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OBM Integrative and Complementary Medicine

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Review

The Beneficial and Adverse Effects of Phytoestrogens

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Academic Editor: James D. Adams

Special Issue: Advantages of Traditional Healing and Use of Plant Medicines

OBM Integrative and Complementary Medicine
2022, volume 7, issue 3
doi:10.21926/obm.icm.2203034

Received: May 19, 2022
Accepted: July 24, 2022
Published: August 08, 2022

Abstract

The most well-known phytoestrogens (flavonoids, isoflavonoids, lignans, coumestans, stilbenes, and prenylflavonoids) are isoflavonoids, which are important active ingredients in medicinal and food plants. They are highly abundant in the Fabaceae family. More than 1,000 types of isoflavonoids have been isolated from nearly 300 kinds of plants, and more are being discovered through modern analytical methods. Glycosides O and C of isoflavonoids are poorly absorbed in the intestine. They are converted by bacterial esterases and/or β-glycosidase enzymes to aglycones, which are absorbed more efficiently. Their bioavailability shows significant differences due to variation in the intestinal microflora of various races. The compounds formed during their biotransformation are structurally similar to estrogens. In Traditional Chinese medicine, several herbs rich in phytoestrogens are used to prevent and cure various diseases, such as osteoporosis, cardiovascular diseases, diabetes mellitus, hypertension, hyperlipidemia, tumors, and inflammation; additionally, 185 herbs are used to treat menopausal symptoms. Some of these herbs can be used to alleviate the unpleasant symptoms of menopause and treat breast and prostate cancer. From a nutritional physiology

perspective, the consumption of *Glycine max* and *Vigna unguiculata* should be emphasized. Soy has been consumed in China for about 5,000 years while it was introduced to Europe nearly 300 years ago. Soybean cultivation in Hungary dates back only 100 years. The assessment of the efficacy of phytoestrogens is unclear. Although several experimental and molecular biology studies have shown favorable results, studies on humans have not shown prominent therapeutic benefits. However, comparing and interpreting the findings of modern studies might elucidate the therapeutic utility of phytoestrogens.

Keywords
Phytotherapy; phytoestrogens; isoflavonoids; flavonoids; lignans; coumestans; stilbenoids

1. Introduction

In the 1930s, sheep that consumed large amounts of clover and alfalfa were found to be infertile in Australia. The cause was later associated with the estrogenic effects of isoflavonoids [1].

Isoflavonoids cause endocrine disruption, i.e., they alter the functions of the endocrine system. They bind to hormone receptors in the body and influence their function, production, hormone synthesis, transport, and breakdown. These effects are also observed in the offspring [2].

Isoflavonoids are a subclass of polyphenolic flavonoids. They are natural compounds with 3-phenylchroman structures that are derived directly from benzo-γ-pyrone. Isoflavonoids are plant-based secondary metabolites, which mainly belong to the Papilionoideae subfamily of Fabaceae or Leguminosae.

In this review, we presented the pharmacological effects of phytoestrogens, including the bioavailability of isoflavonoids, by describing some important medicinal and food plants.

2. Materials and Methods

Scientific publications were studied via the intranet available at Semmelweis University. We reviewed the Web of Science multidisciplinary bibliographic database provided by Clarivate Analytics (previously: Thomson Reuters), PubMed free, journal indexing bibliographic medical database, and Scopus multidisciplinary bibliographic database provided by Elsevier.

3. Structure and Grouping of Estrogens and Phytoestrogens

Estrogens (17β-estradiol, estrone, and estriol) are sterane compounds, where the third carbon atom of the aromatic ring A has a hydroxyl group, and the carbon atom of the ring D has a hydroxyl or oxo group. These sterane compounds are found in animals. Estrogens and isoflavonoids are structurally very similar (Figure 1).

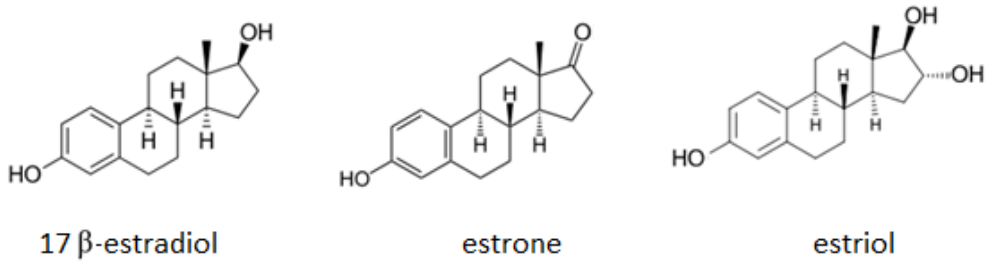


Figure 1 The structural formula of estrogens.

The biological activity of estrone, estriol, and 17 β-estradiol is approximately 1:7:100. The compound 17β-estradiol is an endogenous major steroid hormone and the primary female sex hormone. It also plays a key role in the etiology of breast, prostate, endometrial, and ovarian cancers [3].

Animal-derived estrogens and plant-derived phytoestrogens can bind to the same estrogen receptors as they have similar molecular structures [4].

Phytoestrogenic compounds include flavonoids, isoflavonoids, lignans, coumestans, prenylflavonoids, and stilbenoids, which belong mainly to the Leguminosae family and other families as well [5].

Within the family of flavonoids, some flavones, flavonols, and flavanones have estrogenic properties.

Flavonoids are synthesized by the phenylpropanoid metabolic pathway from phenylalanine to form p-coumaroyl-CoA (4-coumarate-CoA) due to the activity of several enzymes, including phenylalanine ammonia-lyase, cinnamate 4-hydroxylase, and 4-coumarate CoA ligase.

Next, it binds to malonyl-CoA due to the activity of chalcone synthase and forms the backbone of the flavonoid molecule. These compounds are known as chalcones and contain two phenyl rings. The chalcone isomerase enzyme closes the ring, which leads to the formation of a three-ring structure.

The metabolic pathway is regulated by the activity of enzymes. For example, flavonols, flavan 3-ols, and other polyphenols (e.g., isoflavonoids) are formed from naringenin due to the activity of isoflavone synthase, whereas genistein, daidzein, and glycitein are formed from liquiritigenin due to the activity of isoflavone synthase.

The stilbene derivate resveratrol is synthesized from 4-coumarate CoA and malonyl CoA (3x) by the action of stilbene synthase [6-8].

Flavonoids, especially isoflavonoids, show high structural variability, and the molecular structure of thousands of their representatives is now known. The variation occurs due to the degree of oxidation and substitution. These compounds highly differ in the number and position of the phenolic hydroxyl groups. The antioxidant effect of flavonoids depends on the location of the functional groups in the molecule; however, these substitution sites are also responsible for frequent toxicity. Thus, redox transitions also influence the reactions [9].

Hundreds of compounds of lignans composed of phenylpropane units occur in nearly 70 plant families. They accumulate in several plant parts (e.g., fruits, seeds, and roots). The metabolites of enterodiol, secoisolariciresinol, enterolactone and matairesinol-type lignans have estrogenic effects. They are enriched in significant amounts in flaxseed, sesame seeds, and wheat but are also found

in beans and pumpkins. Besides containing daidzin, genistin, daidzein, genistein, formononetin, and biochanin A, green coffee also contains three lignans, secoisolariciresinol, matairesinol, and lariciresinol. Flavonolignan (silychristine, silybinine, and isosilybinin) is found in the milk thistle fruit [10].

In general, the bioavailability of plant-originated polyphenols is determined by their chemical structure. The lignans that have low intestinal absorption are metabolized quickly. Lignans are demethylated and deoxygenated in the gastrointestinal tract by the gut microbiota.

If 17β-estradiol levels are normal in the body, lignans act as estrogen antagonists. However, if the 17β-estradiol levels are low, lignans can exhibit a weak estrogenic effect [11].

Nearly 300 plants contain isoflavonoids, many of which have estrogenic effects. Their most important members occur in legumes belonging to the Fabaceae family. Soy, *Glycine max* (L.) Merril is extremely nutritious.

More than 1,000 types of isoflavonoids have been isolated.

Coumestans are derivatives of isoflavonoids and are common in legumes. Their concentration is the highest in alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), and soybean germ (*Glycine max*).

The main source of resveratrol is red grapes. Resveratrol treatment can reduce the risk of menopausal cardiovascular diseases and decrease the progression of osteoporosis [12].

Mention should also be made of the Janus-faced property of resveratrol (antioxidant, prooxidant, and affects transmethylation), as the compound is highly advertised.

Resveratrol can also bind to nuclear estrogen receptors and modify its genomic activity. It also interacts with membrane estrogen receptors and affects non-genomic estrogen activity. The compound interferes with steroidogenesis and inhibits the steroid biosynthesis pathway. Resveratrol inhibits the hepatic and intestinal metabolism of estrogen. Chronic exposure is dangerous because it might cause endocrine disorders [13].

Resveratrol weakly stimulates estrogen receptors. Phytoestrogenic resveratrol might be used for treating hormonal disorders associated with polycystic ovary syndrome (PCOS). Female Sprague-Dawley (SD) rats treated orally with letrozole showed normal ovarian morphology after treatment with resveratrol [14].

Some studies have found that resveratrol can bind to methyl groups and affect transmethylation; therefore, its role is not favorable [15].

The transmethylation capacity of cancer patients is poor. Tumor anemia is strongly affected by globin methylation. Uncontrollable anemia can be alleviated by methyl donor compounds [16].

Phytoestrogens have been studied extensively in the last decade [17].

The structural formulae of phytoestrogenic compounds are shown in Figure 2 and are based on the review article of Gencel et al. [5].

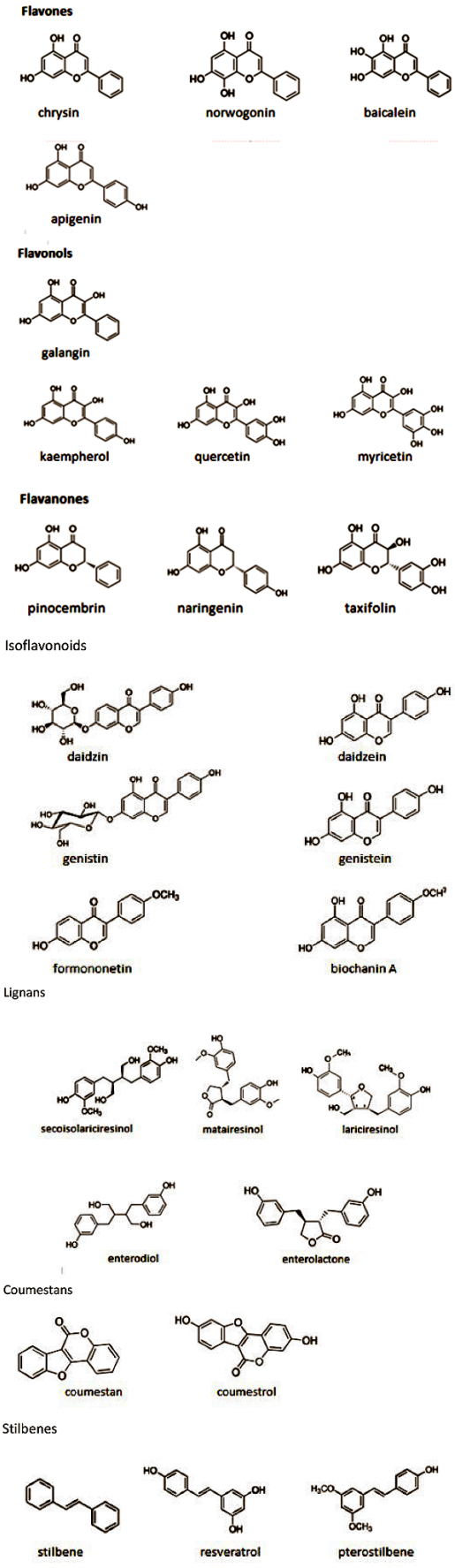


Figure 2 Phytoestrogenic compounds.

Mammalian lignans, such as enterodiol and enterolactone, are diphenolic compounds. They are formed from plant precursors due to the actions of bacterial enzymes in the colon. Mammalian lignanes can help to prevent cancer [18-20].

