of two hydroxyl groups in estradiol and isoflavones

Fig. 15.3 Genistein



Genistein

Genistein (5,7,4′-trihydroxyisoflavone) (Fig. 15.3) is an isoflavone first isolated from the dyer's broom, *Genista tinctoria*, and is naturally found in a number of plants including soybeans, lupin, fava beans, kudzu, and other legumes and commonly exists as inactive glucoside genistein [17]. It is derived from a precursor molecule, biochanin A, that is converted to genistein after breakdown by intestinal glucosidases.

Genistein is one of the phytoestrogens to show potent receptor binding affinity with the estrogen receptors. It can prop up tissue specific as agonist or antagonist depending on the estrogen receptor and concentration of circulating endogenous estrogens [18]. Despite the similarity, genistein can bind to ERα with relative binding affinity of only 0.05–1% to the binding affinity of 17β-estradiol, while it's greater in case of ERβ [19]. This differential binding affinity of genistein (estrogen receptor-β seven times more than that of estrogen receptor-α) makes it a selective estrogen receptor modulator [20] and thus promotes multiple effects on the target tissue.

Synthetic derivatives have been prepared to test the potency of genistein. Among numerous biological effects displayed by genistein, the most pronounced one is anticancer property. It has been reported to exert antiproliferative properties on different types of cancer. Several mechanisms for the anticancer effects have been proposed including inhibition of angiogenesis, affecting cellular transduction signaling pathways inhibiting carcinogenesis, inactivation of nuclear factor kappa B

(NF-κB), inhibition of metastasis of cancer cells by controlling proteolytic enzymes matrix metalloproteinases (MMPs), topoisomerase, tyrosine kinase activity, and antioxidant properties. Genistein has also been known to cause cell cycle arrest in various human cancer cell lines as described below.

Cancer

The proliferative (estrogenic) and antiproliferative (antiestrogenic) effects in human breast cancer cell lines due to genistein made it the phytoestrogen of greatest interest. It imparts biphasic effect in estrogen receptor (ER)-positive MCF-7 breast cancer cell line which is concentration-dependent, with stimulation of cell growth at lower concentrations between 10 and 1.0 nM but at higher doses between 1 and 10 μM, and above inhibition is observed in both ER-positive and ER-negative cells. At lower concentrations, genistein competes with estradiol for binding to the ER and thus mimic as an estrogen agonist [21]. The antiproliferative effects are clearly non-ER mediated. It induces apoptosis in both ER-positive MCF7 cell lines and ER-negative MDA-MB-468 cancer cell lines. It inhibits the growth of cancer cells by intervening in cell cycle progression (affecting CDKs), which ultimately results in cessation of cell proliferation. PTKs (protein tyrosine kinases) that are involved in tumorigenesis are inhibited by genistein action, and this efficacy has led many laboratories to investigate its therapeutic potential against breast and prostate cancer [22]. In addition to PTKs, other mechanisms by which genistein exhibits anticarcinogenic activity are inhibition of angiogenesis (inhibits TGF-β signaling and the suppression of growth factor receptor VEGF-mediated signaling pathway), BRCA1 modulation, induction of apoptosis through inhibitory effect on NF-κB signaling and Akt pathway, inhibition of aromatase enzyme, and inhibiting DNA replication enzymes DNA topoisomerases I and II.

Androgens are crucial in prostate cancer genesis and act *via* activation of androgen receptor (AR). AR-responsive gene, prostate-specific antigen (PSA) acts as major key factor in prognosis and progression in prostate cancer patients. Genistein, at high concentrations, inhibits the growth of androgen-dependent and independent cells in human prostate cancer cell lines by inhibiting PSA synthesis in prostate cancer cells [23]. It displays anti-metastatic efficacy in prostrate tissue in phase I and phase II clinical trial patients [24]. Genistein inhibit cancer progression in colon cells by inducing apoptosis or inhibiting proliferation, upregulating LDL receptors [25], and affecting epidermal growth factors [26].

Genistein in combination with other chemotherapeutic agents such as centchroman, *cis*platin, gemcitabine, docetaxel, and 5-fluorouracil potentiates their antitumor activity [27–30]. Several other combination therapies such as curcumin-genistein [31], terazosin-genistein [32], and genistein-cetuximab [33] have proved to be effective in various cancer cells. A derivative of genistein, phenoxodiol ((3-(4-hydroxyphenyl)-2H-chromen-7-ol, Fig. 15.4), belonging to the family of drugs called signal transduction inhibitors can arrest several cancer cell lines in the G1 stage of the cell cycle [34]. It stimulates apoptosis *via* multiple pathways. It is a molecule with broad antitumor activity and high specificity for tumor cells. Its biochemical tendencies are predominantly well-matched to reversal of

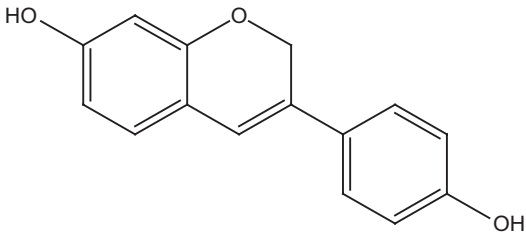


Fig. 15.4 Phenoxodiol [(3-(4-hydroxyphenyl)-2H-chromen-7-ol]

chemoresistance. It is being developed as a chemo-sensitizer of standard chemotherapeutics in solid cancers [35].

