Phytoestrogens in Botanical Dietary Supplements: Implications for Cancer

Colleen E. Piersen, PhD

Phytoestrogens are plant constituents that possess either estrogenic or antiestrogenic activity. Although their activities are weak as compared with human endogenous estrogens, the consumption of phytoestrogens may have clinically significant consequences. A number of botanicals, or the compounds contained therein, have been identified as putative estrogenic agents, but consensus in the biomedical community has been hampered by conflicting data from various in vitro and in vivo models of estrogenic activity. Phytoestrogens may serve as chemopreventive agents while at the same time being capable of promoting growth in estrogen receptor positive cancer cell lines. Furthermore, they may exert their estrogenic influence through receptor-dependent and/or receptor-independent mechanisms. These findings have led to speculation that phytoestrogen intake might be ill advised for patients at an increased risk for hormone-dependent cancers, cancer patients, or cancer survivors. This article will attempt to sort out discrepancies between various experimental models and establish whether certain herbs possess estrogenic activity. The review will focus on 5 popular botanical dietary supplements: *Trifolium pratense* (red clover), *Cimicifuga racemosa* (black cohosh), *Humulus lupulus* (hops), *Angelica sinensis* (dong quai), and *Glycyrrhiza glabra* (licorice). It will address their mechanisms of action, clinical evidence bases, and implications for use in cancer.

Keywords: *phytoestrogen; isoflavone; breast cancer; menopause; estrogen receptor; antioxidant; chemopreventive; botanical; herb*

Phytoestrogens are nonsteroid plant constituents that possess either estrogenic or antiestrogenic activity. More broadly defined, the phytoestrogen class may include estrogenic compounds that are produced by the microfloral metabolism of inactive plant precursors in the original plant material or human gut. Further complicating the picture, phytoestrogens may be metabolized within the body to inactive compounds or other phytoestrogens with different estrogen receptor (ER) binding profiles. Phytoestrogens are typically weak ligands as compared with the body's main estrogens, 17-β-estradiol (E2), estrone (E1), and estriol (E3). However, high therapeutic indices generally mean that large doses may be administered and high concentrations achieved in the body. Especially in estrogen-deprived individuals, the consumption of phytoestrogens may be physiologically relevant and lead to measurable changes in clinical parameters.

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The phenolic phytoestrogens fall into 3 major classes: coumestans, isoflavones, and lignans. Table 1 summarizes the principal phytochemical members and plant sources of each. The triterpenoids, such as are found in black cohosh and licorice, are sometimes classified as phytoestrogens but meet neither a strict structural nor a rigorous functional definition. Whereas soy, flax, and legumes may be consumed as components of a well-balanced diet, red clover and black cohosh are typically consumed in the form of extracts. These extracts, defined as botanical dietary supplements by the 1994 Dietary Supplements Health Education Act, often concentrate the putative active phytochemicals. Thus, both the potential risks and benefits of dietary supplement intake may be elevated over those of phytoestrogen-rich foods. The 3 strongest phytoestrogens are coumestrol, genistein, and equol, a metabolic derivative of daidzein, in that order.

Phytoestrogens exert their (anti)estrogenic effects through ER-dependent and/or ER-independent mechanisms. Direct receptor interaction occurs through ER-α and ER-β, which are members of the nuclear hormone receptor superfamily.[1] These 2 subclasses have differential tissue distributions, although there is some overlap. ER-α is concentrated in reproductive organs such as the uterus and breast but also found in the brain, liver, and kidney. ER-β is also expressed in the reproductive organs but more widely

CEP is at the UIC/NIH Center for Botanical Dietary Supplements Research in the Program for Collaborative Research in the Pharmaceutical Sciences, University of Illinois at Chicago, College of Pharmacy.

Correspondence: Colleen E. Piersen, PCRPS, 833 South Wood Street, M/C 877, Chicago, IL 60612. E-mail: cpiersen@uic.edu.

Table 1. Phytoestrogen Classes: Plant Sources, Major Plant Compounds, and Human Actives

Phytoestrogen Class	*Plant Sources*	*Major Plant Compounds*	*Major Corresponding (Metabolic) Active(s) in Human*
Coumestan	clovers	coumestrol	coumestrol
	soy sprouts	4'-methoxycoumestrol	4'-methoxycoumestrol
	alfalfa sprouts		
Isoflavone	soy	genistein/genistin	genistein
	red clover	daidzein/daidzin	daidzein/equol
	lentils	biochanin A/sissotrin	genistein
	legumes	formononetin/ononin	daidzein
		glycitein/glycetin	glycitein
		prunetin	genistein
Lignan	flaxseed	secoisolariciresinol	enterodiol/enterolactone
	seed oils	matairesinol	enterolactone
	grains	pinoresinol	enterodiol/enterolactone
	berries	lariciresinol	enterodiol/enterolactone
	green tea	syringaresinol	enterodiol/enterolactone

distributed in nonreproductive tissues such as bone, brain, vasculature, and the urinary and gastrointestinal tracts. Phytoestrogens tend to bind preferentially to ER-β, hence their frequent description as natural selective estrogen receptor modulators (SERMs). This class of agents is thought to be beneficial to postmenopausal women in whom agonist action is desirable in the bone, brain, and cardiovascular system for the maintenance of function. Agonist action in the breast or endometrium is considered undesirable because of the risks of cancer induction or promotion. A phytoestrogen's mode of action will depend upon a woman's estrogen status and the relative concentration(s) of active(s) in the bloodstream. To illustrate, a weak phytoestrogen might act as an agonist in a menopausal woman but a partial agonist or antagonist (by direct competition with endogenous estrogens) in a premenopausal woman. Receptor-independent mechanisms of (anti)estrogenic action include aromatase inhibition, stimulation of sex hormone binding globulin (SHBG) production in the liver, alterations in estrogen metabolism, and inhibition of the enterohepatic recycling of endogenous and xenobiotic estrogens.

The use of complementary and alternative medicine (CAM), including dietary supplement consumption, has grown in popularity in recent years, and this trend is consistent in both healthy and cancer populations.[2-4] It is interesting to note that breast cancer patients often fit the same demographic profile as the typical botanical user: female and middle-aged. Healthy patients may be motivated to consume phytoestrogens in the belief that these compounds may reduce the risk of cancer. This belief is based on a large body of suggestive epidemiological data. For example, the incidences of and mortality rates for breast, prostate, and colorectal cancers are higher in the Western world than in Asian countries, suggesting possible links with diet and lifestyle.[5] Cancer patients may be motivated to increase their phytoestrogen intake in the belief that such dietary changes will increase their quality of life, ameliorate the side effects of cancer treatment, complement conventional cancer therapy, or reduce the incidence of recurrence. For the majority of herbals in use, the validity of these hypotheses remains unproven. These questions are particularly relevant for those at elevated risk for estrogen-dependent cancers, those being treated for cancer, or cancer survivors. Estrogen-influenced cancers include those of the breast, uterus, bone, endometrium, colon, prostate, and thyroid.

The UIC/NIH Center for Botanical Dietary Supplements Research was established in the fall of 1999 to address the issues of standardization, quality, safety, and efficacy of botanical dietary supplements. The center's basic and clinical research programs focus on botanicals with potential benefits for women's health, evaluating those that are reported to alleviate the symptoms of menopause and could serve as natural alternatives to hormone replacement therapy (HRT). Women have experimented with herbal remedies for gynecological complaints for centuries, but few systematic investigations have been carried out in the United States. The center is conducting research on the following botanicals: *Cimicifuga racemosa* (L.) Nutt (Ranunculaceae) (black cohosh), *Trifolium pratense* L. (Fabaceae) (red clover), *Vitex agnus-castus* L. (Verbenaceae) (chaste berry), *Humulus lupulus* L. (Cannabaceae) (hops), *Vaccinium macrocarpon* Ait. (Ericaceae) (cranberry), *Viburnum prunifolium* L. (Caprifoliaceae) (black haw), *Angelica sinensis* (Oliv.), Diels (Apiaceae) (dong quai), *Panax ginseng* C. A. Mey. (Araliaceae) (Asian ginseng), *Ginkgo biloba* L. (Ginkgoaceae) (ginkgo), *Glycyrrhiza glabra* L. (Fabaceae) (licorice),

and *Valeriana officinalis* L. (Valerianaceae) (valerian). A 1-year, randomized, double-blinded, placebo-controlled phase II clinical trial of standardized extracts of red clover and black cohosh for the relief of menopausal hot flashes and other symptoms is in progress. This review will focus on 5 botanicals with putative estrogenic activities that have been suggested as alternative therapies for menopause: *Trifolium pratense*, *Cimicifuga racemosa*, *Humulus lupulus, Angelica sinensis* , and *Glycyrrhiza glabra*. It will explore the evidence for their individual estrogenic activities and rationale bases for clinical use.

