INTRODUCTION

Phytoestrogens are a diverse group of plant-derived compounds that structurally or functionally mimic mammalian estrogens and show potential benefits for human health. The number of articles published on phytoestrogens has risen dramatically in the past couple decades. Further research continues to demonstrate the biological complexity of phytoestrogens, which belong to several different chemical classes and act through diverse mechanisms. This paper discusses the classification of phytoestrogens, methods of identification, their proposed mechanisms of action and botanical sources for phytoestrogens. The effects of phytoestrogens on breast and prostate cancers, cardiovascular disease, menopausal symptoms and osteoporosis will also be examined including research on benefits and risks. Copyright © 2003 John Wiley & Sons, Ltd.

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PHYTOESTROGENS

Phytoestrogens defined functionally are substances that promote estrogenic actions in mammals and structurally are similar to mammalian estrogen 17β-oestradiol (E2) (Price and Fenwick, 1985; Knight and Eden, 1996). Other mammalian endogenous estrogens are oestradiol and estrone, which are weakly estrogenic compared with their mammalian counterpart, E2 (Gruber et al., 2002) (Fig. 1). The diverse biological activity of phytoestrogens is due in part to their ability to act estrogenically as estrogen agonists and anti-estrogenically as antagonists. As estrogen agonists, phytoestrogens mimic endogenous estrogens and cause estrogenic effects. As estrogen antagonists, they may block or alter estrogen receptors (ER) and prevent estrogenic activity, causing anti-estrogenic effects (Brzezinski and Debi, 1999).

As estrogen agonists and antagonists, phytoestrogens can also be classified as selective estrogen receptor modulators (SERMs) (Brzezinski and Debi, 1999). SERMs are non-steroidal chemicals with a similar structure to E2 and an affinity toward estrogen receptors (Riggs and Hartmann, 2003). They are unique in that they can function as agonists or antagonists depending on the tissue, ER and concentration of circulating endogenous estrogens (Gruber et al., 2002). Tamoxifen and raloxifene are well-known SERMs. Tamoxifen has been used in clinical practice for breast cancer patients because it acts as an estrogen antagonist in breast tissue, slowing cancer cell proliferation and an estrogen agonist in bone tissue and in the cardiovascular system to prevent osteoporosis and heart disease. However, tamoxifen has shown estrogenic activity in the uterus and therefore may increase the risk of endometrial cancer (Poulet et al., 1997; Pukkala et al., 2002).

Mechanistically phytoestrogens have been shown to bind to two types of estrogen receptors: estrogen receptor α (ERα), which was cloned in 1986, and estrogen receptor β (ERβ) cloned in rats (Kuiper et al., 1996) and in humans (Mosselman et al., 1996). The two receptors differ in their tissue distribution and affinity to ligands, yet there is some overlap. In rats, ERα and ERβ both are clearly expressed in ovary and uterus tissue (Kuiper et al., 1997). ERβ has been shown to have ligand specificity toward phytoestrogens and is distributed in humans in ovary, spleen, testis and thymus tissue (Mosselman et al., 1996) and in rats in bladder, brain, lung, ovary, prostate, testis and uterus tissue (Kuiper et al., 1997). Phytoestrogens show a lower binding affinity than E2 and some show a higher binding affinity for ERβ than for ERα, which may suggest different pathways for their actions and explain tissue-specific variability of phytoestrogenic action (Kuiper et al., 1998; Setchell, 1998). The complexity of phytoestrogens and ERs appears to be further compounded because different transcriptional activities in vitro are activated depending on the ligands, as well as the environment of the promoter region of specific genes for translated ERα and ERβ receptors (Paech et al., 1997). Recent research in teleost fish (Atlantic croaker Micropogonias undulates) identified a third estrogen receptor, ERγ, which is found in various tissues (Hawkins et al., 2000).

MECHANISMS OF ACTION

Both genomic and nongenomic mechanisms have been proposed to explain phytoestrogenic effects on human health (Anderson et al., 1999). Phytoestrogens are able to interact with enzymes and receptors, and because of their stable structure and low molecular weight they can pass through cell membranes (Adlercreutz, 1999b).
These interactions allow them to bind to ERs, induce specific estrogen-responsive gene products, stimulate ER-positive breast cancer cell growth (Kurzer and Xu, 1997), interfere with steroid hormone metabolism or action (Adlercreutz, 1998b) and alter ER structure and affect transcription (Santti et al., 1998). Some genomic mechanisms of action include estrogenic and antiestrogenic effects on ERs, while other effects may not involve direct interaction with ERs (Messina and Loprinzi, 2001). Nonomic mechanisms that do not involve ERs include: induction of cancer cell differentiation, inhibition of tyrosine kinase and DNA topoisomerase activities, suppression of angiogenesis and antioxidant effects of phytoestrogens (Kurzer and Xu, 1997). Other effects can take place at the cellular and molecular level and potentially influence the biosynthesis and metabolism of steroids and fatty acids, the serum steroid carrier proteins (sex steroid binding proteins and α-fetoprotein), and the intracellular and transmembrane transfer of hormones to a membrane and to nuclear receptors (Benassayag et al., 2002). Phytoestrogens inhibit the enzymes needed for hormone conversions, which may reduce cancers by lowering the biological activity of sex hormones in target organs (Adlercreutz, 1998b). As estrogen-like compounds, some phytoestrogens are able to induce estrus in mammals (Mineta et al., 2001).

The different activities and the bioavailability of phytoestrogens vary depending on such factors as the form of administration, dosage, individual metabolism and the ingestion of other pharmacological substances (Kelly et al., 1995; Xu et al., 1995; Wiseman, 1999). Target tissue, concentration dependency, number and type of ER, and the presence or absence of endogenous estrogens also influence the effect of phytoestrogens (Glazier and Bowman, 2001).

Not only do phytoestrogens differ in their biological activity, but they also differ structurally because they come from diverse chemical classes, which may affect their influence on tissues and receptors (Lieberman, 1996). Due to the diversity of chemicals that show estrogenic effects, it appears that estrogenic activity is often emphasized over chemical structure in defining phytoestrogens.