Osteoporosis

Osteoporosis is a bone disease causing brittleness in bone due to a reduction in bone tissue. Osteoclasts responsible for bone destruction are dependent on tyrosine kinase enzyme. Estrogen deficiency causes an abundance of the osteoclast. Genistein is a well-known tyrosine kinase inhibitor and can be used as the antiosteoporotic agent. Genistein along with other phytoestrogens play a favorable effect in maintaining a balance between adipogenesis and osteogenesis. It inhibits the expansion of osteoclasts while stimulates the growth of osteoblasts [36, 37]. Antigenotoxic effects of genistein in postmenopausal women leading to positive effects on bone suggest the fact that genistein can be used for the prevention of bone loss in postmenopausal women without getting significant adverse effects [38]. However, later studies suggested that treatment with genistein to ovariectomized rats resulted in improved biomechanical results and enhancement of morphologic parameters but risk involved with the uterus and mammary glands was unclear [39].

Antidiabetic Effects

Diabetes is a metabolic disorder either caused by defective insulin secretion or insulin action or sometimes both play a role. Genistein preserves pancreatic β-cells, ameliorates glucose and lipid metabolisms, and elevates insulin levels [40]. It decreases insulin-induced lipid synthesis from glucose in isolated adipocytes and also inhibits insulin-stimulated glucose oxidation [41]. The antidiabetic potential is mediated by counteracting reactive oxygen species and chelating metals [42]. Since oxidative stress inflammation also plays a role in promoting metabolic disorders, genistein in the dose of 1 mg/kg/day in 30% dimethyl sulfoxide for 45 days ameliorates the inflammatory state to endorse its antidiabetic efficacy [43].

Neuroprotective Effects

Neurodegenerative disorders mainly comprise of Alzheimer's disease (AD) and Parkinson's disease (PD) where oxidative stress-induced neuron cellular apoptosis is believed to be involved in the development of both these diseases. Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic neurons. PD primary pathological events leading to dopaminergic neurons degeneration is supposed to occur *via* oxidative stress. Increased lipid peroxidation and DNA damage

are also the result of overproduction of reactive oxygen species (ROS). So, genistein being a potent antioxidant attenuates the neuronal damage and loss through counteracting oxidative stress. Genistein has the potential to suppress PTK expression, which is interpreted to be neuroprotective [44]. It has neuroprotective effects on dopaminergic neurons in the nigrostriatal system, and this effect may be accredited to enhancing Bcl-2 gene expression [45]. Activation of the IGF-I receptor signaling pathway is another neuroprotective effects associated with genistein [46].

Alzheimer's disease (AD) is manifested by different cellular and molecular events and primarily involves deposition of the β-amyloid peptide into senile plaques which initiate oxidative stress and accelerates the degeneration of neurons [47].

Genistein has antioxidant and neuroprotective effects on Alzheimer's disease (AD). It mediates protective effect against Aβ-induced toxicity *via* estrogen receptors. This action stimulates MAP kinases, activates the NF-κB signaling pathway, and thereby induces overexpression of antioxidant manganese superoxide dismutase (MnSOD) [48]. Acute genistein treatment has been suggested to be useful in improving memory deficits in ovariectomized rats [49]. Genistein (10mg/kg) improved the memory of Aβ-injected rats due to the estrogen-like activity instead of antioxidant effect [50]. It has been found that genistein increased PPARg (peroxisome proliferator-activated receptor gamma) levels, which in turn will make overexpressing apolipoprotein E (apoE) thus mediates the degradation of amyloid β (Aβ). Therefore, a clinical trial is undergoing, which studies the role of genistein on treatment of AD *via* activation of PPARg levels [51]. Some of the genistein-polyamine conjugates possessed momentous cholinesterases (ChEs) inhibitory activity with an IC50 value of 2.75 μmol/L which was better than that of rivastigmine (5.60 μmol/L) [52].

Daidzein

Daidzein (7,4'-dihydroxyisoflavone) (Fig. 15.5) is commonly found in *Pueraria mirifica* and Kudzu or *Pueraria lobata*. It commonly exists as inactive glucoside daidzein. It is also obtained from precursor formononetin, which on breakdown by intestinal glucosidases is converted to daidzein. Daidzein is further partially metabolized to equol and *O*-desmethylangiolensin (*O*-DMA).

Daidzein is a comprehensively studied phytoestrogen with respect to its skeletal effects. It can promote various therapeutic effects such as prevention of cancer, cardiovascular diseases, etc. Daidzein inhibits the growth of human prostate, colon,

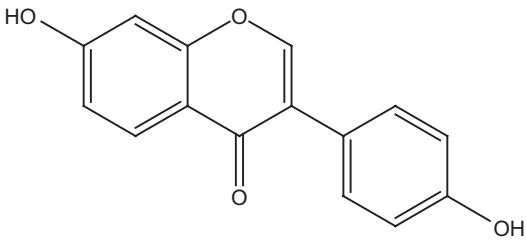


Fig. 15.5 Daidzein (7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)

breast, and ovarian carcinomas [53, 54]. It causes cell cycle arrest at the G1 and G2/M phases in human breast cancer cells [40], induces apoptosis [55], and affects angiogenesis/metastasis [56]. Carboxymethyl derivatives of daidzein act as a carrier for daunomycin (anticancer drug used for ovarian cancer) enhancing anticancer potential in human model of ovarian cancer cell lines [57].

