Botanical Modulation of Menopausal Symptoms: Mechanisms of Action?

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Abstract

Menopausal women suffer from a variety of symptoms, including hot flashes and night sweats, which can affect quality of life. Although it has been the treatment of choice for relieving these symptoms, hormone therapy has been associated with increased breast cancer risk leading many women to search for natural, efficacious, and safe alternatives such as botanical supplements. Data from clinical trials suggesting that botanicals have efficacy for menopausal symptom relief have been controversial, and several mechanisms of action have been proposed including estrogenic, progestogenic, and serotonergic pathways. Plant extracts with potential estrogenic activities include soy, red clover, kudzu, hops, licorice, rhubarb, yam, and chasteberry. Botanicals with reported progestogenic activities are red clover, hops, yam, and chasteberry. Serotonergic mechanisms have also been proposed since women taking antidepressants often report a reduction in hot flashes and night sweats. Black cohosh, kudzu, kava, licorice, and dong quai all either have reported 5-hydroxytryptamine receptor 7 ligands or inhibit serotonin reuptake, therefore have potential serotonergic activities. Understanding the mechanisms of action of these natural remedies used for women's health could lead to more efficacious formulations and to the isolation of active components which have the potential of becoming effective medications in the future.

Abbreviations

AP-1:	activator protein 1
E_2:	17β-estradiol
ER:	estrogen receptor
ERE:	estrogen responsive element
GPER:	G-protein coupled estrogen receptor
GPR30:	G-protein coupled estrogen receptor subtype 30
HT:	hormone therapy
5-HT:	5-hydroxytryptamine (serotonin)
5-HT_7:	5-hydroxytryptamine receptor 7
MAPKs:	mitogen-activated protein kinases
PI3K:	phosphatidylinositol 3-kinase
8-PN	8-prenylnaringenin
P_4:	progesterone
PR:	progesterone receptor
PRE:	progesterone responsive element
SERT:	serotonin transporter
SSRI:	selective serotonin reuptake inhibitor
WHI:	Women's Health Initiative

Introduction

Women potentially spend the last third of their lives in postmenopause, due to their increased life expectancy. Menopausal women suffer from a variety of symptoms, such as hot flashes, night sweats, mood swings, insomnia, vaginal dryness, in addition to long-term complications such as osteoporosis [1,2]. These symptoms arise primarily as a response to the drastic decline in circulating endogenous estrogens [3]. In order to relieve menopausal symptoms, traditional HT (estrogen or estrogen plus progestin), was designed to supplement the hormones. However, the WHI demonstrated an increased risk of developing breast cancer associated with HT [4] leading women to search for natural alternatives such as botanical supplements [5,6].

Botanicals, which are generally perceived as safe due to their natural origin, have a long history of use for female complaints, particularly in traditional Chinese medicine [7]. The fact that Asian

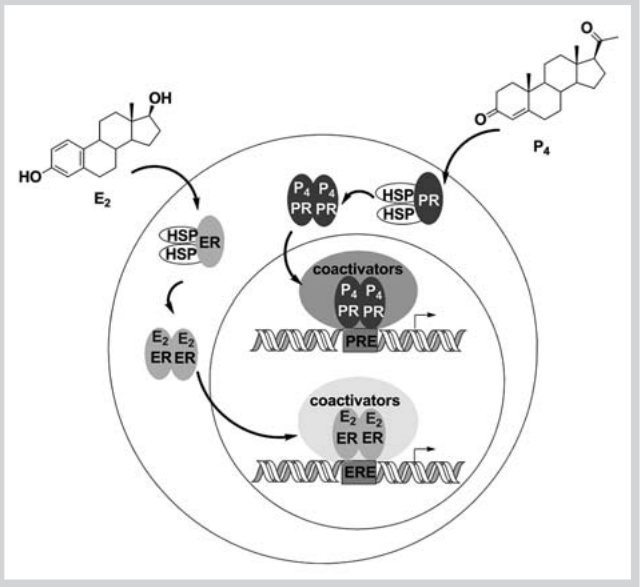

Fig. 1 Classical mechanisms of the estrogenic and progestogenic activities.

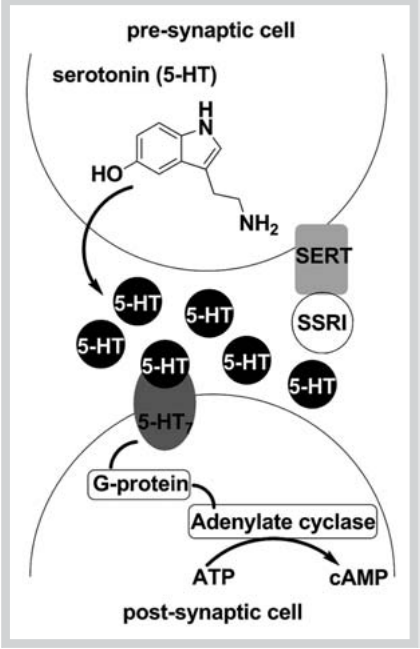

Fig. 2 Mechanism of serotonergic activity. Serotonin is released in the synapses and binds to its receptor (5-HT_7) in the post-synaptic cells. A serotonin receptor, coupled with a G-protein, activates adenylate cyclase, resulting in production of cAMP and activation of enzymatic cascades leading to serotonergic effects.

women have less frequent and severe hot flashes suggests that this effect could be associated with their flavonoid-rich diet and that botanicals with high flavonoid content could be effective in managing menopausal symptoms [8]. As a result, many women turn to botanical dietary supplements for alleviation of menopausal symptoms, specifically for the reduction of hot flashes [5, 6].

Botanical supplements could act through a number of different mechanisms including estrogenic, progestogenic, and/or serotonergic pathways (**Figs. 1** and **2**) [9–13]. It is thought that botanicals with estrogenic activity might mimic the actions of estrogens. The importance of estrogen in homeostatic regulation of many cellular and biochemical events is well illustrated by the pathophysiological changes that occur with estrogen deficiency [14–17]. Endogenous estrogen (estradiol, E_2) is actively involved in the development of the mammary gland and uterus, in maintaining pregnancy and bone density, in protection from cardiovascular diseases, and in relieving menopausal symptoms [16–19]. Estrogens mainly exert their biological effects through binding to ERs including ER*α* and ER*β*, followed by dimerization of ERs and interaction with EREs at the promoter of the estrogen responsive genes, thus activating transcription and generating estrogenic responses which are crucial for normal physiological functions (**Fig. 1**) [20–22]. In humans around one-third of the genes that are regulated by ERs do not contain ERE-like sequences [22, 23]. ERs can also tether to other transcription factors such as Fos and Jun that are directly bound to DNA through their respective responsive elements such as AP1 binding sites to regulate transcription of the related genes [22, 23]. Estrogen also activates rapid signaling pathways such as MAPKs and PI3K pathways, which, in turn, can modulate transcription and proliferation [22, 24]. Studies have revealed another type of ER, namely GPER or GPR30, that is involved in different signaling pathways [25, 26]. It is also known that mechanisms of E_2 actions depend on the ligand, the cell type, and the receptor subtype [22, 23]. It is believed that ER*α* induction is responsible for the proliferative effects of estrogens, while ER*β* activation balances the ER*α*-dependent responses [27–29].

Botanicals and specifically their phytoestrogens, such as genistein and daidzein, preferentially bind and activate ER*β*, thus may exert a safe estrogenic activity [15, 30]. However, the use of botanicals with only plant-derived estrogens in the absence of progestins might increase the risk of developing endometrial hyperplasia and cancer similar to conventional estrogen-alone HT [31, 32]. It is known that women with an intact uterus who take HT to treat estrogen-deficient menopausal symptoms must take a combination of estrogens and progestins, and the same is likely true for phytoestrogens and phytoprogestins. P_4, the precursor of many steroid hormones, plays a crucial role in the normal physiology of the uterus, ovaries, mammary gland, cardiovascular system, bone, brain, and central nervous system [33]. Its biological function is mainly mediated through its binding to PRs, including PRA and PRB, followed by the receptor dimerization, translocation to the nucleus, and interaction with PREs, thus regulating transcription of downstream genes (**Fig. 1**) [34]. Animal models partially suggest that PRA induction is protective in the uterus, while PRB induction might increase breast proliferation [35–37]. Botanicals containing natural progestins, which can activate progesterone-dependent pathways, in addition to estrogenic compounds are preferred.