Models of Estrogenic Action

To interpret the phytoestrogen literature, it is important to understand the various in vitro, animal, and human models in which estrogenicity is assessed. This section describes the common models of estrogenic action and highlights their limitations. It also emphasizes the difficulties and dangers associated with the extrapolation of in vitro phytoestrogen research to animal models or clinical practice.

In Vitro Assays

As a first step to check for direct estrogenic action at the molecular level, phytoestrogens or plant extracts are typically screened for their ability to bind to ER-α and ER-β in ligand-binding assays. These assays may be carried out on intact cells or on isolated receptor preparations in either the test tube or by a pulsed ultrafiltration-mass spectrometric (PUF-MS) procedure that has been developed by UIC Botanical Center investigators.[6,7] The advantage of this last method is that it can identify (the) novel ligand(s) from natural product extracts, thereby accelerating the process of "reverse pharmacology." Cell or tissue studies may be designed to measure the induction of such estrogen-related genes as androgen receptor (*AR*), estrogen receptor (*ER*), progesterone receptor (*PR*), presenilin (*pS2*), clusterin (*CLU*), complement 2 (*C3*), and glyceraldehyde phosphate dehydrogenase (*GAPDH*). Diel et al surveyed a number of natural and synthetic estrogens for 6 of these markers and showed that each agent produced a characteristic fingerprint of uterine gene expression.[8] It would be simplistic, therefore, to evaluate the estrogenic nature of a compound based on a single reporter assay.

At the cellular level, phytoestrogens are routinely tested for proliferative effects on MCF-7 (ER-α+ > ER-β+, PR–), T-47D (ER-α+, ER-β–, PR+), MDA-MB-231 (ER-α–, ER-β–, PR–), and S30 (MBA-MB-231 cells stably transfected with ER-α; ER-α+, ER-β–, PR–) breast cancer cell lines. In ER+ lines, investigators interpret the stimulation of proliferation in the absence of estrogen as estrogenic and the inhibition of proliferation in the presence of estrogen as either estrogenic (partial agonist action) or antiestrogenic. Many studies have illustrated a biphasic effect for phytoestrogen stimulation of breast cancer cells in culture; low concentrations stimulate cell proliferation, whereas high, perhaps supraphysiological, concentrations inhibit growth. Clearly, the relative concentrations of estrogen and phytoestrogen are important to the outcome of these assays. If a strong phytoestrogen (eg, genistein) and estrogen are both present at physiological concentrations, the phytoestrogen will antagonize the action of estrogen to reduce cellular proliferation. The (anti)estrogenic and cytotoxic properties of phytoestrogens can be assayed in the Ishikawa endometrial adenocarcinoma cell line derived from a glandular epithelial cell line.[9] Estrogenic agents induce alkaline phosphatase (AP) activity, whereas antiestrogens inhibit AP activity. The E-SCREEN[10] in MCF-7 cells, a variant of the E-SCREEN in ER+ human ovarian cell line BG-1,[11] and ER-transformed yeast[12,13] models, may also be used to assess (anti)estrogenic activity. Modes of ligand-independent activation of ER function and ER-independent mechanisms with or without (anti)estrogenic outcomes will be summarized in the section titled "Other Mechanisms That May Reduce Cancer Risks."

Test tube or tissue culture assays have inherent limitations. Unless experiments incorporate in vitro-metabolized extracts, they potentially sidestep issues related to metabolism and cannot begin to address bioavailability limitations. As a result, phytoestrogens typically exhibit much higher activities in vitro than in vivo. The bioavailability of phytoestrogens can be examined in the Caco-2 monolayer model. At the UIC/NIH Center for Botanical Dietary Supplements Research, efforts are under way to understand the permeability and bioavailability of various active and marker compounds in black cohosh, red clover, and hops.[14,15]

In Vivo Studies

Phytoestrogenic effects were first observed in domesticated, grazing animals in the form of infertility.[16,17] Today, the most common animal models of estrogenic action are the rodent uterotrophic and vaginal histology assays and estrogen-sensitive tumor models. In comparing such studies, one must first consider the estrogen status of the test population: Are the animals immature, hypophysectomized, ovariectomized, or healthy or cancerous? The best-designed studies will examine estrogenic parameters in more than one tissue and tease out agonist versus antagonist effects by examining the test compound in combination with

known estrogens and antiestrogens.[18] Diet is another variable to be considered; rodent chow may contain isoflavones or other dietary phytoestrogens with the potential to modulate responses to test agents.[19] Animal models are most convincing when coupled with molecular methods. Diel et al, for instance, showed a degree of correlation between the dose-dependency of estrogen-sensitive gene expression in the uterus and uterine weight increase in DA/Han rats exposed to xeno- or phytoestrogens.[8] Recent studies have found that soy phytoestrogens mimic estrogen in their ability to alter sexually dimorphic brain regions, anxiety, learning, and working memory. Moreover, these investigations demonstrated the feasibility of measuring such phytoestrogen-induced changes in a rat model.[20,21]

Animal studies represent a step up from cell or tissue-based assays, but their applicability to the human situation is not always clear. Route of administration (eg, diet, gavage, subcutaneous) can influence outcomes, and dosing equivalency can be difficult. Interspecies differences in anatomy, physiology (eg, blood-brain barrier), and metabolism also become critical issues. In the phytoestrogen field, there are several situations in which animal findings do not fully carry over to the human experience. For instance, high dietary intake of phytoestrogens can be associated with infertility in sheep, cattle, and other species, but this does not appear to be a concern in humans. Soy studies on mammary carcinogenesis in adult female rats have yielded conflicting outcomes, sometimes disagreeing with the patterns of chemoprevention suggested by human epidemiologic data.[22]

Comprehensive or long-term human trials of phyto(estrogens) usually monitor the following estrogenic endpoints: serum sex hormone and SHBG levels; proliferation of endometrial tissue via transvaginal ultrasound or biopsy; proliferation of the vaginal epithelium via cytological assessment; alterations in breast density via mammogram; and bone density via DEXA or blood chemistry. Clinical markers with direct relevance to breast cancer development include examination of breast tissue for intraepithelial neoplasia by biopsy or nipple aspiration, mammographic breast density, serum levels of insulin-like growth factor 1 (IGF-1), and serum estrogen levels in postmenopausal women not on HRT.[23] Studies that require sampling of breast tissue, especially on healthy women, are uncommon but needed to explore effects of phytoestrogens on breast tissue. In one study of healthy, premenopausal women on isoflavone supplementation, Hargreaves et al observed increased levels of pS2 protein in nipple aspiration fluid.[24]

Human studies are confounded by a number of factors related to interindividual variability: composition of gut flora, intestinal transit time, variability in redox potential of colon, and genetic differences in metabolism. Gut flora composition and differences in metabolism are particularly relevant to this discussion of phytoestrogens. In perhaps the most striking example of the importance of the intestinal bacterial composition, only 30% to 40% of individuals can metabolize daidzein to equol. Several soy studies have analyzed these 2 subpopulations and observed significantly stronger clinical responses in the equol producers.[25] In regard to breast cancer risk, equol production is associated with plasma hormone patterns[26] and estrogen metabolite ratios[27] that are consistent with lower overall breast cancer risk. The theoretical implication would be that an assessment of equol-producer status, perhaps coupled with genetic breast cancer risk, would enable caregivers to provide rational advice regarding phytoestrogen supplementation.