Phytoestrogens are also found in algae, cyanobacteria, and fungi [21].

Molds belonging to the genus *Fusarium* also contain estrogenic compounds, but they are toxic resorcilic acid lactones (Figure 3).

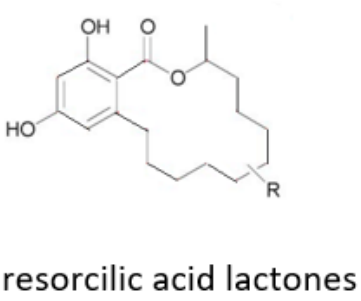


Figure 3 The structural formula of an estrogenic compound of *Fusarium*.

Resorcylic acid lactones (RALs) are potent irreversible inhibitors of the HSP90 chaperone protein or MEK and TAK kinases, which are involved in several signaling cascades. These compounds inhibit the VGFR (vascular endothelial growth factor receptor protein). RALs are highly effective chemotherapeutic agents and might be suitable for treating inflammation. (Effects of RALs: radicicol and pochonin D are HSP90 inhibitors, hypothemycin is a MAP kinase inhibitor, LL-783, 277 is a MEK inhibitor, pochonin C is an HSV inhibitor, and LL-Z1640–2 is a TAK –1 inhibitor) [22].

4. Bioavailability of Isoflavonoids

Phytoestrogens have a beneficial effect on endothelial cells, the smooth muscle cells of the vessel wall, and the extracellular matrix. Isoflavonoids can help to reduce the risk of cardiovascular diseases and type 2 diabetes mellitus due to their scavenger, lipid, and anti-inflammatory properties [5].

The anticarcinogenic effect of these molecules can be detected in cancer cell lines *in vitro* [5, 23].

The glycosides of isoflavonoids are poorly absorbed in the intestinal tract due to their molecular weight and hydrophilic properties. Isoflavonoids can be O and C glycosides. In the intestinal tract, they are converted by esterases and/or β-glycosidase enzymes to aglycones, which are absorbed more efficiently (Figure 4).

After adsorption via the intestinal epithelium, they are incorporated into kilomicrons. Kilomicron remnants enter the liver, where isoflavonoids are metabolized mainly by glucuronidation and to a lesser extent by sulfation. After metabolism, they are excreted with bile, where the metabolites become a part of enterohepatic circulation.

The enzymes of intestinal bacteria cleave glucuronic acid, and isoflavonoids are reabsorbed into the systemic circulation. Most of them are excreted in the feces, but 10–30% are present in the urine.

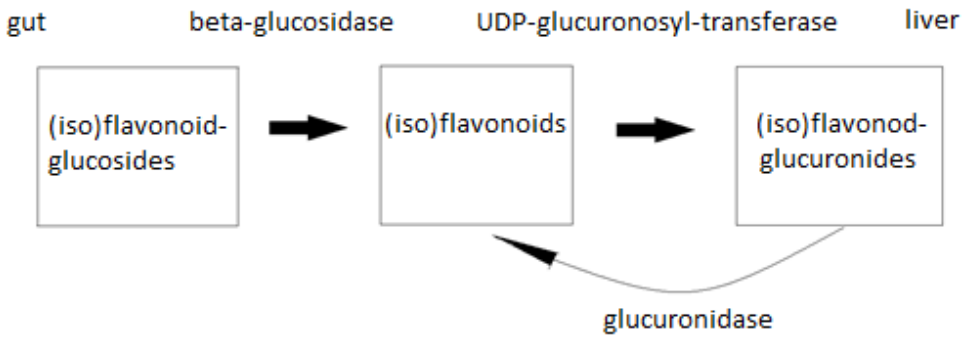


Figure 4 Biotransformation of iso(flavonoids).

In the gastrointestinal tract, (iso)flavonoid glycosides are hydrolyzed into bioactive aglycones at the brush border membrane due to the action of bacterial β-glucosidases. After being absorbed, (iso)flavonoids are metabolized to β-glucuronides and sulfate esters in the intestinal mucosa cells [24].

The isoflavonoid type daidzein, genistein, and several structurally related compounds can inhibit 17β-estradiol-induced hormone-dependent tumors. Isoflavones might influence the glucuronidation of 17β-estradiol in human hepatic microsomes [25, 26].

Phytoestrogenic flavones, flavonols, flavanones, and isoflavonoids are the most effective anti-inflammatory flavonoid derivatives. These compounds are effective in arachidonic acid-induced inflammation. Some of the compounds include naringenin, chrysin, apigenin, galangin, quercetin, kaempferol, and biochanin A.

They mainly attack phospholipase A2, cyclooxygenase-2 (COX2), lipoxygenase (LOX), and NO synthase. They also affect signaling pathways [27].

The formation of prostaglandins and leukotrienes is initiated by free radicals. Free radicals also increase the activity of phospholipase A2. Both LTB4 (Leukotriene B4) and IL-2 (interleukin-2) enhance the activity of NK (natural killer) cells [28].

Extracts containing the phytoestrogen baicalein of *Scutellaria baicalensis* and catechin of *Acacia catechu* are effective inhibitors of COX-1, COX-2 (Cyclooxygenase-1, 2), and 5-LOX (5-lipoxygenase); thus, they can reduce the synthesis of inflammatory eicosanoids and edema in an animal model of inflammation (*in vivo*) [29].

Formulations made from them (e.g., Limbrel/USA; Ato Formula/South Korea) have been used successfully for treating osteoarthritis [27].

The effect of soy isoflavonoids has been studied *in vitro* and on different experimental inflammatory models. The results showed that these compounds have anti-inflammatory activity as structural analogs of 17 β-estradiol.

The effect of genistein was investigated on cell-mediated inflammatory responses *in vivo* and also on mouse models of oxazolone-induced delayed-type hypersensitivity reaction (dependent on T cells/macrophages) and olive oil-induced inflammatory response (granulocyte-dependent but T cell/macrophage independent). Soy isoflavone reduced inflammation and inhibited the activity of granulocytes, monocytes, and lymphocytes. Thus, genistein was found to suppress cell-mediated immune responses [30].

Isoflavonoids can interact with M cells, dendritic cells, mast cells, and other cells in the gut to influence the immune system. Several immune cells are found in the lymphoid tissue of the intestinal mucosa and the GALT (gut-associated lymphoid tissue). The highly specialized M cells are located here, while T and B lymphocytes and macrophages are present in their pockets. These cells carry the antigens to the lymphoid tissue. Peyer's patches in the ileum are secondary lymphatic follicles in which antigens activate B cells and convert them to IgA-producing plasma cells [31-33].

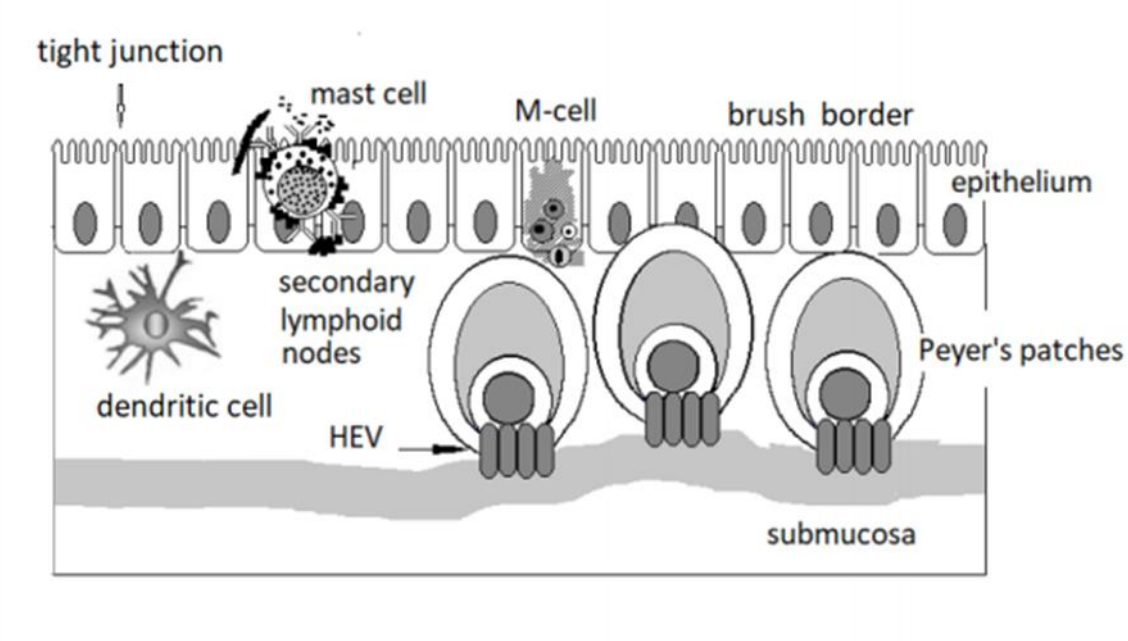


Figure 5 A schematic representation of the gastrointestinal mucosa.

The cells involved in the physiological response to antigens are shown in Figure 5 (based on a study by Perdue 1992) [34].

Inflammatory cytokines include IFN-α, β, γ, IL-1α, β, IL-6, IL-10, IL-12, TNF-α, β, MIF, and chemokines. They are mainly synthesized by mononuclear phagocytes, γ/δ T cells, granulocytes, mast cells, dendritic cells, fibroblasts, and endothelial cells. These cytokines cause inflammatory responses, initiate the activation of specific immune cells, and regulate their differentiation [28].

Dendritic cells facilitate communication between the innate and adaptive immune systems. They present antigens to T cells. Their Toll-like receptors recognize pathogens and dendritic cells phagocytose them. During maturation, these fragments are presented on their surface using the major histocompatibility complex (MHC). After maturation, several molecules such as CD40, CD80, CD86, CD58, and MHC are upregulated on the cell surface significantly increasing their ability to activate T cells. The activated dendritic cells release cytokines, prostaglandins, and NO and promote inflammation by directing cellular migration. Dendritic cells can activate memory and naive T cells. The dendritic cells, M cells, and mast cells in the gut can come into contact with the intestinal lumen, and thus, the isoflavonoids in food have a direct effect on these cells.

Genistein, daidzein, equol, biochanin A, and formononetin act in different modes on the maturation and activation of dendritic cells. Daidzein can significantly inhibit the expression of CD40, CD80, and CD86 (costimulatory molecule) maturation-associated cell surface markers and major histocompatibility complex class II (I-Ab) in a dose-dependent manner. Daidzein can suppress pro-inflammatory cytokines but does not affect IL-10 and IL-1β expression.

Daidzein, genistein, and equol can inhibit lipopolysaccharide (LPS)-stimulated dendritic cells with the downregulation of the expression of CD86.

These molecules have immunosuppressive effects and can suppress the maturation of dendritic cells and, consequently, the production of pro-inflammatory cytokines [35].

Genistein significantly decreased IL-6 and IL-1 beta production during PMA/A23187 (Phorbol myristate acetate/Ca^{2+} ionophore A23187)-induced mast cell activation [36].

Apigenin, quercetin, genistein, and (+/-) equol inhibit rat and human 11β-HSD1 and 11β-HSD2 enzymes in a concentration-dependent manner and thus, have antidiabetic activity. The potency of the effect follows the order: apigenin > quercetin > genistein >> (+/-) equol on human 11β-HSD1 reductase. These compounds bind to the steroid-binding site of human 11β-HSD1 and function as non-competitive inhibitors of 11β-HSD1 when steroid substrates are used. Apigenin is a selective inhibitor of two human 11β-HSD isoforms. Genistein can also inhibit rat 11β-HSD1 reductase, while the other three chemicals have no effects on the enzyme activity at low concentrations [37].

The bi-functional 11β-hydroxysteroid dehydrogenase (11β-HSD) isozyme 11β-HSD1 converts cortisone to the active hormone cortisol, and 11β-HSD2 converts cortisol to inactive cortisone. Thus, they play an important role in diabetes mellitus [37].

Cortisol increases hepatic glucose output by activating the transcription of phosphoenol pyruvate kinase (PEPCK). This effect is independent of insulin, glucagon, and fatty acids [38, 39].