Estrogen withdrawal during menopause results in the decline in the release of neurotransmitters, primarily norepinephrine and serotonin (5-HT), which will lead to a change in thermoregulation in the hypothalamus [38]. This effect ultimately results in frequent sweating and increased peripheral circulation as heat-loss mechanisms generating hot flashes and night sweats. Increase in the amount of serotonin and activation of certain 5-HT receptors as well as inhibition of serotonin reuptake in synapses through the blocking of SERTs are possible approaches in preventing hot flashes (**Fig. 2**). In order to avoid hormonal approaches, some women choose SSRIs to manage menopausal discomforts, particularly vasomotor symptoms [39]. However, there are also a number of undesirable outcomes such as sexual dys-

Table 1 Estrogenic potency of phytoestrogens in competitive ER binding assay[a].

Compound[b]	Plant	References	IC_{50} (μM) reported range ER binding	
			ERα	ERβ
17β-estradiol (E_2)	–	[89, 101, 185]	0.00 001–0.003	0.0014–0.0038
Genistein	soy, red clover, kudzu	[50, 101, 185]	0.59–1.145	0.025–0.09
Daidzein	soy, red clover, kudzu	[9, 185, 186]	0.96–17	0.1–1.20
S-equol	soy, red clover, kudzu	[50, 138, 185]	0.208–1.02	0.016–0.11
Kaempferol	red clover	[80, 186]	8.2	0.05–50
Puerarin	kudzu	[187]	0.87	ND[c]
Miroestrol	kudzu	[89]	0.0003	ND[c]
8-prenylnaringenin	hops	[9, 101, 188]	0.057–0.51	0.068–1.7
Liquiritigenin	licorice	[189]	2.80	0.41
Glabridin	licorice	[113]	5.00	ND[c]
Glabrene	licorice	[190]	1.00	ND[c]
Lindleyin	rhubarb	[124]	225–435	ND[c]
Trans-rhapontigenin	rhubarb	[129]	12	5.6
Desoxyrhapontigenin	rhubarb	[129]	26	28
Apigenin	chasteberry	[138, 139, 186]	7.88	0.08–1.00
Penduletin	chasteberry	[139]	ND[c]	0.31

[a] The values are from different studies and are included for qualitative comparison. Different methods were employed: radiometric binding assay using purified human ER [9, 50, 101, 185], fluorescence polarization assay using purified human ER [124, 129, 138, 189], radiometric binding assay in cells or tissues [113, 187, 188, 190], inhibition ELISA using purified human ER [186], and dextran-coated charcoal method in cells [89]. [b] Some compounds might not be plant-specific. [c] ND: not determined

function, nausea, weight gain, and sleep disturbances associated with these remedies [40, 41]. Therefore, some botanicals have been investigated for their potential serotonergic effects including activation of serotonin receptors, mainly 5-HT_7, or inhibition of serotonin reuptake through SERTs. What follows is a review of the potential mechanisms (estrogenic, progestogenic, serotonergic) of common botanicals for managing menopausal symptoms.

Botanicals with Estrogenic Activity

Due to the importance of estrogens in the alleviation of menopausal symptoms, particularly for the reduction of hot flashes, several popular botanicals have been studied for estrogenic activity including soy, red clover, kudzu, hops, licorice, rhubarb, yam, chasteberry, dong quai, and black cohosh (**Table 1**). *In vitro* and *in vivo* experiments are summarized below describing elucidation of potential estrogenic activity of the extracts and isolation and characterization of their active principles.

Soy (*Glycine max*, Fabaceae) is often consumed as an alternative to HT by menopausal women [42]. Genistein and daidzein (**Table 1**, **Fig. 3B**) are the most abundant estrogenic compounds in soy [43]. They are mainly glycosylated in the extract and are activated upon hydrolysis *in vivo,* contributing to their estrogenic activity [44–46]. It has been shown that these flavonoids preferentially bind and activate ERβ in cell-based assays and that daidzein had weaker estrogenic activity compared to genistein (**Table 1**) [9, 47–49]. It has also been reported that daidzein metabolism by the gut microflora results in the formation of S-equol (**Table 1**, **Fig. 3B**) which had a stronger estrogenic activity than daidzein in ER binding and transcriptional activation assays in HEC-1 cells [50]. S-equol activity was more significant with ERβ, (Ki [ERβ] = 16 nM; β/α = 13 fold), being comparable to that of genistein (Ki [ERβ] = 6.7 nM; β/α = 16) [50, 51]. It has also been reported that gut microflora variability and differences in the metabolism of soy flavonoids could lead to individual variation in the amount of S-equol formed resulting in distinct biological outcomes [51–54].

Genistein, daidzein, and S-equol can also activate ERα-dependent responses such as MCF-7 (ERα+) cell proliferation [55–61]. It has been demonstrated that activation of ERα-dependent responses by genistein is associated with high concentrations of this isoflavone [48, 62, 63], while ERβ-dependent responses are mainly observed at low concentrations [9, 62]. Wober et al. [64] observed a dose-dependent induction of alkaline phosphatase with genistein and daidzein in the endometrial adenocarcinoma cell line, Ishikawa (ERα+), an effect which was inhibited by the antiestrogen ICI 182,780, demonstrating an ER-dependent estrogenic activity. Similarly, Kayisli et al. [65] reported a weak but dose-dependent estrogenic activity with soy isoflavones in Ishikawa cells when they studied cell proliferation and alkaline phosphatase activity. In the presence of E_2, these compounds had antiestrogenic effects [65].

Consistent with the observed *in vitro* effects, subcutaneous injection of genistein (250 mg/kg/day) in ovariectomized Sprague-Dawley rats for two weeks significantly increased uterine weight, uterine-to-body weight ratio, femur weight, and femur-to-body weight ratio, all of which are likely ERα-dependent effects [10]. Cimafranca et al. [66] also showed that 2 μL/g body weight of genistein (25 mg/mL) induced an increase in uterine weight, downregulated the progesterone receptor in uterine epithelium, increased multioocyte follicles, and decreased thymus weight relative to body weight in neonatal mice after 5 days. Some of the effects including increased multioocyte follicles and abnormal estrous cycle were also seen after 6 months. Legette et al. [67] observed an increased uterine weight and enhanced uterine proliferative index in ovariectomized Sprague-Dawley rats receiving 200 mg/kg dietary equol, demonstrating ERα-dependent effects *in vivo*.

Similarly as in the isoflavone studies, soy extract bound to ERβ at 100 μg/mL and activated ERβ-dependent transcription in HEK-293 cells at 0.1–100 μg/mL, while it did not have any proliferative effects in MCF-7 (ERα+) cells [68]. However, Vieira et al. [69] showed that different commercially available soy supplements increased uterine weight in immature female rats when applied at increasing serial doses (125–4150 mg/kg/day) for 3 days. The