**IDENTIFICATION OF PHYTOESTROGENS**

The ability of plant substances to cause estrus in animals was documented in the mid-1920s (Costello and Lynn, 1950; Bradbury and White, 1954). The Allen-Doisy technique was one of the first bioassays to detect estrogenic activity in ovariecromized rats and mice (Allen and Doisy, 1923). In this bioassay the uterine weight of the test animal was used to measure estrogenic activity. A review of plants tested with this technique has been published and includes a variety of plants from different botanical families such as beets (*Beta vulgaris* L., Chenopodiaceae), parsley (*Petroselinum crispum* (Mill.) Nyman ex A. W. Hill, Apiaceae), plum and cherry (*Prunus spp.*, Rosaceae), potato (*Solanum tuberosum* L., Solanaceae) and rice (*Oryza sativa* L., Poaceae) (Bradbury and White, 1954). Later, steroid estrogens were detected in plants, such as estrone found in pomegranate seeds (*Punica granatum* L., Punicaceae) and the seeds of the date palm (*Phoenix dactylifera* L., Arecaceae), although the presence of these compounds has been questioned (Heftmann, 1967). Although phytoestrogens were originally noticed because they induced estrus in animals, not all plants that show estrogenic activity induce estrus. In addition, there are plant substances that induce estrus that are not phytoestrogens. Since the early 1920s additional *in vivo* and *in vitro* methods have been developed to test estrogenic and antiestrogenic activity of plants.

Some *in vivo* tests used to evaluate estrogenic activity are: the degree of cornification of vaginal epithelium in rats and mice, uterotrophic assays that measure uterine wet weight in immature or ovariecromized rats or mice, and proliferative effects in the female genital tract (Galey et al., 1993; Diel et al., 2002). Estrogen activity based on bioassay methods should be viewed with caution when extrapolating to humans because phytoestrogens have been shown to metabolize differently in different animal species (Reinli and Block, 1996). In addition, many variables can affect the results in animal studies such as the model species, dosage, length of study and routes of administration (Yang and Bittner, 2002). In clinical and epidemiological studies, the same principle applies as studies vary by type of intervention (extracts, purified compounds), dosage, age of subject, and length of study, among other variables.

Some *in vitro* assays used to investigate estrogenic activity are: the receptor binding assay in which the binding affinity to the ERs is measured, E-screen assay which tests the ability of a substance to stimulate growth of estrogen sensitive cells such as human breast cancer cells, the reporter gene assay which measures the ability of a substance to activate transcription via an estrogen sensitive promoter, and the analysis of gene expression which evaluates the regulation of estrogen sensitive genes in cell culture (Diel et al., 1999; Mueller, 2002). Some assays are more sensitive to phytoestrogens than others (Dixon-Shanies and Shaikh, 1999). Other examples of *in vitro* assays are the transient gene expression or co-transfection assay (Miksieck, 1993; Miksiec, 1994) and the Ishikawa cell line, an organ specific model that uses estrogen responsive human endometrial adenocarcinoma cells (Wobet et al., 2003). Measuring quantitative structure–activity relationships (QSAR) has been recommended as an initial screening technique for estrogen receptor binding affinity prior to *in vitro* and *in vivo* assays (Hu and Aizawa, 2003).

In the mid-1980s the primary method for phytoestrogen analysis was gas chromatography coupled with a mass spectrometer (GC-MS), which involved extensive purification procedures for either plants or physiological samples before analysis (Wang et al., 2002). It is still valuable for physiological samples such as urine and blood containing low concentrations of phytoestrogens although it is labor intensive and new techniques have been developed (Wang et al., 2002). High pressure liquid chromatography (HPLC) was first used to detect flavonoids in 1976 by Fisher and Wheaton (1976). It is one of the most common analytical methods used for phytoestrogen identification because limited sample preparation is involved and both glycosides and aglycones can be analysed directly (Wang et al., 2002). HPLC coupled with a mass spectrometer (LC-MS) is especially useful for this type of analysis. Franke and coworkers (1995) have
developed reversed-phase HPLC methods that are tailored for phytoestrogen analysis. Acid hydrolysis is part of sample preparation, which converts glycosides into their respective aglycones. The solvent system they used was a linear gradient of acetonitrile and acetic acid and water with a photodiode array (PDA) detector. This group recently reported a new technique for determining phytoestrogens (Franke et al., 2002). One drawback of using LC-MS is the ineffective isomer differentiation and the inability to produce molecular ions for some flavonoids (Wang et al., 2002). Additional reports discuss methods used to detect polyphenolic phytoestrogens in foods and biological fluids (Joannou et al., 1995; Wilkinson et al., 2002). Other non-chromatographic methods have also been used for phytoestrogen analysis, such as immunosay techniques, deconvolution spectroscopy and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Wang et al., 2002).

Comparing phytoestrogen levels and the content of foodstuffs and plants is not always possible because various methods are used to extract and quantify phytoestrogens. Different varieties of plants and brands of food as well as diverse forms of processing need to be considered when evaluating phytoestrogen levels (Mazur, 1998). In addition, individual plants may show different levels of phytoestrogens due to microbial or insect damage prior to analysis and seasonal variation (Price and Fenwick, 1985). Instability and easy degradation of some phytoestrogens during processing and analysis may explain varying analytical results (Ibarreta et al., 2001).

CLASSIFICATION OF PHYTOESTROGENS

There are several classes of phytoestrogens: steroidal estrogens, found in few plants and the more ubiquitous phenolic estrogens, isoflavones, coumestans and lignans which will be the focus of this review. Other classes of phytoestrogens that have been reported include: anthraquinones (Matsuda et al., 2001), chalcones (Rafi et al., 2000), flavones (Milligan et al., 1999), prenyllavonoids (Kitaoaka et al., 1998) and saponins (Chan et al., 2002). Examples of plant steroids are estrone found in palm (Elaeis guineensis (Raf) found in palm (2002). Examples of plant steroids are estrone et al. (Anstead, 2002). Other classes of phytoestrogens are aromatic ring and a hydroxyl group is important for binding effectiveness and the remainder of the ER will accept hydrophobic groups (Anstead et al., 1997). Important features that enable chemicals to bind to an ER are the steric and hydrophobic properties of a compound, as well as the hydrogen bonding between the phenolic hydroxyl group and the ER binding site (Hu and Aizawa, 2003). Estrogenic flavonoids are similar in structure to E2. They are composed of a planar ring system that includes a p-hydroxy-substituted aromatic ring that is approximately 12 Å away from a second in-plane hydroxyl group (Hu and Aizawa, 2003). Two ring structures separated with two carbon atoms as well as spacing between hydrophobic and hydrogen bond interactions are also important in binding affinity to ERs (Brzozowski et al., 1997). Other characteristics for ER-binding affinity of a chemical are the degree and size of branching of the alkyl group and its location on the phenolic ring and the distribution range of electron density on the A ring (Hu and Aizawa, 2003).