Antiosteoporotic functions of daidzein include stimulation of osteoblast and inhibition of osteoclast through ERs and stimulation of protein synthesis in osteoblasts [58] that are further enhanced in synthetic analogs [59].

Ipriflavone, a synthetic isoflavone (prenyl isoflavone derivative) derived from daidzein, has been developed as an oral treatment for acute leukemias [60]. It showed best results for prevention of osteoporosis suggesting that it is a useful and safe alternative to ERT in treating osteoporosis in postmenopausal women [61].

Daidzein displays good antioxidant activity by upregulating the antioxidant enzyme catalase and is thereby linked to chemopreventive potential in many human diseases, including cancer and heart disease [62]. Daidzein along with genistein is among one of the most potent modulators of immune functions [63], but daidzein is also involved in immunosuppressive effects [64]. Daidzein along with genistein can protect against oxidative stress-induced cell apoptosis and cell proliferation inhibition in the diabetic endothelial cell model. This combination therapy may thereby prove to be beneficial for diabetes and chronic lung diseases [65]. Moreover, daidzein anti-oxidative potential imparts neuroprotective effects against Alzheimer's disease [66]. Other beneficial effects of daidzein include decreased LDL oxidation and lipoprotein disorders [67], anti-obesity, and metabolic disorders [68].

Glycitein

Glycitein is commonly found in *Glycine max* (L.) and *Pueraria montana* and accounts for 5–10% of the total isoflavones in soy food products. Glycitein has a structural similarity to those of genistein and daidzein. It has weak estrogenic activity, comparable to that of the other soy isoflavones but much lower than that of diethylstilbestrol (DES) and 17β-estradiol [69]. Glycitein prominently has therapeutic potential for prevention of neurodegenerative disorders [70]. It helps in lowering cholesterol and LDL contents [71] and has preventive measures in cardiovascular diseases [72].

Formononetin

Formononetin is an isoflavonoid found abundantly in *Astragalus mongholicus* Bunge and *Trifolium pratense* L. (red clover). The extracts of these herbs have been used clinically to treat different diseases including cardiovascular diseases since long time. In addition, it has been reported to exert anti-oxidative and estrogenic effects [73] along with hypolipidemic properties. Formononetin has also been found to be beneficial in cardiovascular disorders as it shows vasorelaxation effects [74].

15.2.1.2 Flavones

Flavonoidal chemical entity comprises of 2-phenyl-benzo[α]pyrane or flavane nucleus, which consists of two benzene rings linked through a heterocyclic pyran

ring. Flavonoids exert a wide spectrum of biological activities in a multitude of disease states such as cancer, cardiovascular disease, and neurodegenerative disorders. There are reports about their beneficial effects as anti-allergic [75], anti-inflammatory [76], antiviral [77], antioxidant [78], and antitumor activities [79]. Apart from this, they are known to possess hypercholesterolemic, anti-hepatotoxic, and antifertility activities [80] including anti-inflammatory potential [81, 82]. Some of them are also thought to act as natural fungicides and UV protectants. Apigenin and naringenin are some of the extensively studied flavonoids.

Apigenin

Apigenin (Fig. 15.6) is commonly found in *Medicago sativa*, *Allium sativum*, and *Acinos suaveolens*. Apigenin is a weak mutagen showing both anticarcinogenic activity [83] and antigenotoxic effect [84].

Apigenin acts as antiproliferative by inducing cell cycle arrest and apoptosis [85]. It enhances the efficacy of standard chemotherapeutic drug gemcitabine by increasing its antitumor effects [86]. It also possesses antioxidant potential [87] and neuroprotective effects [88]. It enhances the effect of chemotherapeutic drug *cis*platin in prostate cancer cell lines [89]. 8-Prenylapigenin displays anti-inflammatory and vascular protective properties [90].

8-Prenylnaringenin

8-Prenylnaringenin (8-PN) contained in the female flower of hops (*Humulus lupulus* L.). It is one of the strongest plant-derived estrogen receptor ligands and regarded as one of the most potent phytoestrogens [91, 92]. It strongly binds to both estrogen receptor (ER) α and β with a relative binding affinity of about 0.01 (estradiol = 1), ten times higher than that of genistein [93]. 8-PN anticancer effects include induction of apoptosis in hormone-dependent breast cancer cell lines in a dose-dependent manner [94] and intervening in cell signaling pathways [95]. Later studies suggested proliferative effects of 8-PN [96]. Platelet aggregation is involved in a number of vascular diseases such as atherosclerosis, myocardial infarction, coronary artery disease, and thrombosis. 8-PN anti-aggregatory and anti-adhesive potency on human platelets leads to prevention of these diseases [97].