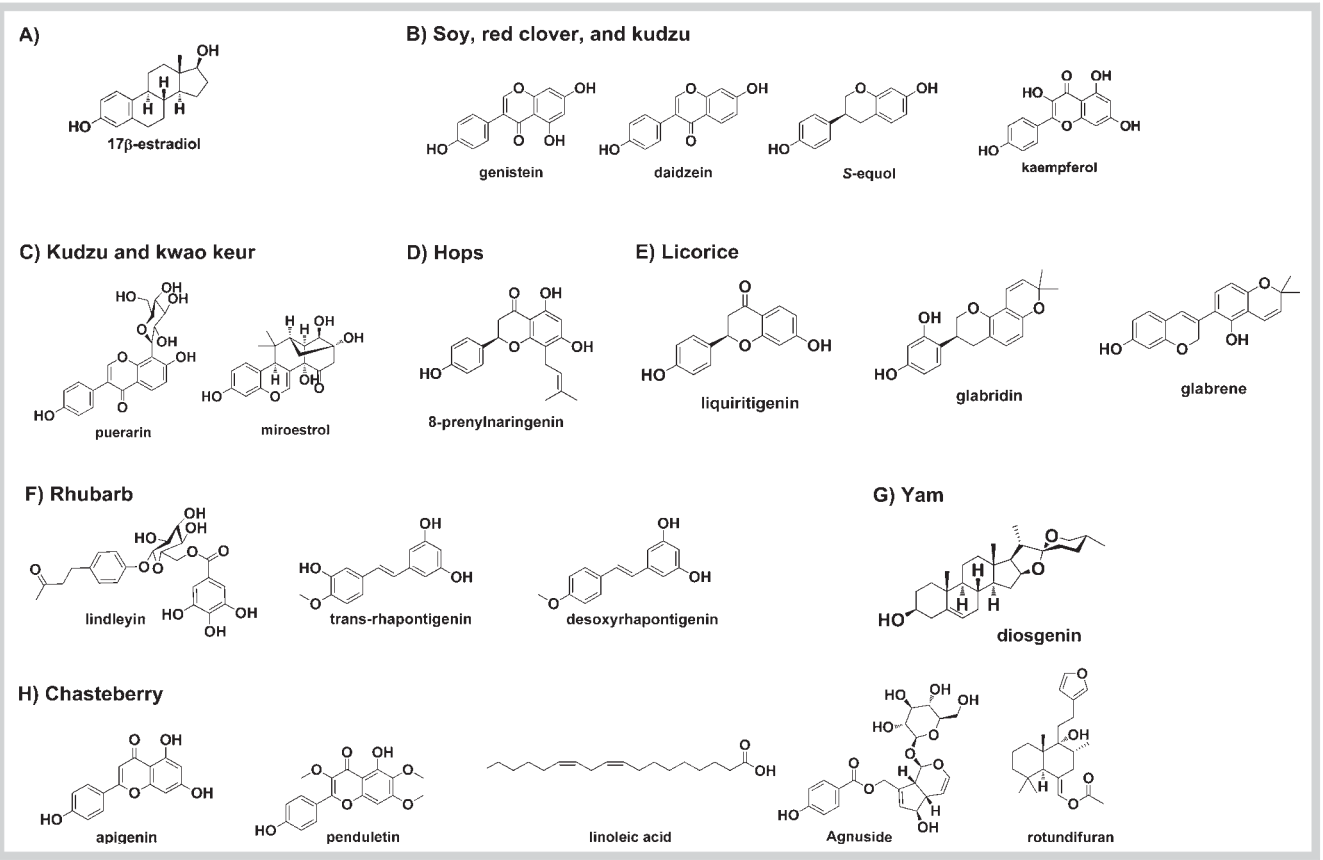

Fig. 3 Chemical structures of phytoestrogens found in the estrogenic botanicals.

observed proliferative effects could be due to the administration of high concentrations of the extract, which can activate ER*α*-dependent pathways, similar to what was observed in experiments with high concentrations of isoflavones. In their studies, the uterotrophic effects were different when extracts from different vendors were used, demonstrating the lack of a unified standardization system in soy extract manufacturing. Allred et al. [70] showed that genistein alone, mixed soy isoflavones, Novasoy, molasses, and soy flour combined with mixed isoflavones have different effects on estrogen-dependent MCF-7 (ER*α*+) tumor growth in athymic mice, demonstrating the effect of the food matrix on the modulation of estrogenic effects of soy and its contents. A thorough review by Hilakivi-Clarke et al. [71] concluded that the estrogenic effects of soy and its isoflavones in animal models are influenced by the dose, the route of administration, the matrix, and the age in which animals receive either whole soy or the isoflavones. In summary, soy contains genistein and daidzein which preferentially bind to and activate ER*β*, but at higher concentrations and depending on the tissue, they can activate ER*α*-dependent responses as well [71–73].

Red clover (*Trifolium pratense*, Fabaceae) is often used for the relief of menopausal symptoms [42], and it contains the same phytoestrogens including genistein and daidzein (**Table 1**, **Fig. 3B**) as discussed above for soy. However, unlike soy, the majority of the isoflavones in red clover are present as the methoxy ethers, formononetin and biochanin A, which require P450-catalyzed metabolism to generate the active phytoestrogens daidzein and genistein, respectively (**Fig. 4A**) [43, 74]. In a chemical and biological evaluation, Booth et al. [75] reported that a standardized red clover extract (0.23% daidzein, 0.41% genistein, 0.07% kaempferol, 14.26% formononetin, 14.47% biochanin A), preferentially bound to ER*β* and induced alkaline phosphatase in Ishikawa cells (EC_{50} = 2.0–2.2 µg/mL). In this study, daidzein and genistein were the most active constituents in the alkaline phosphatase induction assay in Ishikawa cells and in the competitive ER binding assay, with a preferential activity with ER*β*, while formononetin, biochanin A, and kaempferol did not have estrogenic effects. Considering the abundance of formononetin and biochanin A in the extract, relative to daidzein and genistein, P450-catalyzed metabolism obviously plays an essential role in generating the estrogenic activity of the extract (**Fig. 4A**) [75]. Hsu et al. [76] reported moderate estrogenic effects by biochanin A in MCF-7 cells. Markiewicz et al. [77] showed that compared to E_2, genistein, and daidzein, induction of alkaline phosphatase by formononetin and biochanin A in Ishikawa cells is weak. Similarly, in a study by Fokialakis et al. [78], biochanin A weakly induced luciferase reporter activity in MCF-7:D5 L and alkaline phosphatase activity in Ishikawa cells as well as proliferation of MCF-7 cells. Halabalaki et al. [79] showed that formononetin moderately bound to ER subtypes. Compared to genistein and daidzein, formononetin weakly activated alkaline phosphatase in Ishikawa cells and luciferase reporter induction in MCF-7:D5 L cells [79]. This might further emphasize the role of metabolism in exerting estrogenic responses by red clover compounds. While Booth et al. [75] did not observe any estrogenic activity for kaempferol (**Fig. 3B**), Zoechling et al. [80] reported a selective binding of kaempferol to ER*β* (**Table 1**).

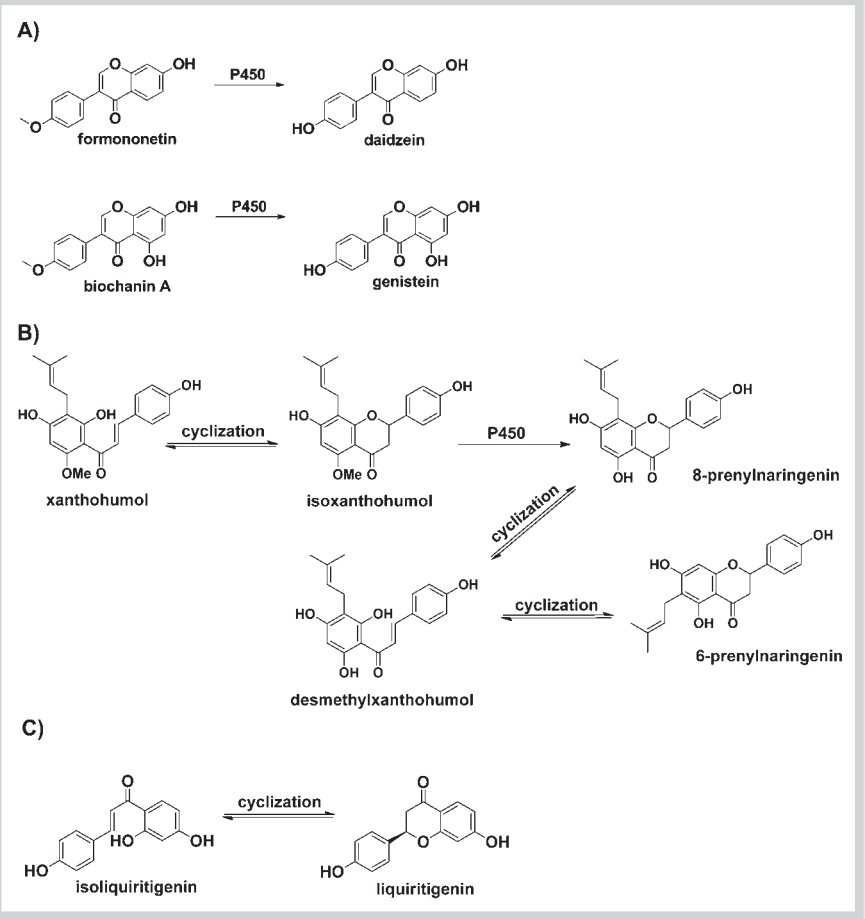

Fig. 4 **A** P450-catalyzed formation of daidzein and genistein from formononetin and biochanin A, respectively. **B** Metabolic formation of 8-PN from its precursors in hops. **C** Metabolic formation of liquiritigenin from isoliquiritigenin in licorice.

In another study, a weak estrogenic activity was reported for a red clover extract through binding and activation of ER*β* in transfected HEK-293 cells but proliferative effects in MCF-7 (ERα+) cells, at concentrations > 30 µg/mL [68]. Interestingly, Overk et al. [9] also showed that red clover extract had binding affinity for both ER subtypes but a greater affinity for ER*β*. They also demonstrated that the extract induced ERE-luciferase and *PgR* mRNA transcription in ERα+ cell lines MCF-7 and Ishikawa as well as activating estrogen responsive alkaline phosphatase in Ishikawa cells (EC_{50}: 2 µg/mL). Red clover phytoestrogens, as well as the extract, have a greater affinity for ER*β*, but they can bind to and activate ERα at high concentrations, especially in cell lines such as MCF-7 and Ishikawa as well as estrogen-sensitive tissues, which have high levels of ERα. Moreover, formation of the ER*β* selective estrogenic constituents, genistein and daidzein, depends on P450-catalyzed metabolism which could influence the results of the cell-based assays, since some cultured cells such as Ishikawa do not metabolize formononetin and biochanin A to the active isoflavones [9].