The biological activity of individual phytoestrogens varies and is often reported as less active than mammal or synthetic estrogens (Knight and Eden, 1996; Tham et al., 1998). Differences in estrogenic activity of similarly classified chemicals may be due to the structural features or deviations in those structures. Some phytoestrogenic compounds may show different estrogenicity due to the bioassay employed (Messina and Loprinzi, 2001) and others may not show estrogenic activity in bioassays because only their metabolized derivatives are hormonally active (Miksicek, 1994). These metabolized derivatives may be active aglycons produced by the removal of the sugar moiety during metabolism or xenobiotics produced through metabolic processes (Adlercreutz et al., 1987; Xu et al., 1995). More research is needed to elucidate the structural-activity relationship that is required for a natural product to be active as a phytoestrogen.

Isoflavones

Isoflavones are the most well known of the phytoestrogens. The recognition of ‘clover disease’ in Australian sheep in the 1940s led to the investigation of estrogenic activity of isoflavones (Kingsbury, 1964). The sheep whose diet was predominately subterranean clover (Trifolium subterraneum L., Fabaceae) suffered from a reproductive disorder that reduced the lambing rates and involved abnormal lactation, changes in the sex organs, permanent infertility, prolapsed uterus and maternal dystocia (Bennetts et al., 1946). Isoflavones are present in green clover and are not present at senescence (Adams, 1995).

Naturally occurring isoflavones that have shown estrogenic activity are: the aglycones, daidzein (4′,7-dihydroxyisoflavone) and genistein (4′,7,8-trihydroxyisoflavone); the glycosides, daidzin and genistin; and biochanin A and formononetin, 4′-methyl ethers of daidzein and genistein (Price and Fenwick, 1985; Kurzer and Xu, 1997) (Fig. 2). In plants, they can often be found as glycosides (Ibarreta et al., 2001). In processing, isolation and analysis, these compounds are readily degraded chemically or enzymically to the aglycone (Price and Fenwick, 1985). Glycitein is another isoflavone reported in soy that has also shown estrogenic activity (Song et al., 1999).

After mammals consume isoflavones, daidzein and genistein are metabolized in the gastrointestinal tract. Biochanin A and formononetin can metabolize to genistein and daidzein respectively. Daidzein may be
Of all the isoflavones, genistein has received the most attention. Estrogenic and antiestrogenic effects of genistein, including effects independent of ERs, have been discussed in relation to breast cancer (Bouker and Hilakivi-Clarke, 2000; Brownson et al., 2002) and other conditions (Dixon and Ferreira, 2002). Several studies have tested genistein with breast cancer cell lines and the results tend to show cell proliferation at low doses and inhibition at high concentrations (Anderson et al., 1999). Genistein has been shown as a powerful antioxidant (Ruiz-Larea et al., 1997). However, other researchers have shown that genistein and other phytoestrogens have low antioxidant ability, suggesting that phytoestrogens may not have such a significant effect as antioxidants (Mitchell et al., 1998).

**Coumestans**

Coumestans are another group of plant phenols that show estrogenic activity. Coumestrol was first reported in 1957 by Bickoff and coworkers as a new phytoestrogen that was isolated from ladino clover (Trifolium repens L., Fabaceae), strawberry clover (Trifolium fragiferum L., Fabaceae) and alfalfa or lucerne (Medicago sativa L., Fabaceae) (Bickoff et al., 1957). Their presence in fodder crops has been associated with disrupting reproductive performances of livestock (Price and Fenwick, 1985; Adams, 1995). For example, feeding cattle haylage containing 37 ppm (mg/kg) or more of coumestrol resulted in negative estrogenic effects such as udder development, bulling of steers and prolapsed vagina, cervix and rectum (Lookhart, 1980). However, few coumestans isolated from plants have shown uterotrophic activity (Price and Fenwick, 1985; Kurzer and Xu, 1997). The main coumestans with phytoestrogenic effects are coumestrol and 4′-methoxycoumestrol (Fig. 4). Coumestrol and genistein have higher binding affinities to ERβ than the other phytoestrogen compounds (Whitten and Naftolin, 1998). *In vitro* coumestrol has been reported to inhibit bone resorption and to stimulate bone mineralization (Tsutsumi, 1995).

Coumestans are less common in the human diet than isoflavones (Ibarreta et al., 2001), yet similar to isoflavones, in that they are also found in legumes, particularly food plants such as sprouts of alfalfa and mung bean (Vigna radiata (L.) R. Wilczek, Fabaceae) (Lookhart, 1980; Adams, 1995; Mazur, 1998) and they are especially high in clover (Franke et al., 1995). Soy sprouts also show high levels of coumestrol (71.1 µg/g wet weight) (Ibarreta et al., 2001).
The coumestrol content in plant material has been reported to vary according to plant variety, stage of growth, cutting, presence of disease, location and insect/fungal attacks (Price and Fenwick, 1985). Attacks by insects and fungus may cause plants, for example alfalfa, to produce coumestrol or 4'-methoxycoumestrol as phytoalexins (Adams, 1995). Phytoalexins are a diverse class of compounds that are often secondary metabolites formed as a result of injury or disruption, which aid plants by protecting them from insects, bacteria and viruses (Ibarreta et al., 2001).

### Lignans

Lignans were first identified in plants and later in biological fluids of mammals. As a class of compounds they contain a dibenzylbutane skeleton and in plants they aid in the formation of lignin used to construct the plant cell wall (Setchell and Adlercreutz, 1988). A cyclic pattern observed in the urinary excretion of these phenolic compounds by humans and animals during the menstrual cycle initiated interest in their physiological role (Adlercreutz et al., 1987). They were thought to be a new class of endogenous hormones. These compounds were elucidated simultaneously by different researchers and identified as unique mammalian lignans (Setchell et al., 1980; Stitch et al., 1980).

The most well known phytoestrogenic lignans are secoisolariciresinol and matairesinol (Fig. 5) which are converted by bacterial action in the gut into enterodiol and enterolactone (Fig. 6). Mammalian lignans not found in plants (Setchell and Adlercreutz, 1988). Enterodiol can be further metabolized to enterolactone (Borriello et al., 1985). The removal of the sugar moiety through metabolism by intestinal bacteria is common in isoflavones and lignans (Adlercreutz et al., 1987). Mammalian lignans, like isoflavones, have a low molecular weight and are considered chemically, biochemically and biologically unique and stable molecules because they have phenolic groups in the meta position of the aromatic rings (Adlercreutz, 1998a). Recently, other enterolactone precursors have been identified: arostigenin, 7-hydroxymatairesinol, lariciresinol, pinore- sinol and syringaresinol (Fig. 5) (Meagher et al., 1999; Heinonen et al., 2001). The last three are found in cereals, specifically whole-grain rye products. However, more studies are needed to evaluate the estrogenic activity of these compounds.