Evidence For or Against Estrogenicity of 5 Popular Dietary Supplements

This review will focus on 5 popular dietary supplements under study in the UIC/NIH Center for Botanical Dietary Supplements Research. All have been described in the literature as estrogenic, but the evidence for black cohosh and angelica is weak or contradictory. Table 2 summarizes the actions of the 5 botanicals in a number of models of estrogenic action. This section outlines the plants' mechanisms of action and clinical evidence bases with an emphasis on implications for use in cancer.

Red Clover

Trifolium pratense (red clover) is native to the regions that border the Mediterranean and Red Seas but is now distributed throughout the globe. It has traditionally been used in Europe and America as an abortifacient, anticancer treatment, antispasmodic, emmenagogue, and wound healer. It has received attention more recently as a possible natural alternative to HRT and for its beneficial effects on the cardiovascular profile. Red clover, like soy, is a rich source of isoflavones. Although there is a large body of evidence supporting the efficacy and safety of the long-term consumption of soy, the data cannot be directly projected to red clover; not only do the phytoestrogen compositions of the 2 plants differ, but soy contains a protein fraction of potential clinical importance. Nonetheless, based on its own history of use, red clover is included on the FDA's GRAS (Generally Recognized as Safe) list. The following discussion will be limited to research on red clover, and to a lesser extent

Table 2. Estrogenic Activities of 5 Common Botanicals

Estrogenic Activities	*Red Clover*	*Black Cohosh*	*Hops*	*Licorice*	*Angelica*
In Vitro Activities					
Competitive estrogen receptor (ER) binding					
ER-α	+	**+/–**	+	+	**+/–**
ER-β	+	–	+	+	+/–
Proliferation of ER+ breast cancer cells					
Stimulation in absence of E	+	**+/–**	+	+/–	**+/–**
Inhibition in presence of E	+[1]	+	+	+/–	+
Induction of E-dependent proteins					
Alkaline phosphatase	+	–	+	–	–
Up-regulation of E-dependent genes					
Presenelin 2 (*pS2*)	+	–	+	+	+
Progesterone receptor (*PR*)	+	–	+	+	+

	Animal	*Human*	*Animal*	*Human*	*Animal*	*Human*	*Animal*	*Human*	*Animal*	*Human*
In Vivo Activities										
Uterotrophic effects	+		**+/–**		**+/–**		+/–		**+/–**	
Endometrial proliferation		–	–	–	+					–
Vaginal cytology	+/–	–	**+/–**	**+/–**	+					–
Mammary gland	–		–		–					
Changes in sex hormone levels		**+/–**	**+/–**	**+/–**				+/–[4]		
Menstrual alterations/disturbances		+[3]				+				
Preservation of bone	+[2]	+	+	+	+					

Bolded symbol indicates predominant result as suggested by literature review.
[1]Biochanin A activity.
[2]Genistein and daidzein activities.
[3]Soy data.
[4]Testosterone in males.

purified isoflavones. In vitro, in vivo, and human studies support the consistent picture of a true estrogenic agent.

Trifolium pratense contains a large number of flavonoids, the isoflavones being the characteristic subclass that is primarily responsible for the plant's estrogenic activity. Genistein and daidzein, the predominant soy isoflavones, are present as well as larger quantities of their methylated precursors, biochanin A and formononetin. These 4 moieties exist in the plant largely in their inactive glucoside conjugate (genistein, genistin; daidzein, daidzin; biochanin A, sissotrin; formononetin, ononin) or glucoside malonate conjugate (biochanin A, biochanin A-7-O-β-D-glucoside-6"-O-malonate; formononetin; formononetin-7-O-β-D-glucoside-6"-O-malonate) forms. The conjugates are readily hydrolyzed by gut bacteria to their bioactive, aglycone counterparts and then absorbed from the intestine. Minor (anti)estrogenic components in red clover include prunetin and kaempferol among others. Commercial preparations of red clover are frequently hydrolyzed to enhance bioavailability.

At the molecular level, research into red clover mechanism of action has focused on the interactions of purified isoflavones with the estrogen receptors. Collins et al first described antiestrogenic, tamoxifen-like activities of biochanin A in an ER-transformed yeast system, emphasizing the differential antagonism of various classes of estrogens.[28] Zava et al observed binding of a 50% hydroethanolic extract of *Trifolium pratense* to estrogen and progesterone receptors in MCF-7 (ER+, PR–) and T-47D (ER+, PR+) breast cancer cell lines, respectively.[29] Center laboratories have shown binding of a crude methanol extract of red clover to human recombinant ER-α and ER-β in both conventional radioligand and PUF-MS binding assays.[6] The extract competed [^{3}H]estradiol binding to ER-α and ER-β with IC_{50} values of 5.6 and 2.5 μg/mL, respectively. Isoflavone standards exhibited competitive binding at both receptor subtypes with the following rank order of potency: genistein > daidzein > biochanin A > formononetin and exhibited a preference for ER-β, consistent with the findings of a previous study.[30] The PUF-MS procedure detected genistein, daidzein, and biochanin A in the crude red clover extract but not the weakest of the isoflavones, formononetin.[6] The tendency of isoflavones to bind preferentially to ER-β over ER-α strengthens the argument that they protect against cancer.

The isoflavones contained in red clover possess a wide range of in vitro bioactivities in addition to their characteristic estrogenic activity, and these activities may manifest themselves at different physiological concentrations. Related to cancer risk, isoflavones,[31] isoflavone metabolites,[32] and crude hydroalcoholic extracts of red clover all possess antioxidant activity. Of possible relevance to estrogenic activity, isoflavones

now appear capable of binding to neurotransmitter and neuromodulator receptors. For instance, genistein and daidzein both inhibit $GABA_A$ receptor through a direct effect, that is, one unrelated to tyrosine kinase inhibition.[33] Genistein and other dietary flavonoids serve as weak adenosine receptor antagonists at both the A_1 and A_3 subtypes.[34,35] A more in-depth discussion of isoflavone-mediated, ER-independent events follows later in this review.

In vitro studies on intact cells further illustrate the (anti)estrogenic actions of red clover and its isoflavone constituents. Center work has demonstrated that a crude methanol extract of *Trifolium pratense* can up-regulate the estrogen-inducible genes *pS2* and *PR* in S30 and Ishikawa cells, respectively, and induce AP activity in Ishikawa cells.[6] Zava et al showed that a 50% hydroethanolic extract of red clover stimulated the proliferation of T-47D cells in steroid-depleted serum but had no effect on the growth of MDA486 cells.[29] Red clover did not stimulate but almost completely blocked progestin-induced alkaline phosphatase activity in T-47D cells, indicative of antiprogestin activity. Red clover also down-regulated ER levels in T-47D cells. This effect was not reversed in the presence of RU486, suggesting an ER- as opposed to a PR-mediated mechanism. Dixon-Shanies and Shaikh showed that genistein, daidzein, and biochanin A inhibited serum-stimulated growth of both T-47D and MCF-7 cells at 10-100 μM.[36] Antiproliferative effects of biochanin A have also been observed on human cancer cell lines established from the gastrointestinal tract.[37]

In vivo animal studies have also implicated the isoflavones as the estrogenic agents in red clover. In a survey of various clover and alfalfa fodders, Saloniemi et al associated isoflavone content with uterotrophic activity in an immature rat model. Biochanin A and formononetin appeared largely responsible for the observed estrogenic activities.[38] In our laboratories, a standardized red clover extract was weakly estrogenic in the ovariectomized rat model. It affected uterotrophic and vaginotrophic outcomes but did not stimulate cell proliferation in the mammary glands.[39] In a rat model of endometrial cancer, genistein increases the expression of estrogen-responsive genes but does not promote tumor growth.[40] Consistent with ER-β activity, isoflavone preparations also improve bone density and protect against cardiovascular disease in the ovariectomized rat and prepubertal rhesus monkey models.[40-45]