Estrogenic activity of red clover has also been studied in animal models. In an *in vivo* study with Sprague-Dawley rats, Burdette et al. [81] showed that a red clover extract, standardized to isoflavones, increased the uterine weight and vaginal cell cornification, demonstrating an estrogenic response in these tissues while they did not observe any mammary gland ductal branching as a sign of estrogenic activity in the breast. This observation showed that red clover can activate ERα-dependent responses *in vivo,* but its effects might be tissue specific. On the other hand, in an *in vivo* study by Overk et al., in which Sprague-Dawley rats were treated with lower doses of red clover extract, no uterotrophic effects, vaginal cell cornification, or increase in the height of uterus luminal epithelial cells were observed [82]. In summary, red clover mainly contains formononetin and biochanin A, which are converted by P450 metabolism to genistein and daidzein and exert estrogenic effects, preferentially through ER*β*. Similar to soy flavonoids, the estrogenic activity of red clover and its flavonoids likely depend on the administered concentration, metabolism of isoflavones, and the target tissue [71–73].

Kudzu (*Pueraria lobata*; Fabaceae) is one of the commonly used botanical supplements in the United States [42]. Isoflavonoids such as formononetin, biochanin A, genistein, daidzein, and puerarin (**Table 1**, **Fig. 3B** and **C**) are among the compounds that were isolated from this plant [83,84]. As discussed above, genistein and daidzein have estrogenic activity preferentially through ER*β*, but activate ERα pathways as well [10,82]. Puerarin is metabolized to daidzein by intestinal bacteria [85,86].

Pueraria lobata extract has been shown to preferentially bind to and activate ER*β* in HEK-293 cells transfected with ER subtypes but surprisingly had a proliferative effect in MCF-7 (ERα+) cells [68]. In this study, it was not mentioned if the kudzu extract also had any effect on the reporter gene activity in ERα transfected HEK-293 cells, and the relative ER subtype selectivity was not clearly stated. These controversial results might also be associated with using two different cell lines and the tissue specific behavior of the extract. As discussed above, the estrogenic constituents of kudzu, such as genistein and daidzein, can activate ERα-dependent estrogenic responses such as proliferation at higher concentrations or in specific hormone responsive tissues. A vari-

ety of studies using the yeast-based estrogenic assays reported various potencies for kudzu extracts [35,36,45]. The observed distinct effects could be related to the different extract preparations and standardization between studies.

Pueraria mirifica, another species of *Pueraria* (commonly called Kwao Keur) which is very popular in Southeast Asia, was shown to significantly promote proliferation of MCF-7 (ERα+) cells at 1 µg/mL, and it was more estrogenic than *Pueraria lobata* extract [87,88]. The active compound was puerarin with a proliferative effect at 1 µM [87]. It was also reported that miroestrol (**Fig. 3C**), another compound in *Pueraria mirifica*, induced ERE-CAT reporter gene activity as well as cell growth in MCF-7 (ERα+) cells, indicating estrogenic activity, mainly through ERα [89]. However, using a yeast-based estrogenic assay with ER subtypes, Boonchird et al. [90] observed an 8.5-fold higher relative potency for the plant extract with ERβ in comparison to ERα. Nevertheless, it was reported that different concentrations of various extracts of *Pueraria mirifica* induced vaginal epithelium cornification, increased uterine weight and thickness, and attenuated body weight in ovariectomized rats [91–95], which are considered ERα effects. Higher concentrations of the extract as well as isoflavone content and the tissue specificity might induce both ERα- and ERβ-dependent responses [30,72,73]. A thorough recent review by Malaivijitnond [96] summarized many biological studies and defined the effects of different cultivars of *Pueraria* plants from various regions and seasons as a potential source of existing discrepancies between different study outcomes. In summary, *Pueraria* species contain formononetin, biochanin A, genistein, daidzein, puerarin, and miroestrol. Miroestrol is a potent ERα ligand while genistein and daidzein preferentially bind to and activate ERβ. However, depending on the concentration and target tissue, ERα-dependent responses could be observed.

Hops (*Humulus lupulus*; Cannabaceae) is a popular botanical for its sleep-inducing effects, especially in Europe [97,98]. It is also present in some dietary supplements for managing menopausal symptoms [42]. Liu et al. [99] reported a moderate estrogenic activity for hops based on competitive ER binding activity, alkaline phosphatase induction, and *PgR* mRNA induction in Ishikawa cells. Overk et al. also reported estrogenic activity for a hops extract in competitive ER binding assays, ERE-luciferase induction in MCF-7 (ERα+) cells, *PgR* mRNA induction in MCF-7 and Ishikawa cells, and induction of alkaline phosphatase enzyme in Ishikawa cells (EC_{50} = 1 µg/mL) [9]. 8-PN has been reported as the estrogenic component of hops, equipotent for both ER subtypes, with an activity greater than that of any of the known phytoestrogens (**Table 1**, **Fig. 3D**) [9,100,101]. Bovee et al. [102] observed estrogenic activity of 8-PN in a yeast-based ER-dependent reporter assay. In this study, the potencies of 8-PN for ERα and ERβ were 100 times and 3900 times less than that of estradiol, respectively. Milligan et al. [103] reported that 8-PN induced alkaline phosphatase in Ishikawa Var I cells (EC_{50} = 4.41 nM) and was active in a yeast-based estrogenic assay. They showed that administration of 8-PN (15.9 mg/kg/day, equivalent to 100 µg/mL) in the drinking water for 72 h increased vaginal mitosis in ovariectomized Swiss albino mice; however, they did not observe a significant increase in the uterine weight and the uterine mitosis response [103]. In contrast, Overk et al. [82] observed that 8-PN increased the uterine weight and the height of uterus luminal epithelial cells in Sprague-Dawley rats significantly; however, an ethanolic extract of hops standardized to its active constituent, 8-PN, and its metabolic precursors, including isoxanthohumol, xanthohumol, and desmethylxanthohumol (**Fig. 4B**), did not induce uterotrophy, vaginal cell cornification, and changes in the height of uterus luminal epithelial cells. Similarly, an *in vivo* study by Diel et al. [104] in ovariectomized Wistar rats showed that subcutaneous administration of 8-PN (10 mg/kg/day) increased the uterine weight, the height of uterine epithelial, and the height of vaginal epithelial cells. Additionally, ERα and clusterin genes were downregulated and complement C3 was upregulated in the uterus, indicating estrogenic activity of 8-PN in this animal model [104].

Bolca et al. [105] showed that disposition of 8-PN in the women's breast tissue, after hop supplementation for 5 days was associated with the dose and the metabolism of the precursor compounds. It is believed that the formation of the estrogenic compound of hops, 8-PN, is closely related to the metabolism of its precursor, isoxanthohumol (**Fig. 4B**), by intestinal microbiota and therefore, subjects with varied microflora could experience different biological outcomes upon hops administration [105–109]. The difference in uterotrophic effects and vaginal histology between hops and its active compound, 8-PN, could be related to the metabolism factor and/or to the other components of hops as an extract. Hops might contain natural progestins which could counteract the estrogenic effects of 8-PN (discussed in the progestogenic effects section) [11]. Moreover, uterotrophy and vaginal cell histology are not the only measures of estrogenicity. Hops as an estrogenic extract might have more pronounced estrogenic effects in other target tissues, such as bone, cardiovascular, and brain which were not evaluated in these studies. In summary, hops flavonoid, 8-PN, is the most potent phytoestrogen known to date and is equipotent for ER subtypes. Since its formation depends on the metabolism of its precursors in hops, the estrogenic activity of hops extract might vary between different subjects depending on their metabolism characteristics.