Lignans are widespread in foodstuff such as cereals, fruits and vegetables and have not been studied as thoroughly as isoflavones and coumestans (Ibarreta et al., 2001). Lignans are commonly found in rye bread (Secale cereale L., Poaceae) and oilseeds such as flaxseed (Linum usitatissimum L., Linaceae) (Thompson et al., 1991). Flaxseed contains the most abundant amount of lignans with about 0.8 mg of secoisolariciresinol/g dry weight (Kurzer and Xu, 1997) and when crushed and defatted the phytoestrogen content rises (Mazur, 1998). Lignans are also found in brewed green and black tea and coffee (Mazur et al., 1998). Grains and cereals show high levels of lignans in the aleurone and pericarp/testa layers (Mazur, 1998). Pumpkin seeds (Cucurbita pepo L., Cucurbitaceae) contain secoisolariciresinol and trace amounts of lariciresinol (Sicilia et al., 2003). Secoisolariciresinol is found in many food and beverage categories, while matairesinol is found in smaller amounts and only trace amounts in food legumes (Mazur, 1998).

Large amounts of lignans have also been reported in coniferous trees, such as the heartwood of Norway spruce (Picea abies (L.) H. Karst, Pinaceae), which has shown greater concentrations of lignans than flax (Saarinen et al., 2000). Hydroxymatairesinol (HMR) was first identified in spruce and was reported as a novel precursor to enterolactone. Additional testing with this compound showed potent antioxidant activity and decreased breast tumors in the rat dimethylben[α]anthracene-induced mammary tumor model; however, HMR did not show estrogenic activity in the uterine growth assay or through transcriptional responses via ERα or ERβ (Saarinen et al., 2000). These results suggest that the antitumor activity of HMR may involve nongenomic mechanisms of activity.

A case-controlled study on metabolized lignan conducted with Finnish men showed a reduced risk of myocardial infarction associated with higher concentrations of serum enterolactone (Vanharanta et al., 1999). Adlercreutz (2003) points out that purified lignans

![Figure 5. Structures of some plant lignans: secoisolariciresinol, matairesinol, lariciresinol, pinoresinol and syringaresinol.](image)

![Figure 6. Structures of mammalian lignans: enterodiol and enterolactone. Plant lignans secoisolariciresinol and matairesinol are converted by human gut bacteria to enterodiol and enterolactone, respectively.](image)
consumed neonatally or prepubertally may have a protective effect on breast tissue by increasing the differentiation of proliferative terminal end bud structures also observed during pregnancy and lactation.

**Other estrogenic compounds**

Other compounds that have shown estrogenic activity are: resveratrol (trans-3,4',5-trihydroxystilbene), diethylstilbestrol (DES) (4,4'-dihydroxy-trans-α, β-diethylstilbene) and mycotoxins such as zearalenol (Fig. 7). Resveratrol, a stilbene, is found in a variety of plants and functions as a phytalexin to protect against fungal infections. Other sources of resveratrol are the skin of grapes (Vitis vinifera L., Vitaceae), wine, as well as other botanicals such as hu-chang (*Polygonum cuspidatum* Siebold & Zucc., Polygonaceae) (Bagchi et al., 2001). Resveratrol has been reported to bind to ERs and stimulate the growth of human breast cancer cells (Ghem et al., 1997) and to increase ovarian weight and disrupt estrous cycles in gonadally intact Sprague-Dawley rats (Henry and Witt, 2002).

In addition to plant estrogens, xenoestrogens and mycotoxins when ingested have an estrogenic response similar to endogenous estrogens. Pesticides and industrial chemicals (i.e. polychlorinated biphenyls), sometimes referred to as endocrine disrupters, are examples of xenoestrogens found in the environment (Belcher and Zsarnovszky, 2001; Ibarreta et al., 2001). Diethylstilbestrol is a synthetic stilbene that has also shown strong estrogenic effects in humans (Setchell, 1998). Mycotoxins are secondary metabolites produced by molds such as *Fusarium* often found on stored crops (Kurzer and Xu, 1997; Ibarreta et al., 2001). Resorcylic acid lactone compounds, such as zearalenone and zearalenol and their derivatives, have been referred to as mycoestrogens, a type of mycotoxin, because they show estrogenic activity but they are not inherent to food plants (Fig. 7).

Zearalenone is a widely distributed mycotoxin that has strong estrogenic effects. It is primarily produced by *Fusarium graminearum*, *F. culmorum* and *F. crookwellense* and is usually associated with corn but can also be found in other cereals such as barley and wheat (IARC, 1993). Reproductive problems such as vulvovaginitis (vaginal swelling), prolonged estrus, reduced sex drive, infertility and abortions have been shown in animals consuming contaminated feed as well as testicular atrophy and enlarged mammary glands in male swine (Schoental, 1985; Visconti and Pascale, 1998). In some cases farmers have exploited the potential of mycotoxins and used it to promote fattening of sheep and cattle (Yang and Bittner, 2002). Methods are being developed to detect mycotoxins in foodstuffs and feedstuffs to safeguard both animal and human health (Rosenberg et al., 1998). The United States Food and Drug Administration (FDA) has not established advisory limits nor action levels for zearalenone, although if this mycotoxin is present usually other mycotoxins for which the FDA has set advisory levels are also present, such as deoxynivalenol produced by *Fusarium* spp.

New sources of estrogenic compounds are attractive for human health because of the numerous effects that endogenous estrogens have on the human body. Yet some synthetic estrogenic compounds have raised concern for human health because of the negative effects they can have on the body. Although phytoestrogens come from plants and are therefore considered 'natural', they may also have negative effects on health that have not been clearly identified. At present much of the research on phytoestrogens is evaluating the beneficial and adverse effects of these compounds on different health conditions.