Kaempferol

Kaempferol is one of the most commonly found dietary flavonoids. It is mainly isolated from grapefruit, tea, and broccoli. It exerts its biological effect not only through estrogen receptors but also through some estrogen-related receptors [98]. Its preventive role in postmenopausal conditions, osteoporosis, and cardiovascular diseases has been established [99]. Anticancer effects are observed in colorectal, lung, and prostate cancers. It can induce apoptosis through inhibiting DNA synthesis, inhibiting kinase activities, inducing nuclear DNA degradation, cell cycle arrest, and telomerase inhibition [100–103]. Recent reports suggest its antiviral potential against influenza virus-induced severe lung damage [104].

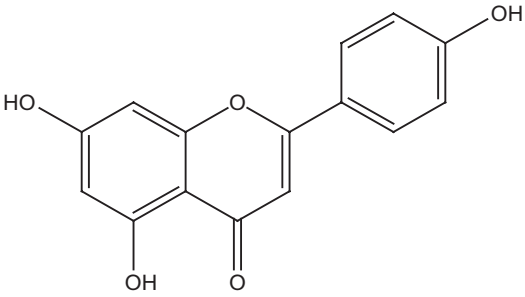


Fig. 15.6 Apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)

Icariin

Icariin is the main active flavonoid glucoside isolated from *Epimedium pubescens*. It is used as antirheumatics (anti-inflammation) and in tonics (for health promotion) [105]. *Epimedium* flavonoids extract containing prominently icariin have a therapeutic effect on osteoporosis [106, 107], and it also enhances bone healing and thus reduces osteoporosis [108]. Recent studies explored its cardioprotective potential [109], antiangiogenesis against liver fibrosis [110], and neuroprotective efficacies [111].

Silymarin

Silymarin is a purified extract from milk thistle (*Silybum marianum*) comprising of a mixture of four isomeric flavonolignans: silibinin (its main, active component), isosilibinin, silydianin, and silychristin [112]. It has been used for centuries as a natural remedy for liver diseases and claimed for clinical applications in the treatment of viral hepatitis [113]. Additionally, silymarin has also been identified for its anti-inflammatory and cytoprotective effects [114], exhibiting antiosteoporotic [115], immunomodulatory [116], and antitumor activities [117] and antiviral potencies [118]. Silymarin is also effective against neurodegeneration and aging-related disorders [119] and fungal infections [120].

Baicalein

Baicalein is a flavonoid extracted from *Scutellaria baicalensis*. It has been reported to have multiple pharmacological activities including anti-inflammatory, anti-allergic, antiviral, and anti-oxidative effects [121–123]. Antitumor effects of baicalein have been widely explored on various human cancer cell lines [124–127]. Silymarin and baicalein in combination have synergistic anticancer effect on human hepatoma HepG2 cells [128]. It also has beneficial effects in treating memory loss and may have potential as therapeutic lead in combating Parkinson's disease [129]. It is also effective in the prevention of noise-induced hearing loss [130].

15.2.1.3 Isoflavan

The isoflavans are a subclass of flavonoids, composed of C6-C3-C6 skeleton with the central heterocyclic pyran ring. Most common example of this class is equol. Other examples include isoflavans glabridin and hispaglabridins A and B.

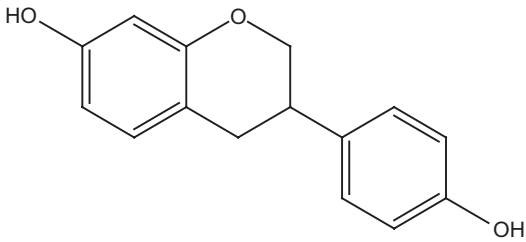


Fig. 15.7 Equol (3,4-dihydro-3-(4-hydroxyphenyl)-2H-chromen-7-ol)

Equol

Equol (Fig. 15.7) is a bioactive metabolite of daidzein that is produced by intestinal bacteria and has estrogenic activity exceeding that of daidzein [131].

It is considered to have therapeutic potential similar to that of the isoflavones genistein and daidzein. Equol being a chiral molecule is profoundly found in nature as enantiomer S-equol. This stereoisomer displays a 13-fold higher relative binding affinity (RBA) for ERβ than ERα which is greater than its parent compound daidzein [132]. Contrary, the R-enantiomer has a stronger RBA for ERα. Equol is known to possess the best antioxidant activity among all isoflavones. It also has anticancerous effects and induces apoptosis in human breast cancer cells [133]. Equol possesses substantial vasodilator [134] and protects cells from H_2O_2-induced cell death to prevent vascular atherosclerosis [135]. To date, few clinical trials have considered equol as a molecule having potential to enhance bone density among postmenopausal women [136, 137]. Studies revealed the fact that ingestion of equol and resveratrol as dietary supplements may improve postmenopausal health problems [138].

15.2.1.4 Flavonol

Any class of flavonoid compounds that are hydroxy derivatives of flavone can be classified as flavonol. One of the most common examples of flavonol is quercetin.

Quercetin (Fig. 15.8) is found in the peel of apples and red onions. Naturally it occurs in form of two glycosides, quercetrin when linked to rhamnose or rutin when linked to rutinose.

Quercetin is presumed to have beneficial health effects such as prevention of cancer and cardiovascular disease [139, 140] and is also a strong antioxidant [141]. Its therapeutic efficacy has been thoroughly investigated for its abilities to express antiproliferative effects by in both prostate cancer and human endometrial adenocarcinoma cell lines [142]. It induces apoptosis in colorectal cancers [143] and in ER-α negative cancer cell lines [144]. Various quercetin derivatives are reported to have DNA topoisomerase activities [145]. Quercetin-amino acid conjugates as safe modulators for multidrug resistance (MDR) [146]. It has protective anti-inflammatory effect against rheumatoid arthritis [147].