The clinical data for red clover reflect mixed estrogenic outcomes. Five trials have addressed the use of red clover for the relief of vasomotor symptoms associated with menopause,[46-50] only 3 of which showed reductions in hot flash frequency.[48-50] Certain of these trials also noted a decrease in night sweats,[49] no changes in sex hormone parameters[46,47,49] with the exception of follicle stimulating hormone (FSH),[50] no changes in SHBG levels,[46,47,49] no changes in the endometrium as assessed by transvaginal ultrasound,[46,49] no changes in vaginal cytology,[46,47] no changes in lipid profiles[49] with the possible exception of high-density lipoprotein (HDL),[47] and no significant side effects. Hale et al examined the effects of a 3-month course of 50 mg of red clover per day on just the endometrium and found no change in the Ki-67 proliferation index.[51] Another stand-alone trial demonstrated benefit of red clover treatment for the relief of pain in cyclical mastalgia,[52] a condition that is improved by the use of antiestrogens such as tamoxifen.[53] Three trials have examined potential cardiovascular health benefits of red clover.[54-56] None of them reported significant changes in plasma lipid levels, although beneficial trends in HDL_3[55] and triglycerides[56] were noted. Nestel et al measured a 23% increase in arterial compliance in menopausal women after 5 weeks of administration of 80 mg isoflavones per day.[57] Two trials have examined the effects of red clover isoflavones on bone metabolism. The first observed significant reductions in loss of spine bone mineral content and density in premenopausal and perimenopausal women after a 1-year course of treatment, which was tentatively attributed to a decrease in the rate of bone resorption.[58] Among postmenopausal women, no improvement was observed in the red clover group over placebo for either parameter. In the second bone trial, an uncontrolled trial, postmenopausal women were randomized to 1 of 3 doses of red clover isoflavones for 6 months. The study showed dose-related increases in the bone mineral density of the proximal radius and ulna, accompanied by increases in HDL cholesterol, reductions in apolipoprotein B, and no changes in endometrial thickness.[59] Although many of the above-mentioned red clover trials contained design flaws, they strongly suggest a constellation of estrogen-related benefits from red clover treatment for female health.

Two recent studies in men have presented conflicting views as to whether red clover isoflavone supplementation should be recommended for prostate health. Jarred et al showed that Trinovin, an isoflavone formulation for men that includes predominantly formononetin and biochanin A, can induce apoptosis in low- to moderate-grade human prostate carcinoma.[60] This supplement did not alter plasma steroid hormone or SHBG levels, with the exception of an increase in dihydrotestosterone, which could be viewed as a detrimental health effect.[61]

Black cohosh

Cimicifuga racemosa (synonym *Actea racemosa* L.) (black cohosh), commonly named black snakeroot, rattleroot, or bugbane, is native to North America and has a history of use by Native American Indians.[62] The rhizomes were traditionally used to treat gender-independent conditions such as colds, kidney ailments, malaise, and rheumatism and female conditions such as uterine disorders, menstrual complaints, and antepartum, intrapartum, and postpartum problems. The Eclectic physicians adopted black cohosh for the palliative care of endometriosis, dysmenorrhea, neuralgia, and rheumatism.[63] The phytomedicine has been used since the early 1940s in Germany for the treatment of menopausal symptoms and is formally approved by The German Commission E for use in premenstrual discomfort, dysmenorrhea, and climacteric neurovegetative complaints.[64] Because of its effectiveness for a variety of neuroendocrine indications, black cohosh has been considered an estrogenic agent. Others have hypothesized a neuroendocrine mode of action at the level of the hypothalamus, the brain structure frequently implicated in hot flash generation. In vitro, in vivo, and human testing of black cohosh extracts has yielded conflicting evidence as to mechanism of action but supports safety of use. Indeed, a recent systematic review surveyed the international literature, black cohosh manufacturers, and adverse event reporting programs and concluded that "although definitive evidence is not available, it would seem that black cohosh is a safe herbal medicine."[65]

The majority of ER binding studies that have been carried out on black cohosh extracts or compounds have reported no activity. In a 2-part report[66,67] and a subsequent follow-up study,[68] Jarry et al (1) demonstrated reductions in serum luteinizing hormone (LH) concentrations at different time points in ovariectomized rat experiments and (2) conducted bioassay-guided fractionation on the bioactive extract to isolate 3 active principles capable of competing with estradiol in a modified ER binding assay. One active was identified as formononetin. Subsequent chemical analyses of different extracts have not confirmed the presence of formononetin in black cohosh.[69,70] In 1998, Zava et al observed no binding in a 50% hydroethanolic extract of *Cimicifuga racemosa* to either estrogen or progesterone receptors in MCF-7 and T47D breast cancer cell lines, respectively.[29] In 2001, Center laboratories showed that a crude methanol extract of black cohosh failed to bind to human recombinant ER-α or ER-β in either conventional radioligand or PUF-MS binding assays.[6] Onorato and Henion employed similar affinity ultrafiltration and liquid chromatography/mass spectrometric procedures to demonstrate that neither 3 black cohosh triterpene glycosides (cimicifugoside, cimiracemoside F, and 27-deoxyacteine) nor their prepared aglycones bind to the ligand-binding domain of ER-β.[71] It would appear, then, that black cohosh likely affects its estrogenic outcomes through an ER-independent mechanism.

In vitro studies at the cellular level provide no consensus as to the estrogenicity of *Cimicifuga racemosa*. Reports of no (anti)estrogenicity follow. First, Zava et al showed that a 50% hydroethanolic extract of black cohosh did not stimulate cell proliferation in T-47D cells in steroid-depleted serum.[29] Amato et al observed no stimulation of MCF-7 cell proliferation in an estrogen-depleted environment or transactivation of ER-α or ER-β in a HeLa cell reporter assay upon treatment with an alcoholic extract.[72] Center laboratories showed that a crude methanol extract of *Cimicifuga racemosa* did not induce mRNA expression of *pS2* or *PR* or induce enzymatic activity of AP in cancer cells.[6] Reports of (anti)estrogenicity follow. Dixon-Shanies and Shaikh showed that a 0.1% ethanolic extract of black cohosh had significant antiproliferative effects on serum-stimulated T-47D cells.[36] Bodinet and Freudenstein demonstrated that an isopropanolic extract of black cohosh inhibited the proliferation of MCF-7 cells under estrogen-deprived conditions and antagonized estrogen-stimulated cell growth. Moreover, black cohosh enhanced the antiproliferative effect of tamoxifen in a dose-dependent manner.[73] Zierau et al observed that ethanolic and isopropanolic extracts of black cohosh antagonized estradiol-induced activities in 3 model systems: proliferation of MCF-7 cells in estrogen-stripped serum, reporter gene expression in the inducible yeast ER assay, and gene expression in estrogen-inducible MVLN cells.[74] Using a serum-depleted MCF-7 protocol, Liu et al observed estrogen-like proliferation at low black cohosh concentrations but antiestrogen-like inhibition at high concentrations.[75,76] The interpretation of cell proliferation data must rely on a thorough understanding of the test system and the relative estrogen/phytoestrogen concentrations present. It should be noted that black cohosh compounds have been observed to interfere with the [3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide] (MTT) assay of cell growth that is frequently employed in proliferation experiments[77] and that serum composition can affect growth outcomes.[36] Fukinolic acid acted in an agonist matter to increase MCF-7 proliferation in an estrogen-free environment.[78] The Liu study also suggested a novel mechanism for black cohosh: up-regulation of ER as observed in MCF-7 cells.[75,76]

Animal studies generally do not support the contention that black cohosh operates through a direct, estrogenic mechanism of action. Published exceptions

include the aforementioned study by Jarry that examined an extract containing formononetin, a compound more characteristic of Leguminosae species.[66] A follow-up study demonstrated that both the estrogenic and nonestrogenic subfractions were able to reduce LH secretion in an ovariectomized rat model.[68] Studies by Foldes, Liu, and Eagon have demonstrated uterotrophic effects and/or prolonged estrus in rodent models.[75,76,79,80] Einer-Jensen et al administered 6, 60, or 600 mg/kg of a 50% ethanolic *Cimicifuga racemosa* extract to immature mice or ovariectomized rats for 3 days. No uterotrophic or vaginotrophic effects were observed.[81] The most thorough animal study to date, a recent murine trial, investigated the safety of a black cohosh isopropanolic extract (Remifemin®) at 1-fold, 10-fold, and 100-fold the human therapeutic dose (0.714 mg/kg, 7.14 mg/kg, or 71.4 mg/kg).[82] The investigators found that black cohosh was safe at all 3 doses with no evidence of estrogenic effects on estrogen-sensitive hormone levels, the endometrium, or the growth of DMBA-induced mammary tumors. Our center has recently completed a study of a 40% isopropanolic black cohosh extract on ovariectomized rats.[83] Black cohosh administration produced no changes in uterine weight or vaginal cell histology and did not antagonize the effects of 17-β-estradiol on these parameters. The last 3 studies would support the safety of use of black cohosh in estrogen-sensitive cancer patients or in women for whom HRT is contraindicated.