### HUMAN HEALTH AND PHYTOESTROGENS

Investigators have proposed the hypothesis that lowered cardiovascular disease, osteoporotic fractures, rates of breast cancer and hot flushes in Asian populations are related to a diet rich in soy, in other words phytoestrogens (Adlercreutz, 1998a; Clarkson and Anthony, 1998; Adlercreutz et al., 2000b; Wagner et al., 2001). Therefore, diet has been evaluated especially in relation to phytoestrogen content. However, when evaluating this relationship, confounding factors such as lifestyle, diet, socio-cultural and morphological differences that distinguish Asian and Western populations must be considered in the analysis (This et al., 2001). Several studies have discussed the potential effects of phytoestrogens in treating breast cancer, endometrial cancer, liver disease and prostate cancer (Fotis et al., 1993; Strom et al., 1999; Adlercreutz et al., 2000b; Messina and Loprinzi, 2001; Lei et al., 2002). Additional research has shown that intestinal bacteria are seen as important in the metabolism of phytoestrogens and have the ability to refine phytoestrogens into compounds similar in structure to E2, that protect against cancer (Xu et al., 1995). Some of the proposed mechanisms by which phytoestrogens may inhibit cancer cells are: inhibition of DNA topoisomerase, suppression of angiogenesis, induction of differentiation in cancer cell lines and induction of apoptosis (Glazier and Bowman, 2001).

As studies continue to evaluate the biological effects of phytoestrogens on human health, the complexity is more evident as estrogenic and antiestrogenic effects are observed as well as a variety of mechanisms of action (Kronenberg and Hughes, 1999). More well designed clinical trials are needed to assess the beneficial effects of phytoestrogens on health (Naftolin and Stanbury, 2002).
Breast cancer

In western countries, breast cancer is the most common cancer affecting women (Parkin, 2001). Historically, the risk of breast cancer was much higher in American women than in Asian women prior to the influence of the western diet in Asian cultures (Bouker and Hilakivi-Clarke, 2000). Epidemiological studies of breast cancer and the dietary intake of soy and lignan have recently been reviewed (Peeters et al., 2003), as well as the mechanisms of phytoestrogenic action in breast tissue (Adlercreutz, 2003). One task has been to find an estrogen replacement therapy for women at risk for breast cancer or who have survived breast cancer. A diet rich in phytoestrogens has been suggested as a preventative agent against breast cancer although there is conflicting evidence (Adlercreutz et al., 2000b; Wagner et al., 2001). Phytoestrogens act as weak estrogens and exhibit estrogenic activity in a low-estrogen environment; therefore it has been postulated that they show antiestrogenic activity in a high-estrogen environment (Messina and Loprinzi, 2001). This explanation suggests that prior to menopause when there is a high-estrogen environment phytoestrogens may protect against breast cancer and after menopause when there is a low-estrogen environment they may stimulate breast cancer (Anderson et al., 1999). This theory is highly debated and many studies show conflicting evidence about the action of phytoestrogens and breast cancer.

Five in vitro studies of phytoestrogens on mammary cells have been reviewed and summarized by This and coauthors (2001). Biphasic effects of genistein in varying concentrations of culture medium were demonstrated on mammary cells (MCF-7 cancer cells). At doses greater than 100 nM/L genistein inhibits receptor age, timing and dose of administration, length of extrapolating conclusions. Studies often differ by subject age, timing and dose of administration, length of study and form of analysis. More research is needed to evaluate the different binding effects of phytoestrogens to ERα and ERβ for breast cancer.

Cardiovascular disease

The leading cause of death in women in industrialized nations is coronary heart disease (CHD). In menopause the risk of CHD greatly increases and it is proposed that this is due to the loss of estrogen (Wroblewski et al., 1997). Evidence from an epidemiological study suggests that a diet low in fat and high in soy proteins, vegetable oils and vegetables rich in carotene is associated with a lowered risk of breast cancer in premenopausal women (Lee and Gourley, 1991). Some methodologies do not determine the exact quantities of phytoestrogens ingested and therefore are problematic for comparisons. Plasma estrogens and breast cancer have been studied more than urinary estrogens because plasma estrogens are easier to identify (Kurzer, 2002). In evaluating various studies, methodologies must be considered when extrapolating conclusions. Studies often differ by subject age, timing and dose of administration, length of study and form of analysis. More research is needed to evaluate the different binding effects of phytoestrogens to ERα and ERβ for breast cancer.

increased bile acid secretion, which aids removal of low density lipoprotein (LDL); affected hepatic metabolism coupled with increased removal of LDL by hepatocytes; and enhanced thyroid function (Wroblewski Lissin and Cooke, 2000). LDL shows increased oxidative resistance when isoflavonoids are incorporated into LDL cholesterol (Vincent and Fitzpatrick, 2000). Additional mechanisms of action have been suggested to explain the effects on plasma lipid concentrations including: action on ERs, reduction of endogenous cholesterol synthesis, and increased activity of cholesterol receptors (Glazier and Bowman, 2001). The isoflavonoid, genistein, has been shown to act as a protein tyrosine kinase inhibitor, which may explain decreased platelet action (Tham et al., 1998).

Studies suggest that isoflavonoids as antioxidants may affect atherogenesis by reducing the oxidation of LDL (Ruiz-Larrea et al., 1997; Wagner et al., 1997). Kurzer and Xu (1997) reviewed antioxidant effects of isoflavonoids from in vitro and in vivo studies and reported that soy isoflavonoids act as antioxidants by directly or indirectly enhancing the activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase enzymes. Hwang and coworkers (2001) reported that extracts of soy, alfalfa and acerola cherry (Malpighia glabra L., Malpighiaceae) may synergistically interact to prevent LDL oxidation. Most of the research has been conducted with soy isoflavonoids and limited research has been conducted to evaluate the effects of lignans and coumestans on cardiovascular disease.

**Prostate cancer**

Prostate cancer is one of the most common cancers for men in the United States, however, little is known about its etiology. In vitro studies using human prostate cancer cells have shown the inhibition of cell growth with high concentrations of phytoestrogens (Adlercreutz et al., 2000a). Rats consuming soy and rye bran had delayed growth of implanted prostate tumors (Landstöm et al., 1998). Further testing with the same phytoestrogens increased apoptosis of the tumors and reduced tumor growth in nude mice implanted with human prostate tumors. However, estrogen has shown controversial effects, such as growth of prostate cancer and benign prostatic hyperplasia and therefore phytoestrogens may have similar effects (Adlercreutz et al., 2000a). Adlercreutz (2002) reviewed some of the more recent studies on prostate cancer and stated that findings support the hypothesis that soy consumption prevents prostate cancer, yet more studies are needed.