The antiviral effects of daidzein, genistein, glycitein, and coumestrol were investigated on the replication of the herpes simplex virus (HSV), and genistein and coumestrol were found to inhibit HSV-1 (acyclovir-sensitive and acyclovir-resistant KOS and 29R strains) and HSV-2 (333 strain) replication at non-cytotoxic concentrations, but daidzein and glycitein showed no antiviral effects. Coumestrol acted in the early stages of viral infection. Genistein and coumestrol strongly inhibited HSV-1 (29R strain) and HSV-2 replication, rather than the replication of HSV-1 (KOS strain). Coumestrol affected the early stages of viral infection, and both genistein and coumestrol reduced HSV-1 protein expression and the spread of HSV-2 between cells [40]. Effective antiviral drugs for COVID-19, caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus), are not available yet. Thus, isoflavonoids were examined in an *in silico* study to determine the effectiveness of these drugs on the ACE2 (angiotensin-converting enzyme 2) cell-entry receptor and on Mpro, the main protease that is essential for viral replication. The researchers investigated whether isoflavonoids could demonstrate antiviral effects on SARS-CoV-2. Molecular docking studies have been performed on 59 isoflavonoids [41].

Drug-likeness properties were calculated using Lipinski's rule. Almost all compounds in the study followed Lipinski's five rules.

In ADMET Studies (absorption, distribution, metabolism, excretion, and toxicity) on the plasma protein binding model, the results showed that the compounds genistein and daidzein could bind to plasma protein more than 95%, while glycitein showed a binding pattern of more than 90% (The target protein crystal structures were obtained from the RCSB Protein Data Bank by the authors.)

The isoflavonoids were investigated through molecular docking studies, and their binding capabilities against ACE-2 and Mpro were determined.

All isoflavonoids were found to bind strongly to the hACE-2 receptor. The binding of isoflavone derivatives daidzein, genistein, and glycitein to the hACE-2 receptor was relatively weaker than the binding of some isoflavone derivatives, but it was stronger than the binding of the pterocarpan derivatives to the hACE-2 receptor. All tested isoflavones could bind to the active site of the

coronavirus Mpro (PDB:6LU7) with one or more hydrogen bonds, as determined by molecular docking studies [41].

5. Properties of Phytoestrogens and Their Role in Signal Transduction

Depending on the concentration of the free radicals released from the antioxidant control contribute to the disruption of vital cell functions, thereby inducing apoptotic or necrotic cell death. Free radicals rapidly activate extracellular signaling-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNK/SAPK), and p38 mitogen-activated protein kinases (MAPK). They also induce the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and Akt (serine/threonine protein kinase) signaling pathways. Thus, free radicals induce apoptosis and proliferation of cells [42, 43].

Free radicals affect signal transduction in several diseases and also affect aging [44, 45].

Recent studies on molecular biology have investigated the role of many plant-active substances in signal transduction. Isoflavonoids have radical scavenging/antioxidant properties, which might help to inhibit signaling pathways. However, these properties do not enhance isoflavonoid function as hormones [43].

Phytoestrogens partly act by binding to estrogen receptors and partly by influencing signaling molecules that initiate signaling pathways. Their receptor affinity is lower than that of estrogens. Phytoestrogens regulate the function of cells by affecting signal transduction pathways.

In animals, genistein, daidzein, and glycitein inhibit carcinogenesis by regulating the cell cycle and apoptosis.

Soy isoflavones, genistein, daidzein, and glycitein have pleiotropic effects on cancer cells. They target multiple cellular signaling pathways, including NF-κB, Akt, MAPK, Wnt (cysteine-rich glycoproteins with highly conserved cell signaling systems), and p53 (tumor suppressor protein) [46].

Most studies related to isoflavones have been performed on genistein. Genistein antagonizes estrogen-mediated and androgen-mediated signal pathways. It has antiproliferative or pro-apoptotic effects and inhibits the growth of cancer cells by inhibiting the PTK (protein-tyrosine kinase), protooncogene HER-2, topoisomerase I, and II, 5α-reductase, protein histidine kinase, as well as, the NF-κB and Akt signaling pathways [47].

Genistein acts as a programmed cell death inducer by downregulating the expression of the Bcl-2 protein (B-cell lymphoma 2), regulating cell death (apoptosis), upregulating the expression of Bax (a subfamily of Bcl2-related proteins), and activating caspase-3 (cysteine-aspartic acid protease), which is a lysosomal enzyme involved in the apoptotic pathway that enhances apoptosis [48]. Genistein exhibits anti-mutagenic and anti-metastatic effects. It inhibits signal transduction pathways and promotes tumor reduction by suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells [49].

Genistein inhibits the activation of the members of the STAT (signal transducer and activator of transcription) protein family, including STAT-3 and STAT-5, IGF-1R (insulin-like growth factor 1 receptor), Ape-1/Ref1 (apurinic/apyrimidinic endonuclease 1/redox effector factor 1), Wnt (cysteine-rich glycoproteins), CREB (cAMP response element-binding protein), and HIF-1α (hypoxia-inducible factor 1-α). It also stimulates the Nrf1, Nrf2 (nuclear factor erythroid 2-related factor-1, nuclear factor erythroid 2–related factor 2), and GADD 153 (endoplasmic reticulum (ER) stress-induced apoptotic pathway) transcription factors. This stimulates the p53 tumor suppressor gene.

Genistein influences the cell cycle (cell cycle progression from G2 to M phase) by downregulating cyclin B1 (involved in mitosis) and cyclin D1 (important regulator of cell cycle progression) regulatory proteins, decreasing the Wee-1 (serine/threonine-protein kinase) nuclear kinase, CDK-1 (cyclin-dependent kinase 1) kinase, and increasing the activity of p21WAF1, p27 KIP1, and p16 INK4a cyclin-dependent kinase inhibitors [50-54].

Biochanin A, genistein, daidzein, glycitein, and formononetin have beneficial effects on altered carbohydrate and lipid metabolism and also on the severe disease-induced complications (cardiovascular diseases, nephropathy, neuropathy, and retinopathy) in type 2 diabetes mellitus.

The PPARs (α and γ) (peroxisome proliferator-activated receptors) are activated by these soya isoflavonoids. These nuclear receptors strongly influence the regulation of insulin sensitivity and glucose homeostasis [39, 55].

Genistein inhibits COX2 enzyme activity by inhibiting the prostaglandin biosynthesis cascade and moderates immune reactions. It can inhibit MMP-9, MMP-2 (matrix metallopeptidases), p38 MAPK, ERK-1/2 mitogen-activated protein kinases, RANK/RANK-L (receptor activator of nuclear factor-κB ligand (RANKL)/RANK/osteoprotegerin (OPG) system, and PTEN (phosphatase and tensin homolog) unique tumor suppressor gene [46].

Genistein can increase the level of GPx (glutathione peroxidase) antioxidant enzyme, kangai-1 (transmembrane glycoprotein that functions as a metastasis suppressor protein), and endoglin (type 1 membrane glycoproteins) on the cell surface; thus, inhibiting angiogenesis, tumor growth, survival, and metastasis of cancer. Due to its antioxidant character, it can also inhibit angiogenesis [47].

Genistein has apoptotic effects on prostate cancer, brain tumor, lung cancer, bladder cancer, ras-dependent bladder cancer, neuroblastoma, leukemia ovarian cancer, gastrointestinal, and colon cancer cell lines, among others. Cytotoxic and apoptotic effects of genistein have been demonstrated on estrogen-dependent/independent breast cancer cell lines [56].

Isoflavonoids of cowpea have also been investigated. The isoflavonoids and vitamin D of cowpea daidzein and genistein together activate the BMP-2/Smad (bone morphogenetic protein-2 growth factor/Smad signal transduction pathways) signaling pathway of osteoblasts in osteogenesis and promote further proliferation and differentiation of osteoblasts. These results showed that isoflavones (daidzein and genistein) isolated from cowpea can be used in the treatment of bone disorders [57, 58].

Genistein can inhibit angiogenesis in several ways, for example, by modifying signaling pathways and influencing gene expression. At concentrations of 5 to 50 μM, it can inhibit the growth of human umbilical vein endothelial cells (HUVECs) and significantly inhibit the expression of VEGF (vascular endothelial growth factor), which is a regulator of angiogenesis.

Genistein might exhibit anti-cancer effects by inhibiting the VEGF-mediated signaling pathway between tumor cells and endothelial cells.

It might also inhibit the activation of the hypoxia-induced factor-1 (HIF-1) protein. Additionally, it inhibits the expression of the VEGF gene in this pathway. Other important proteins involved in angiogenesis are also inhibited in genistein-treated cells, such as platelet-derived growth factor (PDGF) and urokinase plasminogen activator (uPa) [59].

6. Advantages and Disadvantages of Hormone Replacement Therapy

Although menopause has been investigated extensively, studies on it before the 19th century were rarely conducted.

The term menopause is attributed to the French physician Charles-Pierre-Louis de Gardanne, who coined it from the words man and pause (break) in 1821.

In Germany, attempts were made in the 1800s to reduce women's sexual complaints by treating the symptoms of menopause by administering the ovarian tissues of cows via injection and oral therapy, which led to significant improvements.

Menopause was described as a deficiency disease, and it was later treated with ovary homogenates or testicle preparations from animals [60, 61].

Papanicolaou (1883–1962) first described the role of estrogen (estrogen and the role of the hypothalamic-pituitary-adrenal (HPA) axis in guinea pigs), and this laid the foundation for hormone replacement therapy [62].

The treatment of menopause has been in the interest of doctors since the 1930s [63].

Estrogens have a significant effect on the reproductive and multiple organ systems of women and men; thus, they affect many physiological functions. Early studies on sex hormones were performed by the biologist and physiologist Edgar Allen, who studied the effect of follicular fluids on uterine weight, vaginal maturation, and sexual susceptibility in 1923. The biochemist Edward A. Doisy in the United States (professor of biochemistry and later director at St. Louis University School of Medicine in 1924) and the German chemist Adolph F.J. Butenandt (Institute of Chemistry at the University of Göttingen) also conducted their research at the same time, independently. Allen gave follicular fluid to Doisy, who extracted and crystallized estrone. He reported his results at the 13th International Physiological Congress in Boston in 1929. Doisy patented his process in 1934. Later, Doisy isolated estriol and estradiol. Butenandt purified and crystallized estrone and published the results together with Leopold Ruzicka before the manuscript of Doisy was published. Thus, they received the Nobel Prize (1939) for their research and the crystallization of testosterone. Butenandt isolated progesterone in 1934, estradiol in 1936 in collaboration with his colleagues, and testosterone in 1939 [64].

Hormone replacement therapy was started in the 1950s. Estrogen was initially purified from the urine of pregnant horses and later was produced synthetically. Initially, it was distributed mainly among popular actresses employed in the American and Western European film industries, and then, its usage gradually spread in civil society. Estrogen treatment can effectively alleviate the unpleasant physical and mental symptoms of menopause.

The Menopause Rating Scale (MRS) summarizes all the symptoms that occur frequently and require medical treatment in severe cases.

The menopause rating scale:

- Psychological symptoms: 0–16 points (four symptoms: depression, irritability, anxiousness, and exhaustion)
- Somato-vegetative symptoms: 0–16 points (four symptoms: sweating/flushing, heart problems, sleep disturbances, and joint and muscle problems)
- Urogenital symptoms: 0–12 points (three symptoms: sexual problems, urinary complaints, and vaginal dryness) [65].

During hormone replacement therapy, estrogens increase salt and water retention, thereby forming edema, increasing breast tension, and increasing the synthesis of blood clotting factors and fibrinogen, which can cause thromboembolism and stroke. Breast and endometrial cancers and an increase in tumor size commonly occur after estrogen therapy. All these problems might be associated with discomfort, hypertension, headache, and depression.

Because estrogens have a proliferative effect on the endometrium, after 1975, estrogen was used along with progesterone (the main metabolic hormone) or progestin to prevent the development of endometrial tumors.

Combined hormone replacement therapy, however, was not safe. The incidence of breast cancer decreased slightly, and progesterone prevented the development of uterine tumors, but vaginal bleeding, bloating, cholelithiasis, breast tenderness, venous thromboembolism, stroke, and depression persisted [66-68].

In 2002, JAMA reported an increase in the risk of thrombosis and stroke as a result of hormone replacement therapy [69].