15.2.1.5 Coumestans

Coumestans (Fig. 15.9) are plant phenols having estrogenic activity most pronounced among all the phytoestrogens.

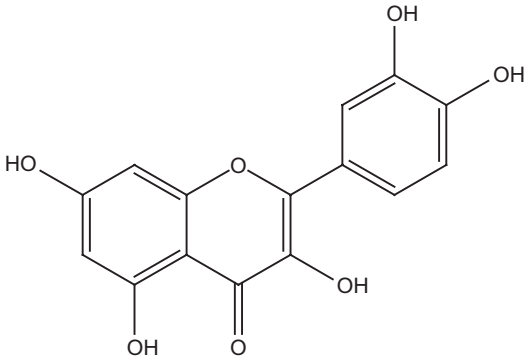


Fig. 15.8 Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-one)

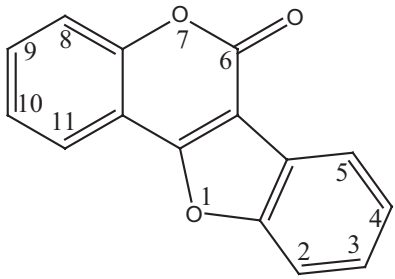


Fig. 15.9 Basic coumestan structure

15.2.1.6 Coumestrol

Coumestrol (Fig. 15.10) is isolated from ladino clover (*Trifolium repens* L.), strawberry clover (*Trifolium fragiferum*), and alfalfa or lucerne (*Medicago sativa* L.). This has a similar structural scaffold to a steroid and thus interacts with estrogen receptors (ERs).

It is reported to have higher binding affinities to ERs than the other phytoestrogen compounds apart from genistein [148], showing similar biphasic effects as genistein on cell growth [149]. Being a phytoestrogen, it displays a wide scale of biological effects on the breast, uterus, and bone. It imparts cytotoxic effects on breast cancer [150] and antitumor effects on prostate cancer [151]. It is a unique molecule as it inhibits resorption of the bone and, at the same time, it stimulates bone mineralization [152]. Since it also has less estrogenic activity than 17β-estradiol, it may prove to be a more potent drug against bone diseases including osteoporosis. Considering the fact, coumestrol and its analogs, coumestrol diacetate and coumestrol dimethyl ether have been evaluated for these effects in vivo with favorable [153]. Psoralidin, a prenylated coumestan and coumestrol, exerts bone-protective effects via osteoblast proliferation and differentiation, thus enhancing bone formation [154]. Coumestrol also displays anticarcinogenic activity [155] and neurobehavioral actions [156] and has neuroprotective effects [157].

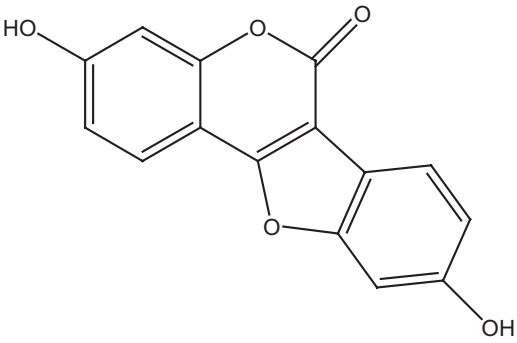


Fig. 15.10 Coumestrol (3,9-dihydroxy-6H-benzofuro[3,2-c] chromen-6-one)

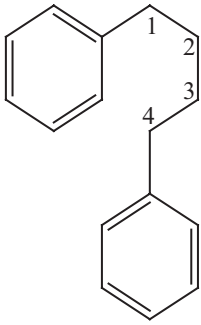


Fig. 15.11 Basic lignan structure

15.2.2 Non-flavonoids

15.2.2.1 Lignans

Lignans (Fig. 15.11) are defined as dimeric phenylpropanoid (C6-C3) compounds containing a dibenzylbutane skeleton and are widely distributed in nature as minor constituents of some plant species.

Lignans occur in high concentration in flaxseed and in lesser concentration in whole grain cereals, vegetables, and fruits. Lignans as they occur in plants are not active estrogens. Such activity is achieved only after the gut flora metabolism of these precursors to the so-called mammalian lignans. They have low molecular weight and with phenolic groups at the *meta* position of the aromatic rings.

As with the isoflavone phytoestrogens, lignans have health-promoting effects particularly in the areas of hormone-dependent cancers and cardiovascular disease [158, 159] and possess estrogenic as well as antiestrogenic activity [160]. Lignans intervene in estrogen metabolism by affecting sex hormone binding globulin (SHBG) [161]. Secoisolariciresinol and matairesinol are the most well-known phytoestrogenic lignans. They are not estrogenic by themselves but on conversion to enterodiol and enterolactone acquire estrogenic action. Enterodiol is further metabolized to enterolactone.