The interaction of phytoestrogens with ERs and ER isoforms such as ERβ2 or ERβx in the prostate as well as other receptors can lead to a variety of biological actions (Morrisey and Watson, 2003). The binding affinity for different ERs and the formation of the ER complex which can bind to ERE may explain the actions observed in the prostate. Other factors that affect whether phytoestrogens have a positive or negative effect on the prostate may be the ER co-factors and phosphorylation status (Morrisey and Watson, 2003). In addition, the effects on growth factor receptors have been suggested because of the inhibition of growth-factor-mediated stimulation of cell proliferation (Adlercreutz, 2002). Other mechanisms may be due to the regulation of steroid receptor pathways, anti-androgenic effects, inhibition of 5α-reductase, 17β-hydroxysteroid dehydrogenase and aromatase activity, inhibition of tyrosine specific protein kinases and DNA topoisomerase II and antioxidant activity (Morrisey and Watson, 2003).

Several epidemiological studies suggest the beneficial use of phytoestrogens in reducing prostate cancer (Severson et al., 1989; Jacobsen et al., 1998; Strom et al., 1999). A human study of 83 cases and 107 controls used a dietary questionnaire to evaluate phytoestrogen consumption for prostate cancer risk (Strom et al., 1999). The results showed slightly protective effects on prostate cancer risk with greater consumption of phytoestrogens. Severson and coworkers (1989) showed that increased tofu consumption was associated with a decreased risk of prostate cancer in men of Japanese ancestry living in Hawaii. Another study reported that Adventist men who consumed soy milk daily were at lower risk for prostate cancer (Jacobsen et al., 1998).

However, this study had a small number of cases and therefore the confidence interval was wide. An herbal mixture including licorice and ginseng as well as six other herbs has shown estrogenic activity and was effective in two cases of hormone-refractory prostate cancer (De la Taille et al., 2000). Other studies have evaluated alternative therapies, such as soy, black cohosh, vitamin E and red clover for their potential use in alleviating hot flashes for prostate cancer patients (Moyad, 2002). A recently published randomized cross-over study on soy food consumption and serum prostate specific antigen (PSA) in men with hyperlipidemia showed lowered LDL, no significant effects on serum PSA, and a reduced calculated risk for CHD (Jenkins et al., 2003).

There is a paucity of research on the effects of phytoestrogens and prostate cancer. Although the present studies show a positive association between phytoestrogens and prostate cancer risk, more clinical studies are needed to confirm this hypothesis.

**Menopausal symptoms**

The symptoms associated with menopause cause many women to seek medical solutions. Hormone replacement therapy has proven effective in the reduction of hot flushes, yet it is still controversial if HRT may be associated with increased risks of breast and endometrial cancer. Initial findings from the WHI randomized controlled trial in which women received a daily dose of conjugated equine estrogen (0.625 mg) and medroxyprogesterone acetate (2.5 mg) have shown an increased risk to benefits ratio (Rossouw et al., 2002). The investigators detected increased risks for invasive breast cancer and CHD with the consumption of the combined hormone preparation after 5.2 years of average follow-up. Due to controversial evidence on HRTs, alternative therapies have been sought, such as phytoestrogens and SERMs.

The mechanisms of action are still poorly understood because of the complex biological actions observed in phytoestrogens. As discussed before, the target tissue, the kinds of ERs and the concentration of endogenous estrogens are all factors that affect phytoestrogen activity at the cellular level. Other non-receptor mechanisms may also explain the biological effects of
phytoestrogens on menopausal symptoms, such as antioxidation, blocking of enzymes involved in the biosynthesis of estrogen, inhibition of protein kinase which is part of intracellular signaling, and inhibition of 5α-reductase and aromatase (Vincent and Fitzpatrick, 2000). The reduction of bioavailability levels of free sex steroids such as estrogen may also be the result of phytoestrogens stimulating sex hormone-binding globulin (SHBG) synthesis (Vincent and Fitzpatrick, 2000).

Some botanicals used in Western countries for menopausal syndromes are black cohosh, dong quai, ginseng, red clover, hops (Humulus lupulus L., Cannabaceae), oil of evening primrose (Oenothera biennis L., Onagraceae) and chasteberry or chaste tree berry (Vitex agnus-castus L., Verbenaceae) (Liu et al., 2001b; Kronenberg and Fugh-Berman, 2002). Several reviews have discussed studies conducted on phytoestrogens and menopausal symptoms and still much contradictory evidence exists as to the benefits of phytoestrogens (Glazier and Bowman, 2001; Merritt, 2001; Kang et al., 2000; Kronenberg and Fugh-Berman, 2002). More research is needed in this area, especially studies examining the long-term effects of phytoestrogens on endometrial tissue and bone loss.

Osteoporosis/bone health

Osteoporosis is often associated with women in menopause. The evidence supports ERT in the prevention of osteoporosis in postmenopausal women and therefore phytoestrogens have been evaluated for their effects on bone mineral density. Researchers hypothesize that a diet rich in isoflavones has a protective effect on bone (Tham et al., 1998). Ipriflavone, a synthetic isoflavone derivative (7-isopropoxy-isoflavone), has been used extensively in animal and human studies to evaluate bone health and phytoestrogens with beneficial results (Scheiber and Rebar, 1999). Vincent and Fitzpatrick (2000) reviewed three animal studies and concluded that genistein has a biphasic effect, lower doses improved bone mineral as opposed to high doses, on bone mineral density in ovariectomized rats. Van der Shouw and coworkers (2000) reviewed three studies in bone mineral density with phytoestrogen consumption that were conducted with postmenopausal women. Two of the studies showed an increase in bone mineral density and the third study, a 10-year follow-up study conducted in the Netherlands, reported a loss of bone associated with higher urinary equol and enterolactone excretion.

Kurzer and Xu (1997) reviewed several other studies that include possible mechanisms of action to explain the beneficial effect of phytoestrogens on bone loss. These mechanisms include preventing urinary calcium loss, beneficial effects on osteoblasts, and influences on the secretion of calcitonin which suppresses bone resorption. In addition, soy foods are considered a good source of calcium. Estrogen receptors have been found in osteoblasts, which may cause alteration in some protein production. The phytoestrogen–ER complex (formed by the binding of phytoestrogens and the ER) may bind to EREs and inhibit or suppress specific gene expression (Anderson et al., 1999). More long-term studies are needed on bone density and fracture rates to determine safety, efficacy and appropriate dosage.