Despite significant advancements in hormone replacement therapy (topical, systemic, and transdermal), most women are skeptical about HRT and even reject medical advice in critical situations, such as in scenarios of high risk of osteoporosis and cardiovascular diseases. In 2018, only 1% of women in Hungary received hormone replacement, although due to unwanted age-related changes, considerably more women would need it under adequate medical control [70]. Women often use natural active ingredients to avoid side effects [71].

7. Hormone Replacement and Phytoestrogens

In the Asian population, 80% of the people can produce S-equol from the main isoflavone of soy, daidzein, while only 25% of the European and American populations are capable of doing so [4]. *Clostridium, Eggerthella, Slackia, Streptococcus strains, Lactococcus garvieae,* and *Enterococcus* initially convert daidzein to dihydrodaidzein, and the conversion of dihydrodaidzein to tetrahydrodaidzein, and than tetrahydrodaidzein to S-equol.

Changes in the intestinal bacterial population, the use of antibiotics, and changes in diet can alter the ability to produce S-equol (Figure 6).

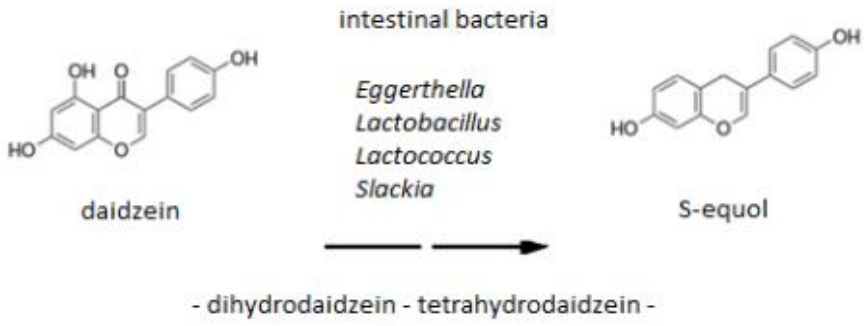
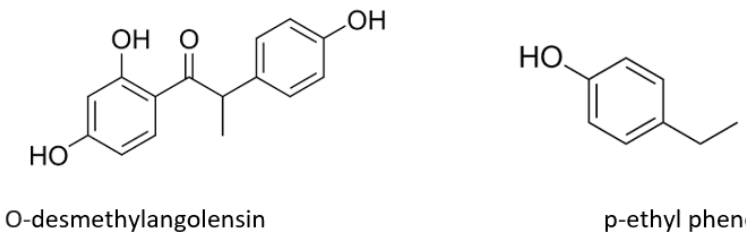


Figure 6 The transformation of daidzein to S-equol.

Antibiotic therapy affects the viability and activity of intestinal bacteria. For example, metronidazole can inhibit S-equol production in 20% of equol-producing individuals [72, 73].

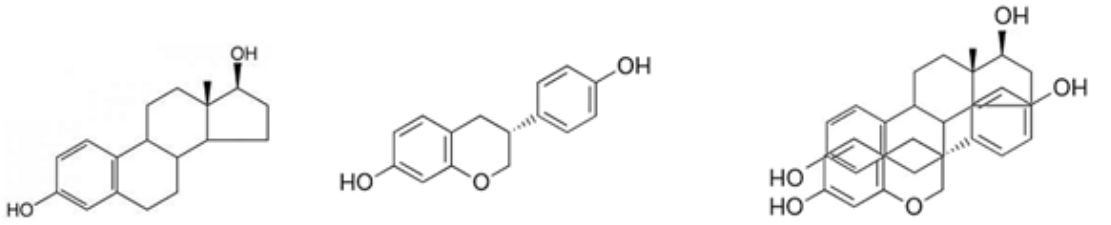
The intestinal microflora of some individuals might convert daidzein to S-equol, and the other molecules, as O-O-desmethylangolensin and genistein to p-ethyl phenol (Figure 7) [74].



O-desmethylangolensin p-ethyl phenol

Figure 7 The chemical structure of isoflavonoid derivates.

The molecular structure and overlap of 17β-estradiol and S-equol formed during the biotransformation of daidzein can be seen in Figure 8 [75].



17β-estradiol S-equol overlap

(3S')-isoflavan -4,7'-diol

Figure 8 The molecular structure and overlap of 17β-estradiol and S-equol.

8. Some Important Herbs that Contain Phytoestrogens

In Traditional Chinese medicine (TCM), several herbs rich in phytoestrogens are used to prevent and cure various diseases such as osteoporosis, cardiovascular diseases, diabetes mellitus, hypertension, hyperlipidemia, tumors, and inflammation. Additionally, 185 herbs are used to treat symptoms of menopause [76].

Significant amounts of phytoestrogen are found in *Agrimonia eupatoria, Angelicae sinensis*, *Arachis hypogaea, Arctium lappa, Astragalus lentiginosus, Carthamus tinctorius, Cimicifuga racemosa, Crotalaria albida, Coffea Arabica (green), Cucurbita pepo, Foeniculum vulgare, Forsythia koreana, Glycine max, Glycyrrhiza glabra, Glycyrrhiza uralensis, Humulus lupulus, Iresine herbstii, Linum usitatissimum, Matricaria recutita, Medicago sativa, Mucuna sempervirens, Ononis spinose, Panax ginseng, Phaseolus vulgaris, Pisonia umbellifera, Pisum sativum, Polygonum cuspidatum,*

Pueraria lobate, Rosmarinus officinalis, Sesamum indicum, Silybum marianum, Spatholobus suberectus, Trifolium incarnatum, Trifolium pratense, Triticum aestivum, Triticum vulgare, *Vigna radiata, Vigna unguiculata, Vitis berlandieri, Vitis riparia,* and *Vitis vinifera.*

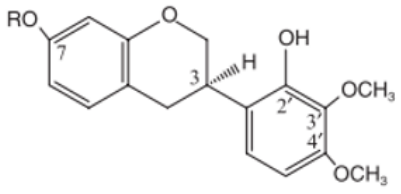
Herbs containing phytoestrogens are important in TCM. In China, Korea, and Japan, herbs have been used for thousands of years to relieve perimenopausal symptoms.

Angelicae sinensis, *Panax ginseng*, *Flemingia macrophylla,* P*ueraria lobata, Trifolium pratense* preparations, *Vigna unguiculata,* and *Glycine max* have special importance in TCM [17, 74, 77].

8.1 Angelicae Sinensis

Angelicae sinensis (Oliv.) Diels belongs to the Apiaceae family. Radix *Angelicae sinensis* is a commonly used Chinese herb, which has traditionally been used to treat dysmenorrhoea and irregular menstruation and as a supportive herb for menopausal complaints. It has an estrogen-like effect on the vaginal mucosa. Its extract competes with estradiol for estrogen-binding and progesterone-binding sites in human myometrial cytosol [35]. The herbal preparation Danggui Buxue Tang was first prescribed by Li Dongyuan in Neiwaishang Bianhuo Lun, in AD 1247 to treat gynecological disorders. This preparation consisted of Radix Astragali (root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao, Leguminosae) and Radix *Angelicae sinensis* (root of *Angelica sinensis* (Oliv.) Diels, Apiaceae). This herbal preparation was widely used [78].

The decoction of Buyang Huanwu consists of radix *Astragali*, radix *Angelicae sinensis*, rhizoma *Ligustici chuanxiong*, radix Paeoniae rubra, flos *Carthami*, semen *Persicae*, and *Lumbricus* (Figure 9). The formulation was analyzed by ultrafast HPLC and DADTOF/MS. In total, 54 major components, four C-glycosylquinocalcones, four flavonoid O-glycosides, 16 isoflavones, six monoterpene glycosides, eight saponins, four organic acids, and five amino acids were identified or experimentally characterized based on their retention times.



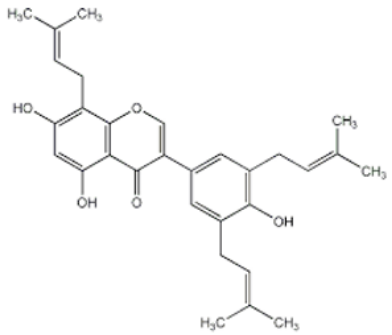
(3R)-2'-hydroxy-3',4'-dimethoxy–isoflavan-7-O- β-D-glycoside R=β-D-glc
(3R)-2'-hydroxy-3',4'-dimethoxy–isoflavan-7-O- β-D-glc-6''-O-malonate
R=6''-O-malonyl-β-D-glc
(3R)-2'-hydroxy-3',4'-dimethoxy–isoflavan-7-O- β-D-glc-6''-O-acetate
R=6''-O-acetyl-β-D-glc
(3 R)-7,2'-dihydroxy-3',4'- dimethoxy–isoflavan R= H

Figure 9 Isoflavan components of the Buyang Huanwu decoction [79].

8.2 Flemingia Macrophylla

*Flemingia macrophylla (*Willd.) Kuntze ex Merr. belongs to the Fabaceae family. The isoflavonoid-rich *Flemingia macrophylla* extract contains typical compounds of Fabaceae and prenylated isoflavones (Figure 10). Among various types of isoflavones, it also contains a new isoflavone component known as flemiphyllin, as well as several flavonoids that were previously unknown.

The extract of *Flemingia macrophylla* inhibits elastase activity, promotes the expression of the protein type I procollagen, and attenuates phosphorylation of mitogen-activated protein (MAP) kinase and the expression of matrix-metalloproteinases (MMP-1, –3, and –9); thus, it has an anti-cancer effect. The antioxidants of the drug have ideal anti-aging properties [80].



flemiphyllin

Figure 10 The isoflavone component of *Flemingia macrophylla.*

8.3 Panax Ginseng

Panax ginseng is a perennial plant in the genus *Panax* of the family Araliaceae. Most ginseng species are native to East Asia. The active components of ginseng include saponins, most of which are glycosides of triterpenoid aglycones and are similar to estradiol. *Panax ginseng* has several phytoestrogens (Figure 11). Most ginsenosides are metabolized by intestinal bacteria. Ginsenoside-Rb1 acts by binding to the estrogen receptor. This was confirmed by the inhibition of its activity by the specific estrogen receptor antagonist ICI 182,780 [81].

Ginsenoside-Rh 1 is also weakly estrogenic and can bind to and activate the estrogen receptor. Ginsenoside-Rh1 increases c-fos and pS2 mRNA levels 24 h after treatment, but the effects are weaker than those of 17β-estradiol on MCF-7 breast cancer cells [82].

The root powder of *Panax quinquefolium* (American Ginseng) is also used to treat breast cancer.

However, in female SENCAR mice with dimethylbenzanthracene (DMBA)-induced mammary carcinoma, the difference between the control group and the American ginseng-treated group was not statistically significant after 52 weeks of treatment. The American ginseng was less effective than the selective estrogen receptor modulators tamoxifen and ospemifene. The Lady 4 combination contained evening primrose oil, damiana, ginseng, and royal jelly, which was beneficial for women with menopausal syndrome after two and four weeks of treatment, as determined by the outcome of the Menopause Rating Scale-II (MRS-II). The MRS-II score in the Lady 4 combination treated group was significantly better than that of the placebo control group [83, 84].

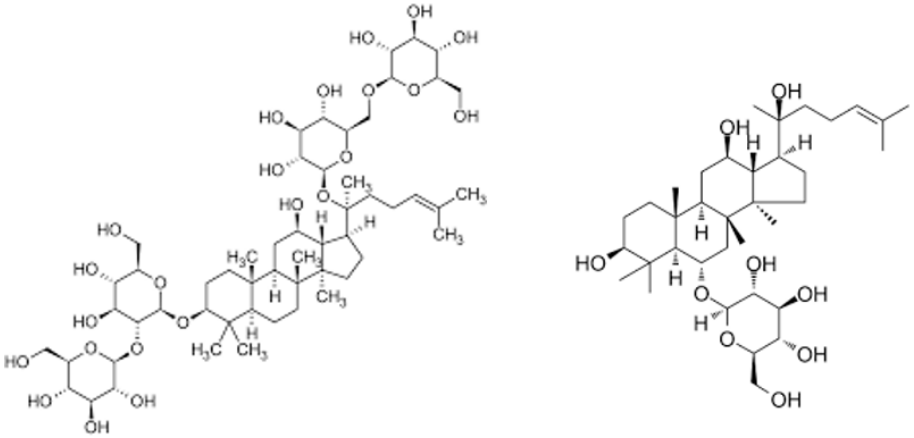


Figure 11 The active ingredients of ginseng.