Licorice (*Glycyrrhiza* species, Fabaceae) is a widely used plant, mainly as a sweetening agent in tobacco, in food and beverages, and in toothpastes. It consists of more than 30 species from which a few have been studied for several biological effects such as antibacterial, antiulcer, anti-inflammation, estrogenic, and chemopreventive [110]. Licorice is a common botanical in menopausal supplements in the United States, either as a single herb or in combination with other herbs [42]. The estrogenic activities of different licorice species and extracts are not the same. For example, Liu et al. [99] did not observe any estrogenic effects with the methanolic extract of *Glycyrrhiza glabra* (European licorice, the most common licorice species) when tested in the competitive ER binding assay, alkaline phosphatase induction in Ishikawa cells, *Tff1* mRNA induction in S30 cells, and *PgR* mRNA induction in Ishikawa cells. However, Dong et al. [111] showed that the boiling water extract of *Glycyrrhiza glabra* stimulated MCF-7 (ERα+) cell growth at concentrations of 0.1–10 µg/mL and enhanced ProAB/luciferase activity in the same cell line at a range of 1–10 µg/mL, which was comparable to the estradiol effect at 10 nM. In this study, the induction of estrogen responsive genes and the activation of rapid signaling pathways through *Erk1/2* and *Akt* in the proliferation of MCF-7 (ERα+) cells was observed at 10 µg/mL of the extract, demonstrating the role of this extract in activating the nonclassical mechanism of estrogenic activity [111]. Simons et al. [112] also observed estrogenic activity for several fractions of an ethyl acetate extract of *Glycyrrhiza glabra* in the yeast-based estrogenic assays. The activity of some fractions was abolished in the presence of either RU58668, a selective ERα antagonist, or (R,R)-5,11-diethyl-5,6,11,12-tetrahydro-2,8-chrysenediol (*R,R*-THC), a selective ERβ antagonist, demonstrating the ER-mediated estrogenic effects. The difference between

the outcomes of these studies could be associated with using different extracts and concentrations tested as well as the various sources of the plant species. Simons et al. [112] also showed that glabrene-rich fractions of *Glycyrrhiza glabra* extract were more estrogenic with a higher potency for ERα, while glabridin (**Table 1**, **Fig. 3E**) had antiestrogenic properties. However, Tamir et al. [113] showed that glabridin bound to ER in T47D cell extract (IC_{50}: 5 µM) stimulated ER-dependent cell growth at concentrations lower than 10 µM and inhibited cell growth at concentrations higher than 15 µM in an ER-independent manner. They also observed increased activation of creatine kinase, a marker of estrogenic activity, in female rat uterus, epiphyseal cartilage, diaphyseal bone, and cardiovascular tissues as well as an increased uterine weight effect comparable to that of E_2. Similarly, Somjen et al. [114] showed that glabridin better than glabrene activated creatine kinase in cultured female human bone cells as well as in female rat skeletal tissues. They also reported the estrogenic activity of glabrene and glabridin in vascular tissues *in vitro* and *in vivo*, with glabrene having selective estrogen receptor modulating-like effects [115].

The other popular species of licorice, *Glycyrrhiza uralensis* (Chinese licorice) was also reported to be estrogenic in yeast-based estrogen receptor activity assays, but the reported activities from different studies were not the same, indicating the lack of a unified standardized extract [116, 117]. *Glycyrrhiza uralensis* extract was reported to stimulate MCF-7 (ERα+) cell growth at concentrations of 10–100 µg/mL with the maximal growth stimulation comparable to that of estradiol at 1 nM [118]. Cell cycle analysis indicated an increased population of cells in the S phase, and Western blots showed increased PCNA levels in response to proliferative concentrations of the extract, confirming an enhanced cell growth. They also demonstrated reduced levels of ERα protein as a marker of estrogenicity and a dose-dependent induction of *pS2* (*Tff1*) and *GREB1* mRNA [118]. These data showed ERα-dependent estrogenic effects by the *Glycyrrhiza uralensis* extract. In contrast, an undefined licorice extract was reported to have no proliferative effects in MCF-7 (ERα+) cells and no uterotrophic effects in animal models, but possessed ERβ selectivity in ERE-luciferase induction in transfected HeLa cells [119]. The contradictory results could be associated with using different extracts which demonstrates the importance of having well-defined standardized licorice extracts.

Studying various species of licorice cultivated in different regions of the world, Kondo et al. [120] reported that *Glycyrrhiza uralensis* and *Glycyrrhiza glabra* had the highest and the lowest amounts of liquiritigenin (**Table 1**, **Fig. 3E**), an estrogenic principle of licorice, respectively. Liquiritigenin was reported to be a highly selective ERβ agonist in the ER binding assay and ERβ-ERE-luciferase induction assay in U2OS cells [121]. This flavonoid did not enhance proliferation of MCF-7 (ERα+) xenograft or induction of uterine weight in nude mice, confirming its better potency for ERβ and the corresponding pathways [121]. Isoliquiritigenin (**Fig. 4C**), the precursor of liquiritigenin, was reported to have estrogenic effects [122]. However, the observed effects could be associated with the conversion of isoliquiritigenin to liquiritigenin. Therefore, *Glycyrrhiza uralensis* is expected to exhibit stronger ERβ-dependent effects, since it contains the highest amount of liquiritigenin. However, activation of ERα-dependent responses such as increased proliferation markers could also be observed in some tissues and/or at higher concentrations [72, 73]. In summary, the most common licorice species in dietary supplements is *Glycyrrhiza glabra* which contains glabridin and glabrene in addition to liquiritigenin, while *Glycyrrhiza uralenis* contains the highest amount of liquiritigenin, an ERβ selective phytoestrogen. More in depth studies are needed to define the estrogenic effects of licorice *in vitro* and *in vivo*.

Rhubarb (*Rheum* species, Polygonaceae) is also a common herb for menopausal symptoms [42]. A variety of estrogenic activities have been reported for rhubarb extracts. For example, a *Rheum undulatum* (rhizome) extract in a yeast-based assay gave an EC_{50} = 80 µg/mL with a relative potency of 100 times lower than that of estradiol [117]. *Rheum palmatum* (root) extracts were reported to have relative potencies of 2500 and 10 000 times lower than that of estradiol in the yeast-based estrogenic assays [116, 123]. It was also shown that a rhubarb extract (species not defined) induced ERE-luciferase in ERα/ERβ transfected TSA201 cells, dose-dependently and the active constituent was lindleyin (**Table 1**, **Fig. 3F**) with a relative binding potency of 20 000 times lower than that of estradiol for ERα. The extract increased vitelogenin (a marker of estrogenic activity) levels in the serum of Japanese Medaka [124]. These studies showed that different rhubarb species might have a weak to moderate ER-dependent estrogenic potential. An extract of *Rheum rhaponticum* (root), which is very popular in Germany, has also been studied. Wober et al. [125] showed that the extract activated reporter gene induction through ERβ in transfected HEC-1B adenocarcinoma cells. Similarly, Moller et al. [126] reported an ERβ activity of the extract in U2OS cells. A three-day *in vivo* study on ovariectomized rats by Papke et al. [127] showed that the rhubarb extract did not induce uterotrophy or markers of proliferation. Interestingly, administration of the extract in the presence of low doses of estradiol (menopausal conditions) suppressed the uterotrophic effects of estradiol, demonstrating an antiestrogenic effect. An extended *in vivo* study for 90 days with ovariectomized rats also confirmed that the rhubarb extract did not induce uterotrophy or markers of proliferation, while it showed no effect on the bone mineral density [128]. Vollmer et al. [129] also reported that different doses of the rhubarb extract did not enhance the uterine wet weight and the proliferation marker genes in ovariectomized rats. Interestingly, when the extract was combined with E_2, it counteracted the uterotrophic effects of E_2, dose-dependently [129]. They reported that the two compounds, *trans*-rhapontigenin and desoxyrhapontigenin (**Table 1**, **Fig. 3F**), from the extract bound to both ER subtypes with a slight preference for ERβ [129]. Activation of ERβ with rhubarb might be the reason that the extract does not show proliferative effects in uterine tissue, and its antiestrogenic effects could be related to its partial agonistic effects for ERs, which manifest as antagonistic activity when the full agonist, estradiol, is present. In summary, rhubarb is mainly an ERβ activating plant, although its reported active compounds, lindleyin, rhapontigenin, and desoxyrhapontigenin, are not ERβ-selective.