Cognition

Cognition and memory functioning have been reported to decrease around menopause, and therefore studies have investigated the association of ERT and cognition, as well as phytoestrogens and cognition (Vincent and Fitzpatrick, 2000). However, limited studies are available on the effects of phytoestrogens on cognitive functioning. A study with female rats proposed that soy phytoestrogens function as estrogen agonists because they increased choline acetyltransferase and nerve growth factor messenger RNA in the frontal cortex and hippocampus (Pan et al., 1999). Other human studies have also suggested improved memory with dietary phytoestrogens (File et al., 2001; File et al., 2002). Contradictory evidence has also been reported. One review paper summarizes two studies on tofu consumption in Hawaii that suggest increased cognitive dysfunction (Sirtori, 2001). The mechanisms are not understood, but it has been suggested that phytoestrogens act as estrogen agonists and may increase spine density and synapse formation in the hippocampus of adults (File et al., 2003). In addition, phytoestrogens may interact with the transcription of neurotrophin genes (File et al., 2003). More research is needed to understand the effect phytoestrogens might have on cognitive functioning.

BOTANICAL SOURCES STUDIED FOR PHYTOESTROGENS

Plant compounds can be limited to specific botanical families that may assist researchers in identifying other species that contain the same compounds. This concept is referred to as chemotaxonomy. For example, there is a higher concentration of phytoestrogens in legume plants even though they are also found in grains, vegetables and fruits distributed across the plant kingdom. The most common phytoestrogens found in legumes are isoflavones. Isoflavones are part of the isoflavonoids, which are almost exclusively limited in distribution to the Fabaceae family and more specifically to the subfamily Papilionaceae (Dewick, 1993). The other subfamilies in Fabaceae, Mimosaceae and Caesalpiniaceae, have shown very few plants with isoflavonoids. A few isolated cases of isoflavonoid derivatives have been reported in other plant families in the dicotyledons. Of the monocotyledons, species of Iris (Iridaceae) are a major source of isoflavonoids. Of the gymnosperm, the genera Juniperus and Podocarpus have been reported to produce isoflavonoids (Dewick, 1993).

Phylogenetic analysis uses chemical, molecular and traditional systematic data (such as plant anatomy) to suggest evolutionary relationships of plant taxa (Daly et al., 2001). This type of analysis provides a useful tool that can guide researchers in more effectively locating specific plant compounds because it elucidates chemotaxonomies (Daly et al., 2001).

Based on the phylogenetic tree of Daly and coworkers (2001), plants that have phytoestrogens tend to be concentrated in an upper evolutionary group that consists of the botanical orders, Fagales, Cucurbitales, Rosales, Fabales and Malpighiales. Cucurbitales contains Cucurbitaceae (pumpkin), Rosales contains Cannabaceae...
Table 1. Plants with estrogen receptor binding activity and activity in estrogen dependent breast cancer cell lines

<table>
<thead>
<tr>
<th>Phytoestrogen (Class)</th>
<th>Phytoestrogen (Flavone)</th>
<th>Phytoestrogen (Isolignan)</th>
<th>Phytoestrogen (Sterol or Isoflavone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Prenylnaringenin</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Genistein, daidzein, formononetin, biochanin A, glycitein</td>
<td>2′,4′,4′-three hydroxyl chalcone</td>
<td>Coumestrol (coumestan), apigenin (flavone), daidzein, genistein (isoflavone), secoisolariciresinol (lignan)</td>
<td>Coumestrol (coumestan), (isoflavone)</td>
</tr>
<tr>
<td>Not identified</td>
<td>Secoisolariciresinol (lignan)</td>
<td>Ginsenosides Rg1 (saponin)</td>
<td>Not identified</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Botanical name (Common name)</th>
<th>Phytoestrogen (Flavone)</th>
<th>Phytoestrogen (Isolignan)</th>
<th>Phytoestrogen (Sterol or Isoflavone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humulus lupulus (hops)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Maclura pomifera</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Morus microphylla</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Prunus africana syn. Pygeum africanum</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Senna obtusifolia syn. Cassia obtusifolia</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Glycine max (soy)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Glycrrhiza glabra (licorice)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Medicago sativa (alfalfa)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Phaseolus vulgaris (bean)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Pueraria lobata (kudzu)</td>
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<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Trifolium pratense (red clover)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Vigna radiata (mung bean)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Linum usitatissimum (flax)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Angelica sinensis (dong qai)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Eleutherococcus senticosus (Siberian ginseng)</td>
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<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Panax notoginseng (San-chi or Tien-chan ginseng)</td>
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<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Mandragora autumnalis (mandrake)</td>
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<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Leonurus cardiaca (motherwort)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Thymus vulgaris (thyme)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Verbena officinalis ( vervain)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>Vitex agnus-castus (chasteberry)</td>
<td>Not identified</td>
<td>Not identified</td>
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### Table 1. (Continued)