8.4 Pueraria Lobata

Pueraria lobata (Willd) (kudzu) belongs to the Leguminosae family and is used in TCM. The flowers of *Pueraria lobata* have two major isoflavones, which include tectoridin and 6”-O-xylosyl-tectoridin, and other isoflavonoids, kakkalide, kakkalidone, puerarin, irisolidone, 6”-O-xylosyl-glycitin, and genistin. It is used to treat intoxication, alcoholic hepatic diseases, and gastrointestinal diseases. The roots of *Pueraria lobata* contain puerarin, daidzin, daidzein, genistin, and genistein and are effective for treating cardiovascular diseases, osteonecrosis, and neurodegradation. They also have antidiabetic, antiviral, and antioxidant effects and can inhibit rectal ulcers and bleeding (Figure 12) [85].

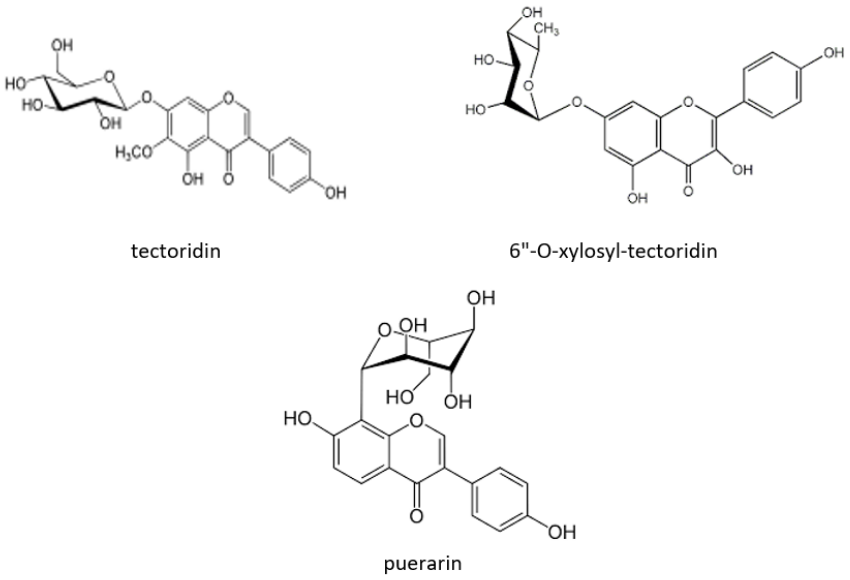


Figure 12 The active ingredients of *Pueraria lobata*.

Tectoridin, 6”-O-xylosyl-tectoridin, kakkalide, and their metabolites have estrogenic effects. Tectoridin metabolites activate estrogen and thyroid hormone receptors [86]. The isoflavonoids from the kudzu root can protect vascular endothelial cells against reactive oxygen species (ROS)-mediated apoptosis and mitochondrial damage [87]. Puerarin is an important isoflavone glycoside. It has low toxicity and strong anticarcinogenic effects. This plant can inhibit the proliferation of different cancer cell lines by modulating different signal transduction pathways, such as NF-κB, PI3K/AKT/mTOR, JNK, MEK/ERK (Raf)-MEK-ERK, and autophagy. The antioxidant molecules produced by the plant can inhibit oxidative stress and inflammation [88].

Daidzin selective aldehyde dehydrogenase (ALDH-2) inhibitor and potential agents for alcohol dependence [89, 90].

8.5 Trifolium Pratense

Trifolium pratense L. belongs to the family Fabaceae. The main active ingredients are biochanin A, formonentin, formononetin, prunetin, genistein, daidzein, isoflavone monogalactosides, and coumestans phytoestrogens [91].

The therapeutic effects of bioactive ingredients of *Trifolium pratense* extract on menopausal symptoms have not been proven. The results of published studies do not sufficiently demonstrate their beneficial effects on the breast and endometrium.

Trifolium pratense also contains coumarin derivatives and should be used with caution; they are contraindicated in anticoagulant therapy [92].

In a study on the properties of *Trifolium pratense*, the relationships between phytoestrogens, antioxidants, and anti-cancer effects were established. The drug positively affects glucose and lipid metabolism; therefore, it has a protective effect on the cardiovascular system.

Other studies, however, did not consider the plant to be a strong candidate for treating the symptoms of menopause. It also did not improve sexual satisfaction. This plant extract has negative effects on sperm parameters [93-95]. Biochanin A is a potent inhibitor of Chlamydia spp. [96]. However, the anti-cancer effect of the extract was reported in several studies [97].

8.6 Vigna Unguiculata

The cowpea is grown in many tropical parts of the world for human consumption and animal feed. The shoots, leaves, unripe pods, and dry ripe seeds of all varieties are harvested. The health effects of the isoflavonoids of edible cowpea (sand beans and chickpeas) have also been studied [57].

Osteoporosis can be delayed by consuming cowpea. Daidzein also has anti-osteoporotic effects. It affects osteoblast function, stimulates cell proliferation, and promotes the differentiation of osteoblasts. Daidzein acts by stimulating the activation of the BMP-2/Smads pathway [58, 98].

Cowpea is a “functional food” and has a positive effect on health. The concentrations of ascorbic acid, chlorophyll, carotenoids, anthocyanins, flavonoids, and phenolic compounds of cowpea change during cooking, but its antioxidant effects still protect against oxidative damage [99].

8.7 Glycine Max

Soy (*Glycine max* (L.) Merril)/Fabaceae) is highly nutritious.

The population of Southeast Asia has been consuming soy for the past 5,000 years; however, it first appeared in Europe in the 17th century. Soybean cultivation in Hungary started only 100 years ago.

In China, Korea, and Japan, the estimated average isoflavone consumption is 20–50 mg/day [100].

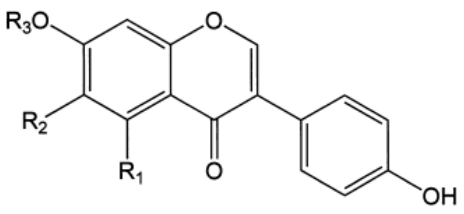
However, not everyone can consume soy. Although soy contains favorable levels of proteins, starch, fatty acids (linolenic acid), simple sugars, crude fiber, amino acids (lysine, methionine, cysteine, threonine, and tryptophan), and isoflavone (daidzein, genistein, and biochanin), it also contains phytic acid and certain proteins that protect against macroorganisms and microorganisms. These proteins, such as β-conglycinin and trypsin inhibitors (Bowman-Birk trypsin-chymotrypsin inhibitor and soybean Kunitz trypsin inhibitor), can cause allergies (atopic dermatitis/eczema) and other side effects (indigestion). Lectins (hemagglutinins) interact with cell surface carbohydrates to damage intestinal mucosal cells and agglutinate red blood cells.

Therefore, special attention should be paid to these proteins while preparing soy foods [101, 102].

Researchers in China compared the isoflavonoid content of different soybeans and found that the isoflavonoid content in Chinese samples was similar to that in Japanese samples but was considerably lower than that in American samples.

The Chinese herbal preparation made from fermented black soybeans or soybeans had higher isoflavone glucosides and aglycones than those prepared from natural soybeans (Figure 13).

Differences in soy products might also cause a lower rate of breast cancer among Asian women [103].



Compounds	R_1	R_2	R_3
DAE	H	H	H
GLE	H	OCH_3	H
GEE	OH	H	H
DAI	H	H	7-*O*-ß-D-glucoside
GLI	H	OCH_3	7-*O*-ß-D-glucoside
GEI	OH	H	7-*O*-ß-D-glucoside
Ac-DAI	H	H	6"-*O*-acetyl-7-*O*-ß-D-glucoside
Ac-GLI	H	OCH_3	6"-*O*-acetyl-7-*O*-ß-D-glucoside
Ac-GEI	OH	H	6"-*O*-acetyl-7-*O*-ß-D-glucoside
Mal-DAI	H	H	6"-*O*-malonyl-7-*O*-ß-D-glucoside
Mal-GLI	H	OCH_3	6"-*O*-malonyl-7-*O*-ß-D-glucoside
Mal-GEI	OH	H	6"-*O*-malonyl-7-*O*-ß-D-glucoside

Figure 13 The list of soy isoflavones [62] [daidzein (DAE), glycitein (GLE), genistein (GEE), daidzin (DAI), glycitin (GLI), genistin (GEI), malonylglucosides, 6-O-malonyl-7-O-β-D-daidzin (Mal-DAI), 6-O-malonyl-7-O-β-D-glycitin (Mal-GLI), 6-O-malonyl-7-O-β-D-genistin (Mal-GEI), acetylglucosides, 6-O-acetyl-7-O-β-D-daidzin (Ac-DAI), 6-O-acetyl-7-O-β-D-glycitin (Ac-GLI), and 6-O-acetyl-7-O-β-D-genistin (Ac-GEI).]

9. Experimental and Clinical Studies with Soy Phytoestrogens

Besides isoflavonoids, the soluble and insoluble dietary fiber, phytochemicals, proteins, and peptides in legumes also have several beneficial physiological effects on the treatment of diabetes, hypertension, hyperlipidemia, and anti-inflammatory therapy.

Besides phytoestrogens, soy proteins also have beneficial effects on the human body. The serum cholesterol levels decreased in elderly women with hypercholesterolemia after the daily intake of 5 g of soy for three months [104].

In a randomized, placebo-controlled study, significant changes occurred in the characteristics of half of the participants with metabolic syndrome compared to the changes in the characteristics of healthy controls. The body weight, body mass index, and LDL-cholesterol levels decreased, and beneficial changes occurred in the lipid profile. Soy consumption significantly reduced the cardiovascular risk and also showed a beneficial effect on the symptoms of metabolic syndrome [105].

However, in another placebo-controlled randomized clinical trial, coronary calcification was shown to be faster in hormone replacement therapy when there was a greater reduction in plasma residual lipoprotein cholesterol and pre-beta-HDL subpopulation. This suggested that they were predictive factors for coronary heart disease [106].

The findings of epidemiological studies suggested that the incidence of breast cancer is significantly lower in Asian countries than in the United States or Europe. This phenomenon has also been associated with higher consumption of soy [107, 108].

Approximately 10% of the total protein consumed by the Asian population is derived from soy. The concentrations of isoflavone in the urine and serum of women and men and the prostate fluid and serum of men were examined, and it was found that isoflavone concentrations were inversely proportional to the development of breast and prostate cancer [47].

Mongoloid-type women who emigrated to the US and adopted American eating habits were found to have a higher risk of developing osteoporosis than their counterparts in Asian countries. The differences in mortality are explained by the Asian diet and the consumption of soy-rich foods [109].

Phytoestrogens reduce bone loss during menopause. In a systematic review and meta-analysis of randomized controlled trials, soy isoflavones were found to have beneficial effects on markers of bone formation; however, extensive multicenter studies are required to confirm these findings, as suggested by the authors [110].

Sathyapalan et al. conducted a randomized, double-blinded trial and showed favorable significant changes in the markers of bone turnover (β-C-telopeptide and procollagen type 1 N-propeptide) after six months of soy consumption [111].

A study published in Clinical Nutrition in 2018 quantified plasma phytoestrogen isoflavones (genistein and daidzein) and lignan (enterolactone) by performing liquid chromatography-mass spectrometry in colon cancer patients of both sexes.

In this case-control study with many Vietnamese and Korean colon cancer patients, high plasma levels of isoflavones were associated with a lower risk of colon cancer regardless of ethnic differences, especially in women.

Genistein concentrations were lower in patients with colon cancer. The level of enterolactone was not associated with the risk of colon cancer [112].

Although several studies have shown the antitumor effect of isoflavones on animals and humans, others have refuted the antitumor effect in recent years [113-115]. Although phytoestrogens have many beneficial effects, several studies have shown the harmful effects of some of these active substances.

The benefits of different soy foods and foods containing soy and other plants (meadow testicles, flaxseed, and beans) in improving menopausal symptoms are not clear. In many cases, no significant differences were found after treatment, but the number of reports of conflicting results was unsatisfactory, based on a summary of the results of clinical studies [67, 116].