The majority of clinical trials and case reports show black cohosh to be effective for the relief of vasomotor symptoms, but an analysis of estrogenic endpoints suggests that black cohosh does not work through a direct, estrogenic mechanism. Most studies to date have been conducted on Remifemin, a commercial isopropanolic formulation standardized to contain 1 mg of triterpenes per 20 mg of extract. Through 2002, at least 19 clinical studies had been performed on black cohosh in menopausal women,[84,85] only 3 of which meet the rigorous standard of being double-blinded, randomized, and placebo-controlled (DB RCTs).[86-88] Six human studies meet the looser definition of an RCT or comparison trial since 1982[68,86-90] and report on the balance (1) no changes in gynecologically relevant hormone parameters (3 negative[87,88,90]: 1 positive[68]); (2) significant reductions in menopausal symptoms including hot flashes, night sweats, nervousness, palpitations, depression, vertigo, and vaginal atrophy as compared to baseline or placebo as demonstrated by validated instruments such as the Kupperman Index (3 positive[86,88,89]: 1 negative[90]: 1 negative by a similar instrument[87]), the Hamilton Anxiety Scale (2 positive[86,89]), or Self-Assessment Depression Scale (2 positive[88,89]); (3) changes in vaginal cytology (2 positive[86,89]: 1 negative[88]); and (4) only mild side effects. Only 1 trial has specifically examined the effectiveness of black cohosh for hot flash relief in women with a history of breast cancer, the majority of whom were on tamoxifen therapy at the time of the study.[87] Jacobson and coauthors found that black cohosh was not significantly more efficacious than placebo against vasomotor symptoms and did not alter FSH or LH levels. Significant improvement was observed only for the symptom of sweating.

At the time that this review was in preparation, a comprehensive series of studies on *Cimicifuga racemosa* BIONORICA extract BNO 1055 was coming to press in the March 2003 supplement to *Mauritas*. The development of this standardized ethanolic extract was chronicled from plant cultivation and collection[91] through in vitro[92] and in vivo testing[93,94] and finally to human trials on efficacy for menopausal symptoms and bone in postmenopausal women[95] and hot flash reduction in female breast cancer survivors (an off-label use).[96] Significantly, a detailed discussion of the product's method of standardization and known chemical actives, if any, was not included. This series of 6 original research articles, accompanied by a review article,[97] presents black cohosh as a SERM alternative to HRT, suggesting safety for breast cancer survivors. Estrogenic endpoints such as increases in markers of bone formation, decreases in markers of bone degradation, maturation of vaginal epithelium, and relief of vasomotor symptoms tested positive in human subjects. The undesirable estrogenic endpoint of endometrial thickening tested negative. Breast outcomes were not addressed, but a dopaminergic D_2 activity was identified. BNO 1055 displaced ^{3}H-estradiol from cytosolic preparations of porcine and human endometrial preparations but failed to compete at recombinant ER-α or, more significantly, ER-β. In an ovariectomized rat model, a 3-month course of treatment reduced loss of bone mineral density, thereby mimicking a classic ER-β-mediated effect, but did not alter uterine weight or uterine expression of estrogen-induced genes, typical ER-α effects.

The characterization of black cohosh as a phyto-SERM, without definitive evidence of specific ER binding at the molecular level, does not truly resolve previous, apparent contradictions in the literature regarding the herb's "estrogenic" mode of action. The authors speculate that (an) as-of-yet-unidentified phyto-SERM(s) in black cohosh may interact with a novel ER subtype. The very fact that black cohosh can mitigate the side effects of tamoxifen in the brain/hypothalamus, but does not share the same receptor-binding profile, could be interpreted as evidence of an ER-independent mode of action. The BNO 1055 body of work is significant, however, in that it allows the

comparison of a standardized extract across a broad spectrum of in vitro, in vivo, and human models.

In recent work at the UIC/NIH Center for Botanical Dietary Supplements, a crude 60% methanolic extract of black cohosh scavenged reactive oxygen species and protected against menadione-induced DNA damage.[98] Ten antioxidant compounds were identified: caffeic acid, methyl caffeate, ferulic acid, isoferulic acid, fukinolic acid, cimicifugic acid A, cimicifugic acid B, cimicifugic acid F, cimiracemate A, and cimiracemate B. Six of these antioxidants reduced menadione-induced DNA damage in cultured S30 breast cancer cells with the following order of potency: methyl caffeate > caffeic acid > ferulic acid > cimiracemate A > cimiracemate B > fukinolic acid. This study offers another mechanism by which black cohosh might reduce cancer risk.

Hops

Humulus lupulus (hops) is native to Asia, Europe, and North America but is now cultivated worldwide. Its traditional therapeutic uses have varied widely between cultures but tend to include a sedative indication. Hops are also used in beer production to impart aroma, flavor, and bitterness. The German Commission E has approved the internal use of hops for mood and sleep disturbances.[64] The estrogenic actions of hops are accounted for by the prenylflavonoids, a distinct class of nonsteroidal phytoestrogens. Hops' mechanism of sedative action has not been fully elucidated.

Extracts of hops consistently test positive in molecular-level assays for estrogen receptor binding. Zava et al observed significant binding of a 50% hydroethanolic extract of *Humulus lupulus* to estrogen but not progesterone receptors in MCF-7 and T-47D breast cancer cell lines, respectively.[29] Eagon et al also observed inhibition by a hops extract in a competitive ER binding assay.[80] The Center bioassay facility has shown binding of a crude methanol extract of hops to human recombinant ER-α and ER-β in conventional radioligand binding assays.[6] The extract competed [^{3}H]estradiol binding to the ER-α and ER-β with IC_{50} values of 30.0 and 27.0 μg/mL, respectively. Polyphenolic hops extracts and the major prenylflavonoids in hops showed no progestogenic or androgenic activities in a series of recombinant yeast screens.[99] 8-Prenylnaringenin, 6-prenylnaringenin, 8-geranylnaringenin, 6, 8-diprenylnaringenin, xanthohunol, and isoxanthohumol all exhibit binding to recombinant human ER-α and ER-β receptors.[99,100] 8-Prenylnaringenin is the most potent of the hops phytoestrogens discovered to date, binding to both receptor subtypes with approximately one tenth the affinity of 17-β-estradiol. The compound exhibits only a slight preference for ER-β over ER-α, and its (R)- and (S)-enantiomers show similar binding profiles.[101]

Cell culture experiments provide further insight into the ER-dependent actions of hops. Zava et al showed that a 50% hydroethanolic extract of hops modestly stimulated the proliferation of T-47D cells in steroid-depleted serum but had no effect on the growth of MDA486 cells.[29] Dixon-Shanies and Shaikh showed that a 0.01% ethanolic extract of hops had significant antiproliferative effects on serum-stimulated T-47D cells.[36] Our work has demonstrated that a crude methanol extract of *Humulus lupulus* can up-regulate the estrogen-inducible genes *pS2* and *PR* in S30 and Ishikawa cells, respectively, and induce AP activity in Ishikawa cells.[6] Milligan et al observed ER-dependent responses to 8-prenylnaringenin in ER-transformed yeast and Ishikawa cell line experiments.[101]