Yam (*Dioscorea* species, Dioscoreaceae) is a common botanical for managing menopausal symptoms [42]. Park et al. [130] showed that yam extract at a high concentration (200 µg/mL) induced *PgR* and *pS2* mRNA in MCF-7 cells after 24 h. These effects were inhibited when the treatment was combined with ICI 182,780 (1 µM), indicating an ER-dependent pathway. Similar to E_2, yam extract reduced the levels of ERα protein and mRNA, measured by Western blot and RT-PCR. This effect was also blocked by ICI 182,780, showing the estrogenic potential of yam extract. On the other hand, the extract was antiproliferative in MCF-7 (ERα+) cells when applied at 20–200 µg/mL for 72 h suggesting that the estrogenic yam extract did not promote estrogen-depen-

dent tumor cell growth [130]. However, the type of the extract and the species of yam was not defined in this study. Our own observations with yam (*Dioscorea villosa*) showed that the methanolic extract was toxic to Ishikawa and MCF-7 (ERα+) cells at concentrations > 5 µg/mL, and therefore the estrogenic activity could not be evaluated (unpublished data). Cheng et al. [131] showed that the ethyl acetate extract of yam (*Dioscorea alata*) at 10 µg/mL weakly induced the transcriptional activation of GAL4-responsive alkaline phosphatase reporter in CHO-K1 cells with either ER subtypes, with a slightly stronger activity with ERα. Similarly, when yam (*Dioscorea alata*) was given to menopausal women at 390 g/day as part of their food for 30 days, a significant increase in serum concentrations of estrone, sex hormone binding globulin (SHBG), and an increase in estradiol was observed, showing that the yam diet enhanced the hormone levels in these subjects [132]. Diosgenin (**Table 1**, **Fig. 3G**) isolated from yam, was used in pharmaceutical industry to synthesize progesterone and cortisone [133] and was shown to have estrogenic activity in an animal model [134]; however, there are few recent reports about its estrogenic activity. The concentration of diosgenin is relatively low in yam species, and it will not biochemically convert to estrogens *in vivo* [131]. Therefore it is not clear, how yam ingestion could lead to increased estrogen levels in menopausal women and which components might be the active principle(s).

Chasteberry (*Vitex agnus-castus*, Lamiaceae) is also a popular botanical added to botanical supplements for women's health [42]. It was shown to have a weak binding affinity for ERs and no alkaline phosphatase induction in Ishikawa cells; however, it induced *PgR* mRNA in this cell line [99]. Activation of *PgR* while other estrogenic markers are negative could be associated with possible progestogenic effects of chasteberry (discussed in progestogenic effects section). Liu et al. [135] reported that the methanolic extract of *Vitex agnus-castus* had a weak binding affinity for ERs (IC_{50} ERα = 46 µg/mL, ERβ = 64 µg/mL) and upregulated ERβ mRNA in T47D:A18 and *PgR* in Ishikawa cells, while inducing alkaline phosphates enzyme in Ishikawa cells. Linoleic acid (**Fig. 3H**) has been found as the "active" estrogenic component of chasteberry based on bioassay-directed fractionation of the crude extract using the ER binding assay. In this study, while linoleic acid induced ERβ in T47D:A18 cells and *PgR* in Ishikawa cells, it did not induce alkaline phosphatase activity in Ishikawa cells. However, linoleic acid is a fatty acid and may contribute to nonspecific binding to ERs and PRs, generating false positive results [135].

Ibrahim et al. [136] showed that an ethanolic extract of *Vitex agnus castus* increased uterine weight in Sprague-Dawley rats in addition to an increase in the plasma levels of progesterone and estrogen and a decrease in LH and prolactin, suggesting an estrogenic effect of chasteberry. It was shown that chasteberry extract had a selective binding to ERβ, and bioassay-guided fractionation of the crude extract led to the isolation of apigenin (**Table 1**, **Fig. 3H**), the most selective ERβ ligand in this plant [137]. Choi et al. [138] also observed ERβ selectivity with apigenin in the competitive binding assay in addition to estrogenic activity in the yeast-based assay and MCF-7 (ERα+) cell growth. Based on these studies, apigenin can preferentially activate ERβ-dependent responses; but can also stimulate ERα-dependent effects at higher concentrations or in certain tissues. Apigenin could also induce progestogenic activity (discussed in progestogenic effects section) [11]. Jarry et al. [139] isolated penduletin (**Table 1**, **Fig. 3H**) from chasteberry, which was also an ERβ selective agonist in the ER binding assay (IC_{50}: 0.31 µM). However, the presence of ERβ ligands in chasteberry in addition to the progestogenic effects of apigenin did not oppose the proliferative responses of chasteberry extract *in vivo* [136], which could be associated with the amounts of these compounds in the plant extract and their insufficient bioavailability.

Hu et al. [140] reported MCF-7 (ERα+) cell proliferation with four different species of *Vitex*. They reported that the essential oil of *Vitex rotundifolia*, which was mainly composed of linoleic acid, strongly stimulated MCF-7 (ERα+) cell proliferation, the effect which was inhibited by ICI 182,780, demonstrating an ERα-dependent activity of the linoleic-rich fractions [141]. Additionally, they found that *Vitex rotundifolia* and its components agnuside (**Fig. 3H**) and rotundifuran (**Fig. 3H**) induced MCF-7 (ERα+) cell proliferation, *EST1* (ERα), *PgR*, and *Tff1* mRNA dose-dependently and the effects were inhibited by ICI 182,780 [142]. Therefore, according to distinct chemical profiles and biological activities of different *Vitex* species, identification of the species is very important, especially for the standardization of the botanical supplements. In summary, *Vitex* species have estrogenic properties, and compounds such as apigenin and penduletin are their ERβ-selective compounds, while rotundifuran and agnuside have been reported to activate ERα-dependent responses.

Dong quai (*Angelica sinensis*, Apiaceae) is another popular botanical for managing menopausal symptoms as well as women's health issues in general [42]. The estrogenic activity of dong quai is still controversial [143]. For example, Amato et al. [119] reported that dong quai had proliferative effects in MCF-7 (ERα+) cells but did not activate ERα/ERβ-dependent luciferase transcription in transfected HeLa cells and did not exert uterotrophic effects in CD-1 mice. However, Circosta et al. [144] observed increased uterine weight, modified vaginal cytology, and reduced luteinizing hormone levels in female Wistar rats treated with an ethanolic extract of dong quai. Similarly, cell based investigations revealed controversial results. Liu et al. [99] reported that dong quai methanolic extract did not bind to ERs, induce alkaline phosphatase activity in Ishikawa cells, or induce estrogen sensitive genes (*PgR* and *Tff1*) mRNA in Ishikawa and S30 cells, respectively. Similarly, Zhang et al. [123] published that an ethanolic extract of dong quai was not estrogenic in a yeast-based assay over the concentration range of 0.1–1000 µg/mL. A recent study showed that wine-processed dong quai extract at 1 mg/mL (very high concentration) had no proliferative effect on MCF-7 (ERα+) cells but it induced ERE-luciferase [145]. On the other hand, Lau et al. [146] observed proliferation of MCF-7 (ERα+) cells with dong quai concentrations > 100 µg/mL, which could be blocked by 4-hydroxytamoxifen, demonstrating a weak ER-dependent estrogenic activity. Interestingly, Rosenberg-Zand et al. [147] showed that an ethanolic extract of dong quai blocked *Tff1* mRNA induction in BT474 breast cancer cells, demonstrating an antiestrogenic effect by this herb. Similarly, Godecke et al. [148] reported that the lipophilic fraction of a methanolic extract (rich in ligustilide) of dong quai at 20 µg/mL significantly inhibited alkaline phosphatase induction in the presence of estradiol in Ishikawa cells, suggesting an antiestrogenic potential. To date, there have been no reports of a purified compound which could be responsible for the observed estrogenic/antiestrogenic properties of dong quai. These studies demonstrate that additional studies with well-defined extracts are needed in order to delineate the estrogenic/antiestrogenic potential of dong quai as well as the active compound. One reason for the contradictory results regarding the estrogenic activities of dong quai could be associated with the instability of its phthalide fractions, in particular

ligustilide [149]. Conclusive data on the relative estrogenic effects of dong quai are currently unavailable.