An increase in pre-existing estrogen-dependent breast cancer was found with genistein. Genistein antagonizes the effects of tamoxifen in cancer therapy, thus reducing its efficacy. Also, its use might be contraindicated in women with breast cancer [117].

A mixture of isoflavonoids isolated from a dietary supplement stimulated the proliferation of MCF-7-E10 (human breast adenocarcinoma cell line) cells *in vitro* [118].

The researchers did not rule out the possibility that the concentration of isoflavonoids in breast tissue might be lower than that in studies conducted *in vitro*, and thus, there might not be an increase in the risk of tumor *in vivo.*

Another *in vitro* study was conducted on the effects of genistein alone, or in combination with estradiol and tamoxifen, on the growth of human dysplastic (MCF-10A(1), MCF-ANeoT, MCF-T(6)3B), and malignant MCF-7, MDA-231, and MDA-435) epithelial breast cell lines [119]. Genistein inhibited the growth of dysplastic and malignant epithelial breast cancer cells *in vitro,* and the addition of tamoxifen had a synergistic/additive inhibitory effect on the proliferation of both cell types. These effects were not modulated by the presence of estrogen receptors [120].

These findings contradicted the findings of Helferich et al., where phytoestrogens were found to cause metastasis by enhancing cell proliferation. In the groups treated with different isoflavonoids, Ki-67 expression (an indicator of proliferation) occurred at a much higher rate than that without isoflavonoid treatment. The rate was 28% for genistein, 35.9% for daidzein, 28.6% for the equol treated group, and 34.3% for those treated with multiple isoflavonoids. These results suggested that people with cancer are at high risk after phytoestrogen consumption [67, 121].

In a 24-week randomized, double-blind study involving few patients, neither soy protein nor isoflavonoids were found to reduce vasomotor symptoms. The differences in the intensity or frequency of hot flushes and night sweats between the groups were not significant. Other symptoms, such as mood swings, vaginal dryness, and decreased libido, occurred; however, the difference between the treatment and placebo groups was not significant [122].

Together, soy preparation and low-dose hormone therapy did not significantly affect LDL and HDL cholesterol concentrations. These biomarkers are important for cardiovascular diseases [123].

Since the use of Chinese herbs is expected to increase among women with menopausal symptoms seeking alternative therapies, studies have been conducted by Chinese researchers using well-known databases, including Gynecology and Fertility Group's Specialized Register of Controlled Trials, Cochrane Central Register of Controlled Trials (CENTRAL; 2015, Issue 3), MEDLINE, Embase, CINAHL, AMED, and PsycINFO (from inception to March 2015). Other methods included Current Control Trials, Citation Indices, conference abstracts in the ISI Web of Knowledge, the LILACS database, PubMed, the OpenSIGLE database, and the China National Knowledge Infrastructure database (CNKI, 1999 to 2015).

However, statistical analyses based on the databases studied did not provide sufficient proof that Chinese herbal preparations were as effective as placebo treatment or hormone replacement therapy in relieving vasomotor symptoms. The safety effects were also inconclusive, and further studies using randomized controlled trials need to be conducted [76].

As no reliable data based on clinical trials are available, future studies should aim to obtain reproducible results regarding menopause and the quality and dose of treatments administered [124].

Herbal preparations are often marketed as food supplements or homeopathic remedies, and thus, clinical trials by manufacturers and distributors are not mandatory for selling them; such clinical trials are generally not performed. Therefore, rigorous and systematic efficacy studies need to be conducted to obtain conclusive results regarding the effects of herbal preparations [125].

10. Discussion

In this review, we summarized the general [6, 11, 20] and specific [41] knowledge regarding phytoestrogens and evaluated the advantages and disadvantages of hormone replacement therapy and phytotherapy in menopausal syndrome. We also briefly summarized the structure and grouping of estrogens and phytoestrogens, especially the bioavailability of isoflavonoids [35, 36]. Here, we also showed the effect of phytoestrogens on signal transduction pathways based on recent studies on molecular biology [23, 44, 45, 49, 51, 58, 98].

Diseases and conditions such as osteoporosis, cardiovascular diseases, diabetes, high blood pressure, hyperlipidemia, and sexual problems impair the quality of life. Thus, information and techniques of medicine and phytotherapy can be applied to alleviate discomfort and even life-threatening conditions [67, 68, 98].

Applied estrogen treatment initially appeared to be a good option, although it caused severe somatic symptoms in many women. Unfortunately, it increased the risk of breast cancer, thromboembolic and cardiovascular events, stroke, and cholecystopathy. A combination treatment using estrogen and progestin was also ineffective [69, 71].

Therefore, women tried phytotherapeutic options, such as traditional Chinese medicine, for natural remedies. The popularity of hormone replacement/alternative therapies has also grown significantly, as indicated by the turnover [76].

The most common drugs in phytoestrogen formulations are *Glycine max*, *Panax ginseng*, *Angelicae sinensis*, *Pueraria lobate*, *Flemingia macrophylla*, *Trifolium pratense*, and *Vigna unguiculata* [74, 77].

However, the biggest problem with phytotherapy treatment is the lack of well-designed randomized clinical trials and information on the efficacy and safety in most cases [124].

Traditional Chinese medicine is an effective healing method based on 3,000 years of constantly evolving experience, which is now gaining popularity not only in the Far East but also in many other parts of the world. More people have started using Chinese phytotherapeutic formulations in Western countries because synthetic drugs and molecular therapies are often ineffective and have severe side effects and cross-reactions; therefore, they cannot be used in polymorbid patients. However, not knowing or misinterpreting the philosophy and practice of traditional Chinese treatments often leads to ineffectiveness and poisoning.

The biggest problem is that the original Chinese descriptions are often poorly translated (e.g., misspelled drug or product names, incorrect preparation methods, and incorrect dose/therapeutic time); without basic knowledge of TCM, it is difficult to interpret and implement therapeutic procedures [126].

Another reason is that although TCM is prevalent, the therapeutic procedure of TCM has not been incorporated into the official health care system in the EU and some regions of North America [127].

The main reason for the fundamental difference between Chinese Medicine formulations and Western medicine is the differences in the starting points, subjects, and techniques of research, the methodological approach, and the theoretical characteristics. These reasons have delayed the development of Chinese Medicine formulations in these regions [126].

In China, over the last two decades, advanced analytical and molecular biological approaches have been used, and efforts have been made to standardize TCM formulations, although some problems persist [126, 128].

For example, studies are being conducted using Chinese herbal active ingredients to decipher the operation of Chinese traditional principles in association with groups from the Eötvös Loránd University (Budapest, Hungary) and the University of Cambridge using Drug Profile Matching, an affinity fingerprint-based *in silico* drug repositioning approach that can quantitatively predict the overall effect profile of compounds through probability scores [129, 130].

It is challenging for practitioners of Western medicine and pharmacognosy to integrate the values of Chinese phytotherapy with the therapy of patients under the pressure of mapping the human genome and the differences in therapeutic ideologies.

It is also challenging for TCM practitioners to combine new molecular biological knowledge with empirically-based and well-functioning approaches. However, research and technical progress can help these different approaches to work together [131].

Interestingly, the American Cancer Institute (AICR) has cited the results of studies on humans that soy consumption is safe for breast cancer survivors. (Content last updated: 8 April 2021) [132].

11. Conclusion

The combination of age-old observations and modern empirical findings might enhance the therapeutic efficacy of phytoestrogens.

Author Contributions

The authors contributed equally to this work.

Competing Interests

The authors declare that they have no conflict of interest.

References

1. Bennetts HW, Underwood EJ, Shier FL. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust Vet J. 1946; 22: 2-12.

2. Csaba G. A hormonális rendszer válsága: az endokrin diszruptorok egészségügyi hatásai (The crisis of the hormonal system: The health-effects of endocrine disruptors). Orv Hetil. 2017; 158: 1443-1451.
3. Henderson BE, Ross RK, Pike MC, Casagrande JT. Endogenous hormones as a major factor in human cancer. Cancer Res. 1982; 42: 3232-3239.
4. Jackson RL, Greiwe JS, Schwen RJ. Emerging evidence of the health benefits of S-equol, an estrogen receptor β agonist. Nutr Rev. 2011; 69: 432-448.
5. Gencel VB, M Benjamin MM, Bahou SN, Khalil RA. Vascular effects of phytoestrogens and alternative menopausal hormone therapy in cardiovascular disease. Mini Rev Med Chem. 2012; 12: 149-174.
6. García-Calderón M, Pérez-Delgado CM, Palove-Balang P, Betti M, Márquez AJ. Flavonoids and isoflavonoids biosynthesis in the model legume lotus japonicus; connections to nitrogen metabolism and photorespiration. Plants. 2020; 9: 774.
7. Jung W, Yu O, Lau SM, O'Keefe DP, Odell J, Fader G, et al. Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. Nat Biotechnol. 2000; 18: 208-212.
8. Yu O, Shi J, Hession AO, Maxwell CA, McGonigle B, Odell JT. Metabolic engineering to increase isoflavone biosynthesis in soybean seed. Phytochemistry. 2003; 63: 753-763.
9. Breinholt V. Desirable vs. harmful levels of intake of flavonoids and phenolic acids. In: Natural antioxidants and anticarcinogens in nutrition, health and disease. Cambridge: The Royal Society of Chemistry; 1999. pp.93-105.
10. Angeloni S, Navarini L, Khamitova G, Maggi F, Sagratini G, Vittori S, et al. A new analytical method for the simultaneous quantification of isoflavones and lignans in 25 green coffee samples by HPLC-MS/MS. Food Chem. 2020; 325: 126924.
11. Álvarez-Caballero JM, Coy-Barrera E. Lignans. In: Antioxidants effects in health, the bright and the dark side. Amsterdam: Elsevier; 2022. pp.387-417.
12. Shah AA, Gourishetti K, Nayak Y. Osteogenic activity of resveratrol in human fetal osteoblast cells. Pharmacogn Mag. 2019; 15: 250-255.
13. Qasem RJ. The estrogenic activity of resveratrol: A comprehensive review of in vitro and in vivo evidence and the potential for endocrine disruption. Crit Rev Toxicol. 2020; 50: 439-462.
14. Zhang N, Zhuang L, Gai S, Shan Y, Wang S, Li F, et al. Beneficial phytoestrogenic effects of resveratrol on polycystic ovary syndrome in rat model. Gynecol Endocrinol. 2021; 37: 337-341.
15. Blázovics A, Fébel H, Bekő G, Kleiner D, Szentmihályi K, Sárdi É. Why do not polyphenols of red wine protect against the harmful effects of alcohol in alcoholism? Acta Aliment. 2019; 48: 358-364.
16. Blázovics A, Nyirády P, Romics I, Szűcs M, Horváth A, Szilvás Á, et al. How can cancer-associated anemia be moderated with nutritional factors and how do Beta vulgaris L. ssp. esculenta var. rubra modify the transmethylation reaction in erythrocytes in cancerous patients? In: Anemia. Rijeka: Tech Open Access Publisher; 2012. pp.93-114.
17. Michel T, Halabalaki M, Skaltsounis AL. New concepts, experimental approaches, and dereplication strategies for the discovery of novel phytoestrogens from natural sources. Planta Med. 2013; 79: 514-532.