Extracts of *Humulus lupulus* appear to exert estrogenic effects on the uterus, vaginal epithelium, bone, and liver at the whole animal level, although the uterine reports are somewhat contradictory. Early studies by Koch and Heim,[102] Zenisek and Bednar,[103] and Churý[104] reported positive outcomes in the Allen-Doisy test, that is, a measure of vaginal cornification in castrated rats. Churý also reported a uterotrophic activity in immature mice for his 95% ethanol, saponified extract of hops. Fenselau and Talalay could not duplicate the Churý result, nor could they discern uterotrophic activity in a number of other fractions of hops.[105] Eagon et al assessed estrogenicity in the ovariectomized rat model and observed no uterotrophic effect after 3 weeks of dietary intervention.[80] However, hops treatment elevated both serum ceruloplasmin and hepatic *c-myc* mRNA levels, indicating an estrogenic effect on the liver. Xanthohumol and humulone inhibited bone resorption in the pit formation assay at IC_{50} concentrations of 1-10 μM and 6 nM, respectively.[106] 8-Prenylnaringenin tested positive for the estrogenic endpoints of increased uterine vascular permeability and stimulation of the vaginal epithelium.[101] 8-Isopentenylnaringenin, an estrogenic isoprenylflavonoid isolated from *Anaxagorea luzonensis* A. Gray (Annonaceae),[107] prevents ovariectomy-induced loss in uterine weight and decrease in bone mineral density in the proximal tibia, and increases in urinary bone resorption markers in a rat model.[108] Although this finding may not translate to the isoprenylflavonoids in hops, it serves as a useful model for study.

A growing literature suggests chemopreventive potential for hops. In 1995, Tagashira et al established antioxidant activities for the hop bitter acids humulones and lupulones.[109] In 1999, Miranda et al tested 6 flavonoids from hops for their antiproliferative activities in human breast cancer and concluded

that xanthohumol and isoxanthohumol have potential chemopreventive activity against breast and ovarian cancer in humans.[110] The next year, the same group established that both prenylflavanones and prenylchalcones, as exemplified by xanthohumol, possess antioxidant activity as measured by the in vitro inhibition of oxidation of human low-density lipoprotein (LDL).[111] Furthermore, they demonstrated that xanthohumol, isoxanthohumol, and 8-prenylnaringenin are potent inhibitors of CYP1A2-mediated metabolic activation of herocyclic amines.[112] In 2001, Rong et al reported that 8-prenylnaringenin up-regulates the function of the E-cadherin/catenin complex in the human MCF-7/6 breast cancer cell line family. Finally, Gerhauser et al confirmed the chemopreventive potential of xanthohumol in a battery of tests designed to look at inhibitory mechanisms by which the agent might interfere with the initiation, promotion, and progression stages of carcinogenesis.[113]

Historically, reports by female hops pickers and brewers of menstrual cycle disturbances and acne, respectively, led to speculation that estrogens might be present in hops.[102] Some European practitioners even recommended hops-sludge baths for the alleviation of various gynecological disorders. Based on the molecular and animal evidence, *Humulus lupulus* may prove useful in menopause because of its estrogenic actions on bone for prevention of osteoporosis and on the brain for relief of vasomotor symptoms. The fact that the prenylflavonoids appear not to exert SERM-like selectivity, however, could produce untoward side effects in menopausal women (or cancer patients). Hops' sedative and possibly antidepressant qualities enhance its appeal as an agent for menopause and strengthen the case for clinical study. One DB RCT has shown a hops-valerian combination drug to be as effective as a benzodiazepam for sleep disorders.[114] It is unknown if this phytomedicine derives its effectiveness from hops, valerian, or both.

Licorice

Glycyrrhiza glabra (synonym *Liquiritae officinalis* Moench) (licorice), commonly named gancao, glycyrrhiza, sweet root, or Yasti-madhu, is consumed both as a food flavoring and a botanical dietary supplement. Traditional systems of medicine incorporate licorice as a demulcent, expectorant, prophylactic and treatment of ulcers, and an anti-allergic and anti-inflammatory agent. The major compound in licorice, glycyrrhizin, is responsible for the plant's sweetness. Its aglycone form, glycyrrhetic acid, and other constituents contribute to its antiulcer activity.[115,116] The German Commission E has approved the internal use of licorice for inflammation of the mucous membrane of the upper respiratory tract and gastric or duodenal ulcers.[64] Licorice was conferred GRAS status in the United States in 1983. Nonetheless, excessive consumption of licorice confers risk for pseudoaldosteronism. Other dose-dependent side effects of licorice occur more frequently in women and with oral contraceptive use.[117]

Licorice contains weak estrogen agonists, and competitive binding experiments suggest that licorice contains phytoprogestins in addition to phytoestrogens. Zava et al observed binding of a 50% hydroethanolic extract of *Glycyrrhiza glabra* to estrogen and progesterone receptors in MCF-7 and T-47D breast cancer cell lines, respectively.[29] Center investigators observed weak binding (IC_{50} > 50 ug/mL) of a crude methanol extract of licorice to human recombinant ER-α and ER-β and therefore reported the extract as inactive.[6] Glycyrrhizin, a triterpenoid; glabrene, an isoflavene; isoliquiritigenin, a chalcone; and glabridin, an isoflavan, are considered the principal (anti) estrogens in licorice. Based on the results of ER binding assays in T-47D cells, the rank order of potency of these compounds at ER-α is isoliquiritigenin (IC_{50} = 0.5 μM) > glabrene (IC_{50} = 1.0 μM) > glabridin (IC_{50} = 5.0 μM).[118] Weaker phytoestrogens in licorice include hispaglabridin A and hispaglabridin B. Maggiolini et al later demonstrated binding of isoliquiritigenin to chimeric proteins composed of the hormone binding domain of either ER-α or ER-β fused to the Gal4 DNA binding domain.[119] Relative selectivity could not be ascertained in the test system. The putative phytoprogestins have not been identified.

Cell culture work on crude extracts generally supports the theory that licorice operates in both an estrogenic and antiprogestinic manner. In one report to the contrary, Amato et al observed no stimulation of MCF-7 cell proliferation in an estrogen-depleted environment or transactivation of ER-α or ER-β in a HeLa cell reporter assay upon treatment with an alcoholic extract.[72] Zava et al showed that a 50% hydroethanolic extract of licorice modestly stimulated (ie, red clover > licorice > hops) the proliferation of T-47D cells in steroid-depleted serum but had no effect on the growth of MDA486 cells.[29] Licorice did not stimulate but almost completely blocked progestin-induced alkaline phosphatase activity in T-47D cells, indicative of antiprogestin activity. Licorice also down-regulated ER levels in T-47D cells. This effect was not reversed in the presence of RU486, suggesting an ER- as opposed to a PR-mediated mechanism. In MCF-7 cells, isoliquiritigenin transactivated endogenous ER-α, induced down-regulation of ER-α, and up-regulated *pS2* mRNA, effects that are consistent with agonist activity.[119] Isoliquiritigenin, glabrene,[118] and glabridin[120] all display biphasic effects on the proliferation of MCF-7 cells. ER-dependent cell proliferation is

observed at low concentrations (10 nM – 10 μM), and ER-independent antiproliferative or cytotoxic effects prevail at high concentrations (10 μM). Center work showed that a crude methanol extract of *Glycyrrhiza glabra* weakly up-regulated *PR* and *pS2* mRNA in S30 and Ishikawa cells, respectively.[6]

At the animal level, licorice often manifests both estrogenic and antiestrogenic properties. In 1967, Kumagai et al provided the first in vivo evidence of the (anti)estrogenicity of glycyrrhizin. At a glycyrrhizin:17-β-estradiol ratio of 1000:1 or less, glycyrrhizin inhibited the actions of 17-β-estradiol on uterine weight and β-glucuronidase activity. At high-dose ratios, reduced inhibition or an additive effect was observed.[121] More recently, glabridin was shown to increase uterine weight in female rats.[120] Both glabridin[120] and glabrene[118] induced the activity of creatine kinase, an immediate early estrogen-induced protein, in the rat model. Induction was tissue selective and occurred at significant levels in the uterus, epiphyseal cartilage, diaphyseal bone, and cardiovascular tissue.