Black cohosh (*Cimicifuga racemosa*, Ranunculaceae) is the most popular botanical for menopausal symptom relief in the United States [42]. However, reports of its estrogenic activities are controversial [150]. Liu et al. [99] showed that black cohosh did not bind to ERs, did not induce alkaline phosphatase in Ishikawa cells, or induce *PgR* and *Tff1* mRNA in Ishikawa and S30 cells, respectively. Amato et al. [119] confirmed these results when they reported that black cohosh did not induce MCF-7 (ERα+) cell proliferation, ERE-luciferase in tranfected HeLa cells, and uterotrophic effects in animal models. Bodinet et al. and Freudenstein et al. [151,152] showed that standardized isopropyl extract of black cohosh inhibited proliferation of estrogen-dependent MCF-7 (ERα+) cells, dose-dependently. Similarly, Gaube et al. [153] showed that a dichloromethane extract of black cohosh inhibited MCF-7 (ERα+) cell proliferation dose-dependently (IC_{50}: 14.7 µg/mL), and the majority of proliferation control and proapoptotic genes were down-regulated. Lupu et al. [154] also did not observe any estrogenic effect with black cohosh in an array of estrogenic assays in estrogen responsive cells. Similarly, Zierau et al. [155] reported that ethanolic and isopropyl extracts of black cohosh did not enhance MCF-7 (ERα+) cell proliferation and did not show estrogenic effects in the yeast-based assay and ERE-luciferase assay in MCF-7 (ERα+) cells. Interestingly, the extracts inhibited estradiol-induced MCF-7 (ERα+) cell proliferation and mRNA expression, demonstrating possible antiestrogenic effects of the black cohosh extracts. It was also shown that isopropyl extract of black cohosh did not influence the uterine weight and vaginal cytology in Sprague-Dawley rats in the presence or absence of estradiol [156]. Mercado-Feliciano et al. [157] also did not observe any estrogenic or antiestrogenic effects in the uterus of female B6C3F1/N mice treated with different doses of an ethanolic extract of black cohosh for 3 days. Another study also demonstrated no classical estrogenic effects in ovariectomized rats after three months of treatment with an ethanolic extract of black cohosh [158]. Ruhlen et al. [159] observed a reduction of hot flashes in women taking a black cohosh extract containing 2.5% triterpenes for 12 weeks. The effect returned to baseline after a 12-week washout period. The extract did not have any effect on serum estrogenic markers, *pS2* expression levels, and cellular morphology in the women's nipple aspirate fluids, demonstrating no detectable estrogenic effect on the breast tissue [159]. In contrast, Liu et al. [160] observed a significant MCF-7 (ERα+) cell growth and ER upregulation in response to a black cohosh treatment, but they did not mention the type of the extract. Bolle et al. [161] also reported a weak estrogenic activity for black cohosh in the yeast estrogenic assay; however, they did not observe a uterotrophic effect with the extract. It is still not clear whether black cohosh has estrogenic activity or other mechanisms of action (i.e., serotonergic discussed below) even though it is currently the most popular dietary supplement used by menopausal women [162].

Botanicals with Progestogenic Activity

Unlike estrogenic reports on botanicals, there are relatively few studies on potential progestogenic effects. Evidence about the progestogenic potential of botanicals including red clover, hops, yam, and chasteberry are summarized.

Red clover (*Trifolium pratense*, Fabaceae) ethanolic extract was shown to induce PRE-luciferase in T47D cells at 20 µg/mL by 4.7-fold. It also bound to PR (IC_{50} = 34 µg/mL) showing a weak progestogenic effect [11]. In the same study, Toh et al. showed that kaempferol (**Fig. 5B**) from red clover at 10 µM induced PRE-luciferase in T47D cells by 5.5-fold, while it bound to PR with an IC_{50} = 1.5 µM. Kaempferol also antagonized progesterone receptor in PRE-luciferase assay in T47D cells in the presence of progesterone (100 nM), suggesting that kaempferol was a PR partial agonist. However, the concentration of kaempferol is low and its PR activity is weak suggesting that there might be other natural as yet unidentified progestins in this botanical. The presence of these progestins might be the reason that Overk et al. [82] did not see uterotrophic effects in Sprague-Dawley rats treated with red clover.

Hops (*Humulus lupulus*, Cannabaceae) did not induce uterine weight in Sprague-Dawley rats, even though its estrogenic compound, 8-PN, had a significant estrogenic effect [82]. These observations could be explained by natural progestins in hops which might oppose the estrogenic activity of 8-PN. An ethanolic extract of hops at 20 µg/mL was reported to weakly induce PRE-luciferase as a marker of progestogenic activity in T47D cells [11]. A progestogenic compound of hops was not defined in this study. These data were inconclusive since the hops extract interfered with the binding data from the fluorescence polarization assay, and the extract was toxic in the PRE-luciferase assay. In contrast, Milligan et al. [100] did not observe any progestogenic effects with hops and its major flavonoids in the yeast-based progestogenic assay.

Yam (*Dioscorea* species, Dioscoreaceae) contains diosgenin (**Fig. 5D**) which could have hormonal effects [163]. Diosgenin (500 mg) was continuously administered to Sprague-Dawley rats for 47 days, and the effects on kidney structure were compared to the intact control group and ovariectomized group [164]. The body weight and kidney wet weight of ovariectomized animals increased compared to control and diosgenin-treated groups. Morphometrical analysis of glomerular length and kidney area between the three groups suggested that diosgenin protected the kidney from morphological changes associated with ovariectomy. The effects were likely due to progestogenic effects produced by the conversion of diosgenin to progesterone [164].

Chasteberry (*Vitex* species, Lamiaceae) was observed to have a weak PRE-luciferase activity at 20 µg/mL in T47D cells [11]. In the same study, Toh et al. also showed a significant (6.5-fold) induction of PRE-luciferase activity by a component of chasteberry, apigenin (**Fig. 5C**), at 10 µM and a good PR binding affinity (IC_{50} = 1 µM), suggesting a possible progestogenic effect [11]. However, the concentration of apigenin in chasteberry is low which explains the weak progestogenic activity of this botanical. Lu et al. [165] also showed that wild female Phayres's leaf monkeys consuming *Vitex* species had an altered endocrine function resulting in higher fecal progestin levels. This in turn could influence the cycle length and reproductive function in these animals. Another study on wild female Chimpanzees showed that consumption of *Vitex fischeri* for 6 weeks caused an abrupt and significant increase in the urinary progesterone levels without any significant influence on estrogen levels [166]. However, none of these studies explored the physiological basis of these endocrine alterations in the studied subjects. Further studies are needed to confirm the progestogenic effects of *Vitex* species in animal models and humans.

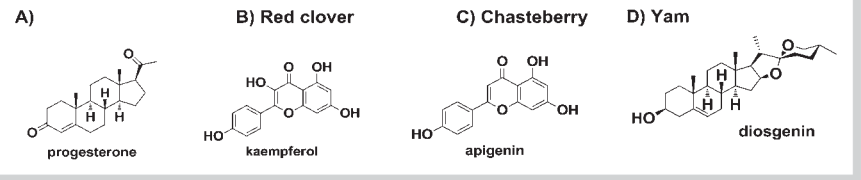

Fig. 5 Chemical structures of phytoprogestins found in botanicals.

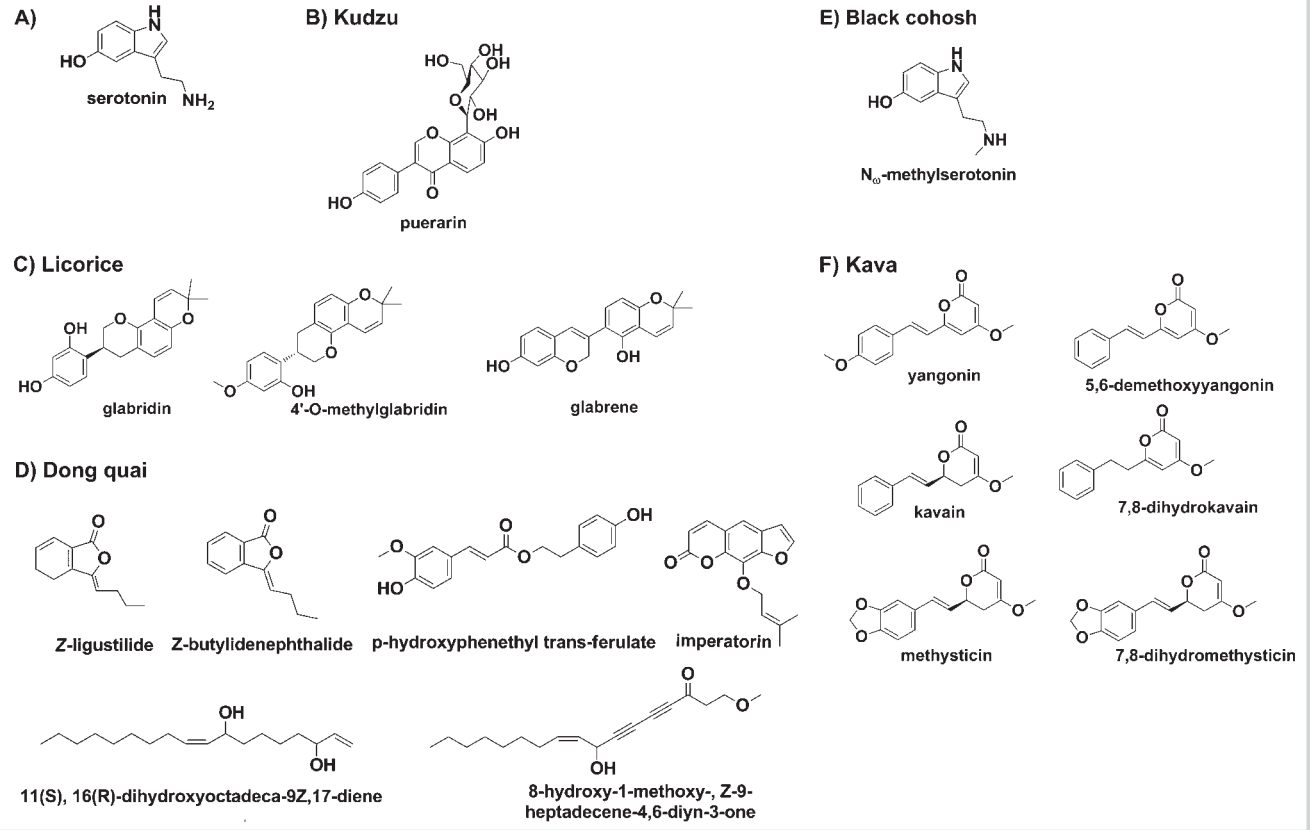

Fig. 6 Chemical structures of serotonergic compounds found in botanicals.