18. Setchell KDR, Adlercreutz H. Mammalian lignans and phyto-estrogens: Recent studies on their formation, metabolism and biological role in health and disease. In: Role of the gut flora in toxicity and cancer. London: Academic Press; 1988. pp.315-345.
19. Thompson LU, Robb P, Serraino M, Cheung F. Mammalian lignan production from various foods. Nutr Cancer. 1991; 16: 43-52.
20. Petrine JC, Del Bianco-Borges B. The influence of phytoestrogens on different physiological and pathological processes: An overview. Phytother Res. 2021; 35: 180-197.
21. Klejdus B, Lojková L, Plaza M, Šnóblová M, Štěrbová D. Hyphenated technique for the extraction and determination of isoflavones in algae: Ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. J Chromatogr A. 2010; 1217: 7956-7965.
22. Winssinger N, Barluenga S. Chemistry and biology of resorcylic acid lactones. Chem Commun. 2007: 22-36. Doi: 10.1039/B610344H.
23. Hwang ST, Yang MH, Baek SH, Um JY, Ahn KS. Genistin attenuates cellular growth and promotes apoptotic cell death breast cancer cells through modulation of ERalpha signaling pathway. Life Sci. 2020; 263: 118594.
24. Barnes S. The biochemistry, chemistry and physiology of the isoflavones in soybeans and their food products. Lymphat Res Biol. 2010; 8: 89-98.
25. Pfeiffer E, Treiling CR, Hoehle SI, Metzler M. Isoflavones modulate the glucuronidation of estradiol in human liver microsomes. Carcinogenesis. 2005; 26: 2172-2178.
26. Zhang Y, Hendrich S, Murphy PA. Glucuronides are the main isoflavone metabolites in women. J Nutr. 2003; 133: 399-404.
27. Gábor M. Pharmacology and dermatopharmacology of mouse ear inflammation. Budapest: Akadémiai Kiadó; 2012.
28. Polya GM, Polya Z, Kweifio-Okai G. Biochemical pharmacology of anti-inflammatory plant secondary metabolites. In: Recent progress in medicinal plants. Houston: SCI TECH Publishing; 2002. pp.1-22.
29. Burnett BP, Jia Q, Zhao Y, Levy RM. A medicinal extract of Scutellaria baicalensis and Acacia catechu acts as a dual inhibitor of cyclooxygenase and 5-lipoxygenase to reduce inflammation. J Med Food. 2007; 10: 442-451.
30. Verdrengh M, Jonsson IM, Holmdahl R, Tarkowski A. Genistein as an anti-inflammatory agent. Inflamm Res. 2003; 52: 341-346.
31. Kanaya T, Williams IR, Ohno H. Intestinal M cells: Tireless samplers of enteric microbiota. Traffic. 2020; 21: 34-44.
32. Baranov V, Hammarström S. Carcinoembryonic antigen (CEA) and CEA-related cell adhesion molecule 1 (CEACAM1), apically expressed on human colonic M cells, are potential receptors for microbial adhesion. Histochem Cell Biol. 2004; 121: 83-89.
33. Lo D, Tynan W, Dickerson J, Scharf M, Cooper J, Byrne D, et al. Cell culture modeling of specialized tissue: Identification of genes expressed specifically by follicle-associated epithelium of Peyer's patch by expression profiling of Caco-2/Raji co-cultures. Int Immunol. 2004; 16: 91-99.
34. Perdue MH, Kosecka U, Crowe S. Antigen-mediated effects on epithelial function. Ann N Y Acad Sci. 1992; 664: 325-334.

35. Yum MK, Jung MY, Cho D, Kim TS. Suppression of dendritic cells' maturation and functions by daidzein, a phytoestrogen. Toxicol Appl Pharmacol. 2011; 257: 174-181.
36. Kim DH, Jung WS, Kim ME, Lee HW, Youn HY, Seon JK, et al. Genistein inhibits pro-inflammatory cytokines in human mast cell activation through the inhibition of the ERK pathway. Int J Mol Med. 2014; 34: 1669-1674.
37. Edgerton DS, Basu R, Ramnanan CJ, Farmer TD, Neal D, Scott M, et al. Effect of 11β-hydroxysteroid dehydrogenase-1 inhibition on hepatic glucose metabolism in the conscious dog. Am J Physiol Endocrinol Metab. 2010; 298: E1019-E1026.
38. Zhu Q, Ge F, Dong Y, Sun W, Wang Z, Shan Y, et al. Comparison of flavonoids and isoflavonoids to inhibit rat and human 11β-hydroxysteroid dehydrogenase 1 and 2. Steroids. 2018; 132: 25-32.
39. Kuryłowicz A. The role of isoflavones in type 2 diabetes prevention and treatment—A narrative review. Int J Mol Sci. 2020; 22: 218.
40. Argenta DF, Silva IT, Bassani VL, Koester LS, Teixeira HF, Simoes CM. Antiherpes evaluation of soybean isoflavonoids. Arch Virol. 2015; 160: 2335-2342.
41. Alesawy MS, Abdallah AE, Taghour MS, Elkaeed EB, H Eissa I, Metwaly AM. In silico studies of some isoflavonoids as potential candidates against COVID-19 targeting human ACE2 (hACE2) and viral main protease (Mpro). Molecules. 2021; 26: 2806.
42. Ramachandiran S, Huang Q, Dong J, Lau SS, Monks TJ. Mitogen-activated protein kinases contribute to reactive oxygen species-induced cell death in renal proximal tubule epithelial cells. Chem Res Toxicol. 2002; 15: 1635-1642.
43. Blazovics A. Redox homeostasis, bioactive agents and transduction therapy. Curr Signal Transduct Ther. 2007; 2: 226-239.
44. Jomova K, Valko M. Free radicals, signal transduction, and human disease. In: Oxidative stress in vertebrates and invertebrates: Molecular aspects of cell signaling. Hoboken: John Wiley & Sons, Inc.; 2012. pp.17-32.
45. Teixeira CP, Florencio-Silva R, Sasso GR, Carbonel AA, Simões RS, Simões MJ. Soy isoflavones protect against oxidative stress and diminish apoptosis in ovary of middle-aged female rats. Gynecol Endocrinol. 2019; 35: 586-590.
46. Li Y, Sarkar FH. Down-regulation of invasion and angiogenesis-related genes identified by cDNA microarray analysis of PC3 prostate cancer cells treated with genistein. Cancer Lett. 2002; 186: 157-164.
47. Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. Cancer Lett. 2008; 269: 226-242.
48. Li Y, Kong D, Bao B, Ahmad A, Sarkar FH. Induction of cancer cell death by isoflavone: The role of multiple signaling pathways. Nutrients. 2011; 3: 877-896.
49. Wang SD, Chen BC, Kao ST, Liu CJ, Yeh CC. Genistein inhibits tumor invasion by suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells. BMC Complement Altern Med. 2014; 14: 26.
50. Kim EK, Kwon KB, Song MY, Seo SW, Park SJ, Ka SO, et al. Genistein protects pancreatic β cells against cytokine-mediated toxicity. Mol Cell Endocrinol. 2007; 278: 18-28.
51. Mukund V, Saddala MS, Farran B, Mannavarapu M, Alam A, Nagaraju GP. Molecular docking studies of angiogenesis target protein HIF-1α and genistein in breast cancer. Gene. 2019; 701: 169-172.

52. Hernandez-Montes E, Pollard SE, Vauzour D, Jofre-Montseny L, Rota C, Rimbach G, et al. Activation of glutathione peroxidase via Nrf1 mediates genistein's protection against oxidative endothelial cell injury. Biochem Biophys Res Commun. 2006; 346: 851-859.
53. Yeh TC, Chiang PC, Li TK, Hsu JL, Lin CJ, Wang SW, et al. Genistein induces apoptosis in human hepatocellular carcinomas via interaction of endoplasmic reticulum stress and mitochondrial insult. Biochem Pharmacol. 2007; 73: 782-792.
54. Touny LH, Banerjee PP. Identification of both Myt-1 and Wee-1 as necessary mediators of the p21-independent inactivation of the Cdc-2/cyclin B1 complex and growth inhibition of TRAMP cancer cells by genistein. Prostate. 2006; 66: 1542-1555.
55. Chiarelli F, Di Marzio D. Peroxisome proliferator-activated receptor-γ agonists and diabetes: Current evidence and future perspectives. Vasc Health Risk Manag. 2008; 4: 297-304.
56. Blazovics A. Small molecules in cancer therapy: Cytotoxics and molecularly targeted agents. Curr Signal Transduct Ther. 2011; 6: 2-19.
57. Jayathilake C, Visvanathan R, Deen A, Bangamuwage R, Jayawardana BC, Nammi S, et al. Cowpea: An overview on its nutritional facts and health benefits. J Sci Food Agric. 2018; 98: 4793-4806.
58. Jadai R, Venna N, Ajumeera R, Challa S. Isoflavones rich cowpea and vitamin D induces the proliferation and differentiation of human osteoblasts via BMP-2/Smad pathway activation: Mechanistic approach. IUBMB Life. 2019; 71: 1794-1805.
59. Varinska L, Gal P, Mojzisova G, Mirossay L, Mojzis J. Soy and breast cancer: Focus on angiogenesis. Int J Mol Sci. 2015; 16: 11728-11749.
60. Singh A, Kaur S, Walia I. A historical perspective on menopause and menopausal age. Bull Indian Inst Hist Med. 2002; 32: 121-135.
61. Kohn GE, Rodriguez KM, Hotaling J, Pastuszak AW. The history of estrogen therapy. Sex Med Rev. 2019; 7: 416-421.
62. Stockard CR, Papanicolaou GN. The existence of a typical oestrous cycle in the guinea-pig—With a study of its histological and physiological changes. Am J Anat. 1917; 22: 225-283.
63. Santen RJ, Simpson E. History of estrogen: Its purification, structure, synthesis, biologic actions, and clinical implications. Endocrinology. 2019; 160: 605-625.
64. Simpson E, Santen RJ. Celebrating 75 years of oestradiol. J Mol Endocrinol. 2015; 55: T1-T20.
65. Schneider HP, Heinemann LA, Rosemeier HP, Potthoff P, Behre HM. The menopause rating scale (MRS): Reliability of scores of menopausal complaints. Climacteric. 2000; 3: 59-64.
66. Jyotsna VP. Postmenopausal hormonal therapy: Current status. Indian J Endocrinol Metab. 2013; 17: S45-S49.
67. Remport J, Blázovics A. Fitoösztrogének a menopauza terápiájában|Phytoestrogens in the treatment of menopause. Orv Hetil. 2017; 158: 1243-1251.
68. Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV, et al. Treatment of symptoms of the menopause: An endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2015; 100: 3975-4011.
69. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. JAMA. 2002; 288: 321-333.

70. Webbeteg. Klimax: A nők alig egy százaléka kap hormonpótlást (Menopause: barely 1 percent of women receive hormone replacement). 2018. Available from: https://www.webbeteg.hu/cikkek/menopauza/23448/klimax-es-hormonpotlas.
71. Velentzis LS, Salagame U, Canfell K. Menopausal hormone therapy: A systematic review of cost-effectiveness evaluations. BMC Health Serv Res. 2017; 17: 326.
72. Setchell KD, Brown NM, Summer S, King EC, Heubi JE, Cole S, et al. Dietary factors influence production of the soy isoflavone metabolite s-(-) equol in healthy adults. J Nutr. 2013; 143: 1950-1958.
73. Wang XL, Kim HJ, Kang SI, Kim SI, Hur HG. Production of phytoestrogen S-equol from daidzein in mixed culture of two anaerobic bacteria. Arch Microbiol. 2007; 187: 155-160.
74. Miadoková E. Isoflavonoids–An overview of their biological activities and potential health benefits. Interdiscip Toxicol. 2009; 2: 211-218.
75. Paula MA, Antony DP. Use of soy isoflavones on hormone replacement therapy during climacteric. Afr J Pharm Pharmacol. 2014; 8: 1071-1078.
76. Zhu X, Liew Y, Liu ZL. Chinese herbal medicine for menopausal symptoms. Cochrane Database Syst Rev. 2016. Doi: 10.1002/14651858.CD009023.pub2.
77. Pitkin J. Alternative and complementary therapies for the menopause. Menopause Int. 2012; 18: 20-27.
78. Su JY, Xie QF, Liu WJ, Lai P, Liu DD, Tang LH, et al. Perimenopause amelioration of a TCM recipe composed of Radix Astragali, Radix Angelicae Sinensis, and Folium Epimedii: An in vivo study on natural aging rat model. Evid Based Complementary Altern Med. 2013; 2013: 747240.
79. Liu EH, Qi LW, Peng YB, Cheng XL, Wu Q, Li P, et al. Rapid separation and identification of 54 major constituents in Buyang Huanwu decoction by ultra-fast HPLC system coupled with DAD-TOF/MS. Biomed Chromatogr. 2009; 23: 828-842.
80. Chiang HM, Chiu HH, Liao ST, Chen YT, Chang HC, Wen KC. Isoflavonoid-rich Flemingia macrophylla extract attenuates UVB-induced skin damage by scavenging reactive oxygen species and inhibiting MAP kinase and MMP expression. Evid Based Complementary Altern Med. 2013; 2013: 696879.
81. Lee YJ, Jin YR, Lim WC, Park WK, Cho JY, Jang S, et al. Ginsenoside-Rb1 acts as a weak phytoestrogen in MCF-7 human breast cancer cells. Arch Pharm Res. 2003; 26: 58-63.
82. Lee Y, Jin Y, Lim W, Ji S, Choi S, Jang S, et al. A ginsenoside-Rh1, a component of ginseng saponin, activates estrogen receptor in human breast carcinoma MCF-7 cells. J Steroid Biochem Mol Biol. 2003; 84: 463-468.
83. Yakoot M, Salem A, Omar AM. Effectiveness of a herbal formula in women with menopausal syndrome. Complement Med Res. 2011; 18: 264-268.
84. Wurz GT, Marchisano-Karpman C, DeGregorio MW. Ineffectiveness of American ginseng in the prevention of dimethylbenzanthracene-induced mammary tumors in mice. Oncol Res. 2006; 16: 251-260.
85. Yao M, Liao Y, Li GQ, Law FC, Tang Y. Quantitative analysis of two isoflavones in Pueraria lobata flowers from eleven Chinese provinces using high performance liquid chromatography. Chin Med. 2010; 5: 14.
86. Shin JE, Bae EA, Lee YC, Ma JY, Kim DH. Estrogenic effect of main components kakkalide and tectoridin of puerariae flos and their metabolites. Biol Pharm Bull. 2006; 29: 1202-1206.