In vitro and in vivo studies clearly document chemopreventive activities for licorice. Much of this work does not address the role of the phytoestrogens per se and is therefore outside the scope of this review. In short, compounds from licorice show promise as chemopreventive agents in models of antimutagenesis, anticarcinogenesis, and tumor suppression.[122] The National Cancer Institute recognizes the chemopreventive value of licorice root and constituents such as glycyrrhizin and various chalcones.[123] The triterpenoids[124] and polyphenols[125,126] (glabridin, hisglabridin A and B, and others) in licorice both contribute to its antioxidant activity. The administration of glabridin to mice resulted in a significant protective effect on LDL cholesterol from oxidation.[127]

In recent years, licorice has received much attention as a potential agent for prostate cancer. In vitro, licorice inhibits 17-β-hydroxysteroid dehydrogenase, the enzyme that catalyzes the conversion of androstenedione to testosterone. One group reported that licorice consumption reduced serum testosterone levels in men,[128] but another group was unable to corroborate this finding when it looked at a correlated parameter, the concentration of testosterone in saliva.[129] Licorice is also a component of PC-SPES, an estrogenic[130] herbal combination product believed by some to be of value in prostate cancer. The phytochemical value of PC-SPES, if any, has been obscured by findings of adulteration of various lots with varying amounts of diethylstilbestrol (estrogen), warfarin, indomethacin, and natural products.[131]

The (anti)estrogenic bioactivities of licorice have not been exploited, or perhaps are not physiologically relevant, in human use. There are no (English language) reports of human RCTs that have specifically examined *Glycyrrhiza glabra* for estrogenic endpoints. Theoretically, the aldosteronic effects of licorice could help compensate for declining adrenal function during menopause and thereby contribute to the relief of climacteric symptoms. Heavy licorice consumption during pregnancy has been associated with shorter gestation and preterm delivery.[132,133] The effects of glycyrrhizin on cortisol and prostaglandin metabolism are thought to explain these findings. The fact that *Glycyrrhiza glabra* often exhibits biphasic, concentration-dependent behavior in both in vitro and in vivo models raises the question of which bioactivities are relevant at safe human serum levels. Additional research into the pharmacokinetics and pharmacodynamics of licorice is warranted to address these questions.

Angelica

Angelica sinensis (angelica), often termed dong quai or danggui, is the most widely used *Angelica* species in China and has no major side effects. Considered a female tonic, the dried root is incorporated into a large number of multibotanical formulations, but there is little evidence-based rationale for gynecological use. In the United States, angelica has been marked for indications such as amenorrhea, premenstrual syndrome, and endometriosis and as an alternative to HRT. This section will review what (English language) literature exists to support the estrogenicity of this popular "women's herb."

A number of in vitro studies, including some in the Chinese literature, have explored the potential mechanism of action of angelica. Zava et al observed no binding of a 50% hydroenthanolic extract of *Angelica sinensis* to either estrogen or progesterone receptors in MCF-7 and T-47D breast cancer cell lines, respectively.[29] In contrast, Eagon et al observed binding of a danggui extract in a competition ER binding assay.[80] Similar to our results for licorice, we also found that angelica bound to human recombinant ER-α and ER-β, albeit quite weakly, and we therefore reported the extract as inactive.[6] Chinese investigators have demonstrated the interaction of angelica with a number of molecular targets. Liao et al reported binding of an aqueous extract of danggui to neurotransmitter receptors for GABA and 5-HT_{1A}.[134] Han et al observed bioactivity in assays of the α-adrenergic, angiotensin II, and calcium channel receptors; the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) and hypoxanthine transferase (HPGT) enzymes, and the cholecystokinin (CCK) peptide.[135]

In vitro cell culture experiments provide conflicting evidence as to the estrogenicity of angelica.

Table 3. ER-Independent or Indirect Mechanisms of Action That Could Theoretically Affect Cancer Risk

	Phytoestrogen Class		
Mechanism of Action	*Coumestan*	*Isoflavone*	*Lignan*
ER-independent mechanisms with (anti)estrogenic effects			
Aromatase inhibtion	√	√	√
5-α-reductase inhibition		√	√
17-β-hydroxysteroid dehydrogenase inhibition	√	√	
Sulphatase and sulphotransferase inhibition		√	
Alterations in SHBG levels		√	√
Alterations in estrogen metabolite ratios		√	√
ER-independent mechanisms without direct (anti)estrogenic effects			
Antioxidant	√	√	√
DNA topoisomerase inhibition	√	√	
Tyrosine kinase inhibition		√	
Inhibition of angiogenesis		√	√
Cell adhesion effects		√	√
Enhancement of immune system function		√	

ER = estrogen receptor.

Reports of no (anti)estrogenicity follow. Zava et al showed that a 50% hydroethanolic extract of dong quai neither stimulated the proliferation of T-47D cells in steroid-depleted serum nor had an effect on the growth of MDA486 cells.[29] Angelica did not stimulate or block progestin-induced alkaline phosphatase activity in T-47D cells, a cell model indication of no progestogenic activity. Reports of (anti)estrogenicity follow. Amato et al observed stimulation of MCF-7 cell proliferation in an estrogen-depleted environment but no transactivation of ER-α or ER-β in a HeLa cell reporter assay upon treatment with an alcoholic extract.[72] Dixon-Shanies and Shaikh showed that a 1.0% ethanolic extract of angelica had weak (hops > black cohosh > dong quai) antiproliferative effects in serum-stimulated T-47D cells.[36] Center work shows that a crude methanol extract of *Angelica sinensis* weakly up-regulates *PR* and *pS2* mRNA in S30 and Ishikawa cells, respectively.[6]

Animal studies of angelica have concentrated on the uterus as a target. Amato et al observed no change in uterine weight in a group of mice that had been fed an alcoholic extract of licorice for a period of 4 days.[72] Another study assessed uterine weight in the ovariectomized rat model and observed a uterotrophic effect after 3 weeks of dietary intervention.[80] Levels of uterine *c-myc* mRNA decreased, however, opposite of the effect that would be predicted for an estrogenic agent. Animal studies in a number of species have demonstrated both uterine relaxant and contractile effects.[136-138] Ligustilide, a volatile oil component, has been suggested as the active for the inhibitory effect. Two other constituents of angelica, tetramethylpyrazine and ferulic acid, independently and synergistically inhibit the spontaneous movement of the rat uterus.[139] One study administered a diet containing 5% crude dong quai to rats for 1 month. DNA synthesis and glucose utilization increased in uterine and ovarian tissues.[140] Dong quai is thought to increase sexual activity and perhaps influence fertility in animals.

Human studies of *Angelica sinensis* do not support an estrogenic mechanism of action. A DB RCT of angelica for vasomotor symptoms in postmenopausal women showed no significant reduction in number of hot flashes or relief of menopausal symptoms as measured by the Kupperman Index.[141] No changes were observed between placebo and treatment groups for either endometrial thickness or vaginal maturation index.

Other Mechanisms That May Affect Cancer Risks

It must be remembered that herbs are not single-entity drugs; rather, each is a complex mixture of hundreds, if not thousands, of compounds. These compounds may exert their biological actions alone or in concert, and each compound may have multiple targets of action. The isoflavones, the most extensively studied of the phytoestrogen molecules, have been ascribed numerous biological activities, and illustrate many of these principles. Thus, even a botanical with classic phytoestrogen activity (eg, red clover or hops) may contain antiestrogens and other compounds with the ability to counteract the effects of the estrogenic principals. This section identifies ER-independent mechanisms of action, with or without (anti)estrogenic outcomes, by which botanicals may alter cancer risk. Table 3 highlights the phytoestrogen classes in which the evidence is consistent with activity. Nonestrogenic phytochemicals may also possess these activities but are not addressed in this review.