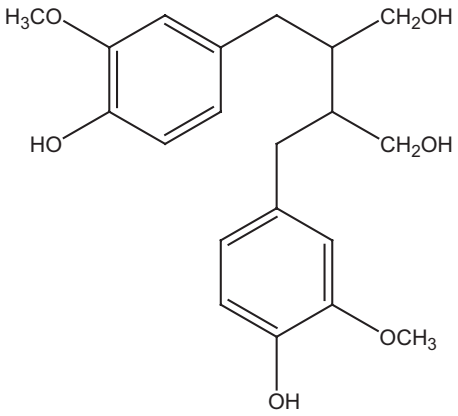


Fig. 15.12 Secoisolariciresinol (4-(4-(4-hydroxy-3-methoxyphenyl)-2,3-bis(hydroxymethyl) butyl)-2-methoxyphenol)

Secoisolariciresinol (Fig. 15.12) is isolated from *Taxus baccata* and *Urtica dioica*. It is effective in lowering serum cholesterol and in preventing the development of diabetes mellitus and has also proved to be good antioxidant along with enterodiol and enterolactone [162]. Enterolactone has a strong protective effect on the breast cancer [163]. It inhibits estradiol proliferative effect on MCF-7 breast cancer cells in culture [164]. Secoisolariciresinol diglucoside (SDG) increases bone mass in offspring in rats when taken during lactation [165]. SDG exerts antidepressant-like effect in mice [166] and is antidiabetic [167].

15.2.2.2 Stilbenes

Stilbenes (Fig. 15.13) are 1,2-diarylethenes. Stilbenoids, hydroxylated derivative of stilbenes, are of pharmaceutical interest. They have been explored for their chemopreventive effects, lipid-lowering and vascular activities, and antidiabetic potential. *Trans*-resveratrol is one the most pronounced and major active compounds of all the monomeric stilbenes.

Resveratrol (Fig. 15.14) is a secondary plant metabolite and exists as *cis* and *trans* isomers. Anticancer properties exhibited by this molecule have drawn considerable attention, since it has a remarkable inhibitory potential in various stages of tumor development.

It may block tumor development at various stages by targeting kinases [168], steroid hormone receptors [169], reactive oxygen species [170], ribonucleotide reductase [171], and DNA polymerases [172]. Resveratrol being structurally similar to the synthetic estrogen diethylstilbestrol (DES: 4,4'-dihydroxy-trans-a,b-diethylstilbene) behaves as a strong estrogen receptor agonist. Unlike other phytoestrogens, it can bind with equal affinity to both the estrogen receptors ERα and ERβ. Among both the enantiomeric forms, the *trans* isomer exerts higher activity in ER-positive cancer cell lines. At lower concentrations (10–25 nM), it displays proliferative effects on MCF-7 cell lines, whereas at concentrations of 0.1 and 1 μM, it

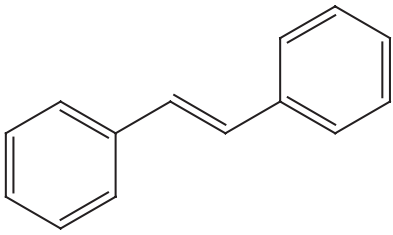


Fig. 15.13 Basic stilbene structure

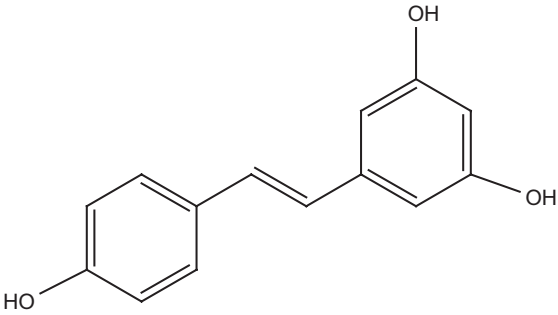


Fig. 15.14 Resveratrol (5-(4-hydroxystyryl) benzene-1,3-diol)

had no effect. At concentrations of 10 μM, resveratrol competes with the endogenous estrogen 17β estradiol and inhibits its binding to ER. This phenomenon can be explained by the fact that in the presence of estrogen resveratrol shows agonist as well as antagonistic effect in ER-positive breast cancer cells, while lack of estradiol leads to induction of ER-dependent transcriptional events by this molecule [173]. Resveratrol anticancer effects include cell cycle arrest (S/G2 phase transition of the cell cycle) and induction of differentiation and apoptosis in several of human cancer cell lines, such as breast, prostate, leukemia, colon, and esophageal cells [174]. Resveratrol in combination therapy with standard chemotherapeutic drugs increases the efficacy of the therapy and concurrently reduces the undesired side effects. Roscovitine (ROSC), a selective cyclin-dependent kinase (CDK) inhibitor in MCF-7 breast cancer cells in combination with resveratrol, enhanced the ROSC-mediated inhibition of cell proliferation and cell cycle arrest [175]. Resveratrol-based aspirin prodrugs exert improved anticancer properties [176]. Apart from combination therapy, resveratrol analogs with improved bioavailability displayed positive results [177]. It has been reviewed extensively as MDR reversion molecule particularly against breast cancer [178]. Considering the above facts, resveratrol might be the most promising candidate for HRT and chemoprevention of breast cancer.