87. Gao Y, Wang X, He C. An isoflavonoid-enriched extract from Pueraria lobata (kudzu) root protects human umbilical vein endothelial cells against oxidative stress induced apoptosis. J Ethnopharmacol. 2016; 193: 524-530.
88. Ahmad B, Khan S, Liu Y, Xue M, Nabi G, Kumar S, et al. Molecular mechanisms of anticancer activities of puerarin. Cancer Manag Res. 2020; 12: 79-90.
89. Rezvani AH, Overstreet DH, Perfumi M, Massi M. Plant derivatives in the treatment of alcohol dependency. Pharmacol Biochem Behav. 2003; 75: 593-606.
90. Keung WM. Anti-dipsotropic isoflavones: The potential therapeutic agents for alcohol dependence. Med Res Rev. 2003; 23: 669-696.
91. Drenin AA, Botirov EK, Turov YP. A new isoflavone glycoside from Trifolium pratense L. Russ J Bioorganic Chem. 2011; 37: 862-865.
92. Fugh-Berman A, Kronenberg F. Red clover (Trifolium pratense) for menopausal women: Current state of knowledge. Menopause. 2001; 8: 333-337.
93. ULKAY MB, AKTAŞ A, Bozkurt HH. The effect of Trifolium pratense L. (red clover) on rat testes. Kafkas Univ Vet Fak Derg. 2008; 14: 151-155.
94. Bakirel T, Keleş O, Bozkurt Hh, Ak Ke. The effect of Trifolium pratense on spermatogenesis and its acute toxicity (LD50) in mice. in mice. Turkish J Vet Anim Sci. 2002; 26: 555-559.
95. Giorno CD, Fonseca AM, Bagnoli VR, Assis JS, Soares Jr JM, Baracat EC. Effects of trifolium pratense on the climacteric and sexual symptoms in postmenopause. Rev Assoc Med Bras. 2010; 56: 558-562.
96. Hanski L, Genina N, Uvell H, Malinovskaja K, Gylfe Å, Laaksonen T, et al. Inhibitory activity of the isoflavone biochanin A on intracellular bacteria of genus Chlamydia and initial development of a buccal formulation. PLoS One. 2014; 9: e115115.
97. Mohsen A, Fatemeh K, Leila N, Mona P, Mohammad Z, Mozafar K. Pharmacological and therapeutic properties of the red clover (Trifolium pratense L.): An overview of the new finding. J Tradit Chin Med. 2021; 41: 642-649.
98. Yu B, Tang DZ, Li SY, Wu Y, Chen M. Daidzein promotes proliferation and differentiation in osteoblastic OCT1 cells via activation of the BMP-2/Smads pathway. Pharmazie. 2017; 72: 35-40.
99. Nderitu AM, Dykes L, Awika JM, Minnaar A, Duodu KG. Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated in vitro gastrointestinal digestion. Food Chem. 2013; 141: 1763-1771.
100. Borbélyné Hunyadi É, Drexler D. Ökológiai szójatermesztés Európában. (Organic soybean production in Europe). ÖMKi: Ökológiai Mezőgazdasági Kutatóintézet Közhasznú Nonprofit Kft; 2016.
101. Rickert DA, Johnson LA, Murphy PA. Improved fractionation of glycinin and β-conglycinin and partitioning of phytochemicals. J Agric Food Chem. 2004; 52: 1726-1734.
102. Medic J, Atkinson C, Hurburgh CR. Current knowledge in soybean composition. J Am Oil Chem Soc. 2014; 91: 363-384.
103. Yuan D, Pan Y, Chen Y, Uno T, Zhang S, Kano Y. An improved method for basic hydrolysis of isoflavone malonylglucosides and quality evaluation of Chinese soy materials. Chem Pharm Bull. 2008; 56: 1-6.

104. Regöly-Mérei A, Biró G, Lászlófi M, Greiner E, Gergely A, Kákossy T, et al. Szójafehérje hatása hospitalizált idős nők lipid és mikroelem statusára. (The effect of soybean protein on lipids and trace elements in elderly hospitalized women). Orv Hetil. 1991; 132: 1523-1526.
105. Ruscica M, Pavanello C, Morlotti B, Gomaraschi M, Vitali C, Bosisio R, et al. Soya-enriched mixed diet significantly improves cardiovascular and metabolic risk factors: A randomized controlled trial. Atherosclerosis. 2016; 252: e210.
106. Lamon-Fava S, Herrington DM, Reboussin DM, Sherman M, Horvath K, Schaefer EJ, et al. Changes in remnant and high-density lipoproteins associated with hormone therapy and progression of coronary artery disease in postmenopausal women. Atherosclerosis. 2009; 205: 325-330.
107. Xie Q, Chen ML, Qin Y, Zhang QY, Xu HX, Zhou Y, et al. Isoflavone consumption and risk of breast cancer: A dose-response meta-analysis of observational studies. Asia Pac J Clin Nutr. 2013; 22: 118-127.
108. Wu AH, Pike MC, Williams LD, Spicer D, Tseng CC, Churchwell MI, et al. Tamoxifen, soy, and lifestyle factors in Asian American women with breast cancer. J Clin Oncol. 2007; 25: 3024-3030.
109. Zheng X, Lee SK, Chun OK. Soy isoflavones and osteoporotic bone loss: A review with an emphasis on modulation of bone remodeling. J Med Food. 2016; 19: 1-14.
110. Kanadys W, Barańska A, Błaszczuk A, Polz-Dacewicz M, Drop B, Malm M, et al. Effects of soy isoflavones on biochemical markers of bone metabolism in postmenopausal women: A systematic review and meta-analysis of randomized controlled trials. Int J Environ Res Public Health. 2021; 18: 5346.
111. Sathyapalan T, Aye M, Rigby AS, Fraser WD, Thatcher NJ, Kilpatrick ES, et al. Soy reduces bone turnover markers in women during early menopause: A randomized controlled trial. J Bone Miner Res. 2017; 32: 157-164.
112. Ko KP, Yeo Y, Yoon JH, Kim CS, Tokudome S, Koriyama C, et al. Plasma phytoestrogens concentration and risk of colorectal cancer in two different Asian populations. Clin Nutr. 2018; 37: 1675-1682.
113. Jacobsen BK, Knutsen SF, Fraser GE. Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States). Cancer Causes Control. 1998; 9: 553-537.
114. Constantinou AI, Mehta R, Husband A. Phenoxodiol, a novel isoflavone derivative, inhibits dimethylbenz [a] anthracene (DMBA)-induced mammary carcinogenesis in female Sprague–Dawley rats. Eur J Cancer. 2003; 39: 1012-1018.
115. Davis JN, Kucuk O, Djuric Z, Sarkar FH. Soy isoflavone supplementation in healthy men prevents NF-κB activation by TNF-α in blood lymphocytes. Free Radic Biol Med. 2001; 30: 1293-1302.
116. Levis S, Griebeler ML. The role of soy foods in the treatment of menopausal symptoms. J Nutr. 2010; 140: 2318S-2321S.
117. Helferich WG, Andrade JE, Hoagland MS. Phytoestrogens and breast cancer: A complex story. Inflammopharmacology. 2008; 16: 219-226.
118. Onoda A, Ueno T, Uchiyama S, Hayashi SI, Kato K, Wake N. Effects of S-equol and natural S-equol supplement (SE5-OH) on the growth of MCF-7 in vitro and as tumors implanted into ovariectomized athymic mice. Food Chem Toxicol. 2011; 49: 2279-2284.

119. Tanos V, Brzezinski A, Drize O, Strauss N, Peretz T. Synergistic inhibitory effects of genistein and tamoxifen on human dysplastic and malignant epithelial breast cells in vitro. Eur J Obstet Gynecol Reprod Biol. 2002; 102: 188-194.
120. Ju YH, Doerge DR, Allred KF, Allred CD, Helferich WG. Dietary genistein negates the inhibitory effect of tamoxifen on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted in athymic mice. Cancer Res. 2002; 62: 2474-2477.
121. Yang X, Belosay A, Hartman JA, Song H, Zhang Y, Wang W, et al. Dietary soy isoflavones increase metastasis to lungs in an experimental model of breast cancer with bone micro-tumors. Clin Exp Metastasis. 2015; 32: 323-333.
122. Germain AS, Peterson CT, Robinson JG, Alekel DL. Isoflavone-rich or isoflavone-poor soy protein does not reduce menopausal symptoms during 24 weeks of treatment. Menopause. 2001; 8: 17-26.
123. Carmignani LO, Pedro AO, Costa-Paiva LH, Pinto-Neto AM. The effect of soy dietary supplement and low dose of hormone therapy on main cardiovascular health biomarkers: A randomized controlled trial. Rev Bras Ginecol Obstet. 2014; 36: 251-258.
124. Chen LR, Ko NY, Chen KH. Isoflavone supplements for menopausal women: A systematic review. Nutrients. 2019; 11: 2649.
125. Blázovics A. Food and food supplement antioxidants: Targets in human antioxidant system and effects on the production of endogenous antioxidants. In: Antioxidants effects in health, the bright and the dark side. Amsterdam: Elsevier; 2022. pp.837-850.
126. Blázovics A. A hagyományos kínai fitoterápia értelmezése és beillesztése a nyugati típusú orvoslásba az emberi genom ismeretének birtokában. (The interpretation and integration of traditional Chinese phytotherapy into Western-type medicine with the possession of knowledge of the human genome). Orv Hetil. 2018; 159: 696-702.
127. Chan K. Chinese medicinal materials and their interface with Western medical concepts. J Ethnopharmacol. 2005; 96: 1-18.
128. Xie PS, Leung AY. Understanding the traditional aspect of Chinese medicine in order to achieve meaningful quality control of Chinese materia medica. J Chromatogr A. 2009; 1216: 1933-1940.
129. Chen Z. A kínai gyógyászat gyógynövényei a XXI. században. (Herbs of Chinese medicine in the 21st century). In: Natural bioactive ingredients in modern medicine. Gödöllő: Szent István Egyetemi Kiadó; 2013. pp.18-22.
130. Végner L, Peragovics A, Tombor L, Jelinek B, Czobor P, Bender A, et al. Experimental confirmation of new drug–target interactions predicted by drug profile matching. J Med Chem. 2013; 56: 8377-8388.
131. Harmsworth K, Lewith GT. Attitudes to traditional Chinese medicine amongst Western trained doctors in the People's Republic of China. Soc Sci Med. 2001; 52: 149-153.
132. American Institute for Cancer Research. The cancer research [Internet]. Arlington: American Institute for Cancer Research; 2021. Available from: https://www.aicr.org/cancer-prevention/food-facts/soy/.

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