Ligand-Independent Activation of ER Function

Nuclear steroid receptors are phosphoproteins, and their function can be regulated by phosphorylation status. Consequently, inhibitors or activators of protein kinases including growth factors can affect ligand-independent activation of ER function. Genistein is one example of a known inhibitor of tyrosine-specific receptor kinases.[142]

ER-Independent Mechanisms With (Anti)estrogenic Effects

Phytoestrogens tend to lower endogenous steroid hormone levels, thereby reducing cancer risk. This effect is achieved in part by interactions with enzymes involved in hormone production. These interactions include the inhibition of aromatase, which converts androgen to estrogen and the inhibition of 5-α reductase, which converts testosterone to the more active dihydrotestosterone.[143] Aromatase expression is up-regulated in tumor cells, and the estrogen produced in situ contributes to tumor promotion. Aromatase inhibition is observed for a wide variety of phytochemicals including flavonoids, flavones, and lignans.[144,145] Thus, natural aromatase inhibitors are being considered as chemopreventive and chemotherapeutic agents for breast cancer prevention and treatment, respectively.[146] Isoflavone and lignan phytoestrogens also stimulate SHBG production by hepatocytes, thereby reducing metabolic clearance of sex hormones and minimizing their bioactivities.[147,148] The urinary excretion rates of isoflavonoids and lignans correlate positively with SHBG levels.[149] Finally, phytoestrogen intake is associated with alterations in estrogen metabolism. At physiologically relevant concentrations, phytoestrogens inhibit the sulphotransferases that sulfate both endogenous and environmental estrogens.[150] Equol producers and flaxseed consumers have elevated urinary ratios of 2-hydroxyestrone to 16α-hydroxyestrone (2OHE1: 16aOHE1),[27,151] a marker that is negatively correlated with cancer risk.

ER-Independent Mechanisms Without (Anti)estrogenic Effects

Phytoestrogens may reduce cancer risk through ER-independent mechanisms that do not relate to hormonal status. In plants, phytoestrogens serve primarily as antioxidants, and this role is preserved in animals. Of potential clinical relevance, equol is the strongest of the isoflavones in terms of antioxidant activity.[25] Phytoestrogen-mediated inhibitions of DNA topoisomerases I and II[152,153] and tyrosine kinases have implications for cellular proliferation. Phytoestrogen-mediated inhibition of angiogenesis,[154] cell adhesion effects, and immune system enhancement may influence tumor development.

There is increasing evidence of phytoestrogen interactions with a number of steroid and nonsteroid receptors. Progesterone receptor activation may be particularly relevant owing to the complicated interplay between the estrogen and progesterone systems. Nonsteroid targets of particular interest include the receptors for neurotransmitters and neuromodulators such as serotonin, dopamine, γ-aminobutyrate (GABA), and adenosine for their potential to modulate endocrine pathways.

Clinical Discussion

Despite a large body of literature on the subject, especially in the soy field, the cancer implications for phytoestrogen consumption in healthy patients remain unclear. Animal studies suggest that neonatal or early stimulation with soy—and perhaps other phytoestrogens by inference—may alter differentiation of breast cells in a way that protects them against later assault by carcinogenic agents.[155] Yet, this protective effect diminishes with age, and dietary isoflavones can promote the growth of existent tumors in vivo.[156] Epidemiologic data also suggest that the chemopreventive potential of phytoestrogens may be related to the timing, forms, and patterns of consumption. These lines of evidence would imply no protection if phytoestrogen consumption begins in the perimenopausal period and possible detrimental effects if consumption is initiated after the induction of a tumor. Urinary patterns of phytoestrogen and phytoestrogen metabolite excretion are generally predictive of cancer risk, but again the results are not clear-cut. Whether or not estrogenic herbs confer chemoprotection to the reproductive/endocrine systems, there is growing evidence of beneficial effects to the cardiovascular system and bone. These benefits include improved lipid profiles (red clover,[54] soy,[157-161] flax[162,163]), arterial compliance (red clover,[59] soy[57,164]), and bone density (red clover,[59] soy[165-167]).

When advising cancer patients on CAM or botanical dietary supplement use, the major concerns must be safety and efficacy.[168] Safety risks include the probability of direct adverse events and risks for interactions with conventional cancer treatments. Efficacy considerations include changes in disease progression, survival rate, or reduction in symptoms. For many phytomedicines, it may be difficult to assess risk:benefit ratio of treatment without reliable data from randomized, double-blinded, placebo-controlled human trials. Other factors also complicate the rational approach: lack of standardized product, unknown

mechanism of action, and patient-specific health status or habits.

At this time, there are no proven herbal treatments for the primary treatment for cancer. Herbals may be useful in conjunction with conventional cancer treatment either as part of a palliative care program or to address other health concerns, but certain caveats apply. First, antioxidant compounds in phytomedicines have the potential to interfere with radiation and chemotherapies. Supplementation may be beneficial in one treatment scenario and detrimental in another; thus, the results from one experimental protocol cannot be safely extrapolated to another.[169] Herbals also have the potential to interact with cytochrome P450 enzymes to alter the metabolism of chemotherapeutic agents.[170] This concern has been heightened by reports that St. John's wort, an inducer of the CYP3A4 and CYP1A2 enzymes, can alter the pharmacokinetics of a number of drugs. In vitro studies indicate that 3 popular botanicals, licorice, hops, and red clover, can inhibit CYP450s,[171,172] but there are no literature reports of clinically significant interactions.

Hot flashes are a major clinical complaint among both healthy menopausal women and male and female cancer patients during and after hormone depletion therapies. HRT controls hot flashes very effectively but poses proven risks. In the most alarming report to date, the Women's Health Initiative concluded that estrogen plus progestin therapy leads to increased incidences of invasive breast cancer, coronary heart disease, stroke, and pulmonary embolism.[173] Steroidal hormone agents are not considered viable treatment options for ER+ cancer patients because of their potential to promote or progress tumors and compete with chemotherapeutic agents such as tamoxifen. Phytoestrogens theoretically pose similar but likely reduced risks. It is unclear whether phytoestrogens would be harmful for ER-α– ER-β+ cancer patients as the role of ER-β has not yet been fully explored.

Nonhormonal, pharmacologic alternatives for the treatment of hot flashes include α-adrenergic agents (menopause,[174] breast cancer treatment,[175] prostate cancer treatment[176]), selective serotonin reuptake inhibitors (SSRIs) (breast cancer survivors[177,178]), and gabapentin (menopause[179]). In the controlled studies cited above, the agents showed efficacy over placebo for the reduction of hot flash frequency and severity, but their practical use is limited by their side effect profiles. The studies' value may ultimately lie in their ability to provide basic researchers with clues as to the etiology/physiology of hot flashes. Well-designed clinical trials of nonpharmacological alternatives—beyond those under study at the UIC Center—such as soy (menopause,[157,180-185] breast cancer patients[186,187]), evening primrose oil (menopause[188]), ginseng (menopause[189]), and vitamin E (menopause,[190] breast cancer survivors[186]) have demonstrated modest to no efficacy. A nonestrogenic botanical such as black cohosh may prove to be the ideal solution. Treatment may be almost as effective as that achieved with a pharmacologic agent but better tolerated, leading to good compliance and a positive clinical outcome.

Cancer patients, and especially breast cancer patients, may be inclined to experiment with phytomedicines, both for cancer-related indications and general health maintenance. The consumption of modest quantities of phytoestrogens, particularly from food sources as part of a well-balanced diet, has general acceptance from the medical community. The consumption of high levels of phytoestrogens, as may occur with botanical dietary supplements, should probably be approached cautiously or discouraged for patients with cancer or at elevated risk for ER+ cancer. Additional research, both basic and clinical, is required to understand the full impact of phytoestrogens on cancer prevention, promotion, progression, and treatment.

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