Other activities of resveratrol include antioxidant and neuroprotective properties showing positive effects on Alzheimer's disease [179] and Parkinson's disease [180]. It is in clinical trials phase II to evaluate its neuroprotective potential in patients with AD [181]. Resveratrol also protects the cardiovascular system by a large number of mechanisms [182], possesses anti-inflammatory activity [183] and antimicrobial effects [184], and demonstrates suppression of oxidative DNA damage [185]. Resveratrol also affects in modulation of lipoprotein metabolism by

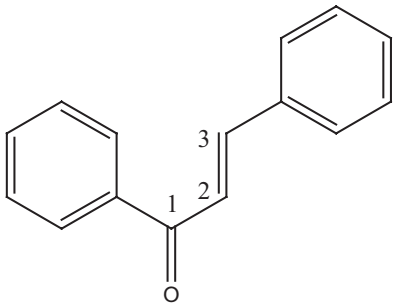


Fig. 15.15 Basic chalcone skeleton

affecting lipogenesis and lipolysis in adipocytes of normal rats [186] and could aid in the treatment of chronic intestinal inflammation [187]. The pure compound is now available in tablets and is recommended as a dietary supplement.

15.2.2.3 Chalcones

Basic chalcone skeleton (Fig. 15.15) comprises of an α, β double bond. Presence of a hydroxyl group at position 2′ and/or 3′ imparts ER-ligand activities, and thus chalcones possess anticarcinogenic potency and antiproliferative effects for different cancer cells [188]. Some chalcone derivatives show biphasic effect in breast cancer cells in a dose-dependent manner. They show proliferative effects at low concentrations but exhibit an ER-independent inhibitory activity at levels ≥10 μM [189]. Two of the commonly found plant chalcones are isoliquiritigenin and licochalcone A.

Isoliquiritigenin

Isoliquiritigenin (Fig. 15.16), a chalcone primarily reported from licorice, has been identified in terms of its anti-oxidative effect [190], anti-inflammatory effect, antiplatelet aggregation [191] and most of all estrogenic properties. Isoliquiritigenin can behave as antitumor agent in several human carcinoma cell lines and induce apoptosis. HL-60 human promyelocytic cell line [192], colon cancer cells [193], human prostate cancer [194], and human hepatoma cells [195] have been found to be inhibited by the action of isoliquiritigenin. Biphasic effects of isoliquiritigenin in MCF-7 breast cancer cells are well-documented. Antitumor mechanism of isoliquiritigenin involves induction of apoptosis [196], inhibition of cell signaling pathways, and inhibition of angiogenesis [197]. Some studies evaluated its antiangiogenetic effects and considered it to be pronounced enough in leukemia cells to be considered as a drug [198]. Not only in cancer, isoliquiritigenin also showed neuroprotection [199], anti-inflammatory potential [200], and bone-protective efficacy [201].

Licochalcone A

Licochalcone A is also a molecule of therapeutic interest and possesses various biological activities including antibacterial [202], anti-parasitic [203], estrogenic,

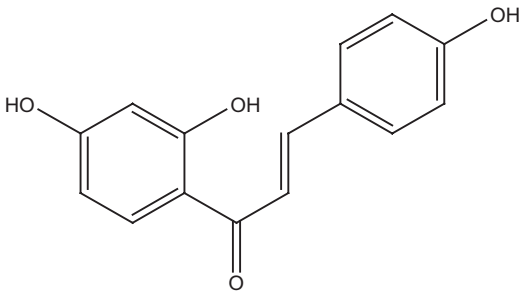


Fig. 15.16 Isoliquiritigenin ((E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one)

and antitumor effects [204]. Its antiproliferative potency has been found to be very pronounced [205]. Like other phytoestrogens, licochalcone induces apoptosis in MCF-7 breast cancer [206] and leukemia cells [207]. It is also effective in prostate cancer [208] and gastric cancer as it can induce apoptosis by causing cell cycle arrest at G2/M transition [209]. Furthermore, licochalcone A is also effective as an anti-obesity molecule [210] and has bone healing effects [211].

15.3 Phytoestrogens as Therapeutic Agents

The therapeutic potential of phytoestrogens cannot be clearly evaluated as the dose of phytoestrogens administered, their dietary composition, and duration of use come into effect and thus makes them difficult to compare. Data regarding considerable health benefits to cardiovascular, breast, bone, and menopausal symptoms are not strong enough to conclude. Thus the degree to which phytoestrogen consumption confers health benefits excluding side effects still remains unresolved.

15.3.1 Menopausal Symptoms and Prevention of Osteoporosis

Phytoestrogen consumption came into effect as a natural alternative to hormone replacement therapy. They were found to relieve not only from vasomotor perimenopausal symptoms including hot flushes but also played a crucial role in prevention of osteoporosis. Most of the phytoestrogens from all the category including coumestrol, resveratrol, genistein, daidzein, and others are reported to have bone-protective effect in vivo. Although their therapeutic potential seemed dependent on the dose, duration, and route of administration and on the animal model employed, their osteoprotective potential is dependent on their effects on the uterus and mammary glands and the risks involved in using these molecules as osteoprotective agents [39]. Although mixed results have been obtained, still dietary supplements containing numerous phytoestrogens continue to be popular as a natural alternative to hormone replacement.