Botanicals with Serotonergic Activity

Some botanicals such as black cohosh are widely used for managing menopausal symptoms, although there is little evidence for their hormonal effects [150,162]. Since there have been reports of relief of hot flash intensity and frequency by SSRIs [167], botanicals have been studied for potentially similar mechanisms.

Black cohosh (*Cimicifuga racemosa*, Ranunculaceae) probably does not have estrogenic activity as discussed above. However, it remains one of the most popular botanical supplements for menopausal symptoms implying alternative efficacy mechanisms. Therefore, black cohosh was studied for its potential serotonergic effects [156]. A black cohosh isopropyl extract was shown to inhibit the binding of [^{3}H] lysergic acid diethylamide to 5-HT$_7$, one 5-HT-subtype that is associated with thermoregulation in the hypothalamus (IC_{50} = 2.4 µg/mL) [156]. In the same study, Burdette et al. showed that a methanolic extract of black cohosh elevated cAMP levels in 5-HT$_7$ transfected HEK 293 cells, suggesting that the extract acted as a partial agonist of the receptor. This effect was reversed in the presence of methiothepin, the antagonist of 5-HT$_7$, suggesting a receptor-mediated process. Powell et al. [12] also showed that the methanolic extract of black cohosh displayed 5-HT$_7$ binding activity and induced cAMP production. Using bioassay-guided fractionation, N$_\omega$-methylserotonin (**Fig. 6E**) was identified as a potent ligand for the 5-HT$_7$ receptor (IC_{50} = 23 pM) which induced cAMP production (EC_{50} = 22 nM) and blocked serotonin reuptake (IC_{50} = 490 nM). Nadaoka et al. [168] observed that black cohosh attenuated the conversion of 5-HT to its metabolite 5-hydroxyindoleacetic acid in the hypothalamus, hippocampus, and cortex of the mice subjected to an acute immobilization stress, demonstrating elevated levels of 5-HT upon black cohosh administration. Although the clinical trials on the efficacy of black cohosh in relieving menopausal symptoms are not conclusive [169,170], the positive effects reported by some women might be attributed to its ability to activate serotonin receptors and block serotonin reuptake, leading to enhanced serotonergic activity. More animal model studies are necessary to provide further evidence for serotonergic activity of black cohosh.

Kudzu (*Pueraria lobata*, Fabaceae) methanolic extract at a high concentration (2 g/kg) as well as puerarin (1–30 mg/kg) (**Fig. 6B**) systemic administration in male Sprague Dawley rats induced hypothermia [13]. The same effect was observed with i.c.v. injection of 50–100 µg of puerarin. A significant correlation between the measured hypothermia and reduced 5-HT efflux in the rat hypothalamus was observed, indicating the role of the serotonergic system in inducing hypothermia by kudzu extract and puerarin as its active compound [13]. The well-known components of kudzu, soy, and red clover, genistein (10 nM – 10 µM), were shown to stimulate [^{3}H] serotonin uptake in transfected COS-7 cells, demonstrating its potential serotonergic effects [171].

Kava (*Piper methysticum*, Piperaceae) was shown to have neurotransmitter-like effects [172]. The extract of kava leaves was reported to have $GABA_A$ receptor binding activity (IC_{50} = 3 µg/mL), dopamine D2, opioid (μ and δ), and histamine (H_1 and H_2) receptor binding activity (IC_{50} = 1–100 µg/mL) as well as a weak binding to serotonin (5-HT_6 and 5-HT_7) and benzodiazepine receptors [172]. The active principles of kava with serotonergic activities were reported to be the kavalactones including, kavain, 7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin, yangonin, and 5,6-demethoxyyangonin (**Fig. 6F**).

Licorice (*Glycyrrhiza glabra*, Fabaceae) also was reported to contain compounds with serotonergic effects. Glabridin, 4′-*O*-methylglabridin, and glabrene (**Fig. 6C**) from *Glycyrrhiza glabra* inhibited the reuptake of radioactive serotonin in HEK-293 cells at 50 µM, with glabridin having a dose-dependent effect [173].

Dong quai (*Angelica sinensis*, Apiaceae) has been shown to have serotonin-like activity in the 5-HT_7 serotonin receptor binding assay, and its most active compound was *p*-hydroxyphenethyl *trans*-ferulate (IC_{50} = 47.6 µM) (**Fig. 6D**) [174]. Deng et al. [174] reported that *p*-hydroxyphenethyl *trans*-ferulate, *Z*-butylidenephtalide, 11(*S*), 16(*R*)-dihydroxyoctadeca-9Z,17-diene, 8-hydroxy-1-methoxy-, *Z*-9-heptadecene-4,6-diyn-3-one, and imperatorin (**Fig. 6D**) isolated from the methanolic extract of dong quai weakly bound to the 5-HT_7 receptor. These data suggest that dong quai might have serotonergic activity.

Summary of Clinical Trial Data of Botanical Supplements Used for Menopausal Symptoms

Clinical evidence supporting the efficacy of most botanicals for alleviating vasomotor symptoms is sparse [169, 175]. A randomized, four-arm, double-blind clinical trial of standardized black cohosh extract, red clover extract, placebo, and equine estrogen plus progestin showed that these remedies did not significantly alter the status of vasomotor symptoms compared to placebo [169]. On the other hand, in a meta-analysis performed on evaluating the efficacy of black cohosh, 6 of 9 eligible clinical trials showed a significant effect in the black cohosh group compared to the placebo group [170]. Black cohosh treatment improved vasomotor symptoms, overall by 26%. However, there was a significant discrepancy between the reviewed trials. Similarly, a recent double-blind, placebo-controlled multicenter clinical study using an isopropanolic extract of black cohosh for managing menopausal symptoms showed some efficacy and no toxicity [176].

A double-blind, randomized, controlled trial of dietary soy consumption by menopausal women showed a significant improvement in reducing somatic symptoms including hot flashes compared to placebo [177]. However, a meta-analysis by Bolanos et al. [178] demonstrated that due to the huge heterogeneity in the studies, having a unified conclusion was not possible, although the overall results had a tendency in favor of soy efficacy. In a single-center, randomized, placebo-controlled, double-blind study, Levis et al. [179] observed that a larger proportion of the women in the soy group experienced hot flashes and constipation compared to the control group, and that there were no significant differences between groups in other outcomes. The North American Menopause Society (October 2010) [180] concluded that while different studies showed mixed efficacy results, soy-based isoflavones seem to be weakly effective in relieving menopausal symptoms, and the effects might be more pronounced in supplements with higher genistein content or increased S-equol. There are many more clinical trials that showed mixed results about the efficacy of botanicals in menopausal women [181–184]. While these equivocal data could be due to the study settings, botanical sources, standardization issues, dosing, and symptom evaluation system, laboratory and preclinical studies have shown some promising effects and clear mechanisms of action for some botanicals supporting their potential for managing vasomotor symptoms.

Conclusions

While HT remains the gold standard for managing vasomotor symptoms, increasing numbers of menopausal women seek alternative nonhormonal remedies including botanicals. The mixed results of different trials might indicate that botanicals can be helpful for some women, especially those with contraindications for HT. Current data are insufficient to suggest botanicals as proven remedies for menopausal symptoms, and additional clinical trials are necessary. Further characterization of well-defined herbal extracts is crucial to better understand their effects and predict the safety issues that might arise due to their increased intake. Such information would be helpful for health-care providers when addressing the menopausal problems of patients, since women continue taking botanicals for managing menopausal symptoms. Finally, understanding the mechanism of action, could also direct researchers to find naturally occurring active components with the potential of becoming effective medications in the future with the desired safety profile for alleviation of menopausal symptoms.

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Conflict of Interest

The authors have no conflict of interest regarding the materials discussed in this manuscript.

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