15.3.2 Cardiovascular Health and Prevention of Heart Disease

Postmenopausal health problems also accompany heart diseases as there is decrease in estradiol levels during menopause [212]. In the current scenario of rise in cardiovascular diseases, soy consumption has been correlated with reduced risk of cardiovascular disorders. Isoflavonoids in particular play a role by increasing plasma level of the good cholesterol (HDL) and deteriorate bad cholesterol (LDL) [213]. Owing to this, phytoestrogens received the great attention and simultaneously stimulated consumption and adoption of soy foods in Western countries. However, there are escalating evidences to suspect the impact of soy consumption on LDL levels, and other cardiovascular diseases might be spurious. Regardless, people at risk for heart disease still consider soy intake as part of their daily meals.

15.3.3 Breast Cancer: Pro or Con?

Role of phytoestrogens in the area of breast cancer as perpetrator or protector has proven to be one of the trickiest questions to be tackled. Although extensive studies regarding this factor have been done, still results have been frustratingly incongruous. The fact that phytoestrogens bind to ERs with relatively high affinity may lead to the idea that high phytoestrogen intake could be the factor behind the increased risk of carcinogenesis. In such a case, breast cancer survivors are put at risk for reoccurrence. For instance, the phytoestrogen genistein enhances multidrug resistance in breast cancer cell [214]. Still, the relatively opposite results with traditionally low cancer rates in Asia stand as evidence of phytoestrogen benefit though this difference could be accounted on the difference in traditional diet of Asian and Western women [215]. On account of these reports, extensive studies have been done taking epidemiological approach to address these concerns, but no concluding results could be obtained due to numerous factors involved. It has been a difficult task to get a clear consensus as to whether or not phytoestrogens are beneficial or harmful particularly in the area of breast cancer. But since the mounting evidences of quantifiable benefits on bone and postmenopausal health, women without grave threat factors for breast cancer or a family history of the same are likely to integrate soy into their diet without momentous concern.

15.3.4 Other Therapeutic Applications

The therapeutic effects of PEs on neurodegenerative disorders have been clearly stated and have extensive effect on CNS. Although recent studies have also suggested negative outcome in case of cognitive functions [216]. Phytoestrogens have benefecial effects in hepatic alterations and have antidiabetic potential as seen with many of the molecules. They display antiaging effect on the skin via estrogen receptors [217]. Their antiviral properties toward a range of exogenous viruses have been clearly established [11]. Phytoestrogens have noteworthy antioxidant potential and

are also good anti-inflammatory agents. They display a plethora of therapeutic properties such as antibacterial, antifungal, antidepressant, and anti-obesity to name a few.

15.3.5 Cons: The Endocrine Disrupting Properties of Phytoestrogens

Although phytoestrogens have been established as source of mediating a possible protective role in the areas of certain diseases such as cardiovascular diseases and some hormone-dependent cancers, conversely they also exert a detrimental effect on normal cells. Mutagenic effects, genotoxicity, and cancer cell-promoting properties are some of the perpetrator effect held by them. Isoflavones fit into the category of being an endocrine disruptor, which can be defined as compounds having ability to alter the function(s)/structure of the endocrine system and causing adverse effects. There have been reports implicating that phytoestrogens consumption during critical timings of development may hamper with the organizational role of estrogen and simultaneously causing myriad of adverse health outcome. This has been related to occurrence of malformations in the reproductive system, cancers, and reduced fertility. This also leads to early puberty and disrupted brain organization [218]. Paradoxically, phytoestrogens being effective antioxidants may also cause oxidative DNA damage and may lead to immunosuppressive effects. Phytoestrogens can influence human endogenous retrovirus expression (HERV) causing injurious effects of HERVs on cancer causing genes.

15.4 Conclusions

Phytoestrogens are intriguing, as although their beneficial effects have dominated the research areas, the potentially adverse effects of these compounds have been overlooked. Clearly some of the phytoestrogens have paradoxical effects, and many key factors could be attributed to it. To begin with the ligand-binding affinity of phytoestrogens, both ER could play a role. Also since they act within ER-mediated and ER-independent pathways, this may lead to additive, synergistic, or possible antagonistic interactions. In in vitro and in vivo, studies deliver varying outcome due to contribution of various factors such as involvement of multiple pathways, dosage, and bioavailability. Phytoestrogenic actions are dependent on concentration, varying with the target tissue and upon number and type of ER behaving as selective estrogen receptor modulators (SERM) which makes them difficult to predict the effect. Other key factors are also involved in the discrepancy obtained in the results. Ethnicity of the patient and bioavailability and genetic polymorphism of the PE metabolizing enzyme vary considerably with ethnicity and region of the population involved. Phytoestrogenic content in the supplements and extracts varies reasonably, and the difference in bioavailability of the compound must also be taken into account [219]. The presence or absence of endogenous estrogens also

influences the effect of phytoestrogens [220]. All these factors can be attributed for the clinical efficacy of phytoestrogens which is also the necessity of the present scenario in the field of chemotherapy, but the undesired effects cannot be neglected unless critically observed and evaluated. As with many other compounds found in nature, there are many pros and cons associated with phytoestrogens, but overall, phytoestrogen supplements have a safe side-effect profile. To conclude, phytoestrogens are worthy of further investigations to achieve maximum beneficial output with a corresponding minimization of their undesired effects.

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