<table>
<thead>
<tr>
<th>Order</th>
<th>Family (Subfamily)</th>
<th>Botanical name (Common name)</th>
<th>Phytoestrogen (Class)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentianales</td>
<td>Rubiaceae</td>
<td><em>Coffea arabica</em> (coffee)</td>
<td>Matairesinol, secoisolariciresinol (lignan)</td>
<td>(Kitts, 1987; Mazur et al., 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Uncaria tomentosa</em> (cat’s claw)</td>
<td>Not identified</td>
<td>(Salazar and Jayme, 1998)</td>
</tr>
<tr>
<td>Caryophyllales</td>
<td>Polygonaceae</td>
<td><em>Fallopia multiflora syn. Polygonum multiflorum</em></td>
<td>Not identified</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Polygonum cuspidatum</em> (hu-chang)</td>
<td>Emodin, emodin 8-O-β-D-glucopyranoside (anthraquinone)</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rheum coreanum</em></td>
<td>(anthraquinone)</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rheum officinarum</em></td>
<td>(anthraquinone)</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rheum palmatum</em></td>
<td>(anthraquinone)</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rheum rhabarbarum</em></td>
<td>(anthraquinone)</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rheum tanguticum</em></td>
<td>(anthraquinone)</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rheum undulatum</em></td>
<td>(anthraquinone)</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rumex acetosella</em> (sheep sorrel)</td>
<td>Not identified</td>
<td>(Zava et al., 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rumex crispus</em> (yellow dock)</td>
<td>Not identified</td>
<td>(Zava et al., 1998)</td>
</tr>
<tr>
<td>Ranunculales</td>
<td>Papaveraceae</td>
<td><em>Sanguinaria canadensis</em> (bloodroot)</td>
<td>Not identified</td>
<td>(Zava et al., 1998)</td>
</tr>
<tr>
<td>Zingiberales</td>
<td>Zingiberaceae</td>
<td><em>Curcuma longa</em> (tumeric)</td>
<td>Not identified</td>
<td>(Zava et al., 1998)</td>
</tr>
<tr>
<td>Poales</td>
<td>Poaceae</td>
<td><em>Avena sativa</em> (oat)</td>
<td>Secoisolariciresinol (lignan)</td>
<td>(Uusitupa et al., 1992; Mazur, 1998)</td>
</tr>
<tr>
<td>Arecales</td>
<td>Areaceae</td>
<td><em>Serenoa repens</em> (saw palmetto)</td>
<td>Not identified</td>
<td>(Di Silverio et al., 1992)</td>
</tr>
<tr>
<td>Liliales</td>
<td>Liliaceae</td>
<td><em>Aloe arborescens</em></td>
<td>Not identified</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Aloe ferox</em></td>
<td>Not identified</td>
<td>(Matsuda et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Dracaena loureiro</em></td>
<td>5,7-Dihydroxy-3-(4-hydroxybenzyl)-4-chromanone (homoisoflavone), 4,4′-dihydroxy-2,5-diethoxydihydrochalcone (retrodihydrochalcone)</td>
<td>(Ichikawa et al., 1997)</td>
</tr>
<tr>
<td>Asparagales</td>
<td>Agavaceae</td>
<td><em>Yucca sp.</em></td>
<td>Not identified</td>
<td>(Zava et al., 1998)</td>
</tr>
<tr>
<td>Magnoliaceae</td>
<td>Annonaceae</td>
<td><em>Anaxagorea luzonensis</em></td>
<td>8-Isopentenylnaringenin (prenylflavonoid)</td>
<td>(Kitaoka et al., 1998)</td>
</tr>
</tbody>
</table>
(hops) and Fabales contains Fabaceae (soy, licorice, red clover) and Malpighiales (flax). Other plant species that have shown estrogenic activity are found in different orders located at basal positions from the initial group discussed: Myrtales (evening primrose), Apiales (dong quai, ginseng) and Lamiales (chasteberry). Although estrogenic activity has been reported for these plant species, specific phytoestrogens have not been identified. Chemotaxonomy offers an innovative way to select potential plant sources for further testing. For example, the phylogenetic relationships suggest that research should be conducted in plant families found within the initial group discussed above. Although the majority of studies on phytoestrogens have been conducted on soy, other botanical species have also been included in this discussion.

**Soy**

Soy or soybean (*Glycine max*) belongs to the Fabaceae family and has long been used as a food plant. Of the bean plants it has one of the highest levels of protein and oil (Duke, 1981). Medicinally it has been reported in ancient Chinese herbals for the healthy functioning of the heart, kidneys, liver and stomach (Duke and Ayensu, 1985). It was domesticated in China around the eleventh century BC and there are many varieties. The Chinese distinguish the different varieties by color. Black seeds are associated with medicine and have been used for strength and vigor as well as in mixtures for post-partum and sexual disorders (Li, 1973). The black bean sprouts have been used as a laxative, for rheumatism and hair growth. A 'bean relish' made of salted fermented beans has been highly valued in Chinese medicine and has multiple uses including colds, headaches, hemorrhaging in abortion, threatened abortion, difficult labor, irritability and fever (Li, 1973). In the 1940s, genestin, the genistein glycoside, was first reported in soybean oil meal and was later shown to have estrogenic activity in mice (Cheng et al., 1953). Soy has been well studied for phytoestrogens and numerous articles have been published on the benefits and risks of soy over the last few years, therefore we will present some of the more recent research.

It is unclear whether soy protein with trace amounts of isoflavones (phytoestrogen-extracted soy protein), phytoestrogen-intact soy protein, or a combination of both causes the beneficial cholesterol effects seen in animal studies (Van der Schouw et al., 2000). Consumption of soy protein has shown a decrease in lipid peroxidation compared with casein consumption in postmenopausal cynomolgus monkeys and lowered atherosclerosis in rabbits (Wagner et al., 1997; Van der Schouw et al., 2000). Isoflavone-intact soy protein has lowered LDL and raised high density lipids (HDL) cholesterol suggesting that the active components are found in the extractable protein portion (Clarkson and Anthony, 1998). When acetycholine was administered to rhesus monkeys fed intact isoflavones, their arteries dilated while the group fed isoflavones extracted from soy constricted them (Honoré et al., 1997). These results are supported by another study with rhesus female monkeys that observed that a diet of soy with intact isoflavones sustained normal coronary blood flow after an intracoronary infusion of collagen possibly due to platelet aggregation being inhibited or because platelets release vasoconstrictors (Williams and Clarkson, 1998). Two studies showed that a diet of soy protein inhibited atherosclerotic plaque formation as opposed to animal protein, yet more research is needed to determine if the benefits are mediated by soy protein or phytoestrogens (Van der Schouw et al., 2000). Further investigations are needed to elucidate the effects of phytoestrogen dietary supplements, which are often isolated from soy and then encapsulated and sold in tablets or capsules.

A meta-analysis of 38 studies reported that a diet containing an average of 47 g soy protein decreased total cholesterol by roughly 9.3%, LDL cholesterol by 12.9%, and triglycerides by 10.5% and increased high-density lipoprotein (HDL) cholesterol by 2% (Anderson et al., 1995). Another review reported that several human studies found evidence that a diet of soy protein reduces LDL (Wroblewski Lissin and Cooke, 2000). In a randomized clinical trial conducted for 9 weeks with hypercholesterolemic men and women, increased isoflavone doses (25 mg, 42 mg or 58 mg) in soy protein showed a lower LDL and total cholesterol concentration suggesting a dose-response relationship (Crouse et al., 1998). A placebo-controlled crossover trial with menopausal and perimenopausal women showed improved systemic arterial compliance with the daily consumption of soy iso flavone supplements (Nestel et al., 1997). However, four small studies showed no statistically significant association between a soy isoflavone diet and an increase in HDL cholesterol for pre-menopausal women; and the effects on LDL cholesterol were conflicting (Cassidy et al., 1995). Further findings showed that isoflavone pills improved arterial compliance but did not show effects on plasma lipids (Clarkson and Anthony, 1998).

A reduction of the relative risk of breast cancer in premenopausal women who consume a soy rich diet has been reported (Ingram et al., 1997) while others indicate a moderate and non-significant reduction of this risk (Petakis et al., 1996; Hargreaves et al., 1999). Kurzer (2002) reviewed hormonal effects, including concentrations of reproductive hormones in the blood, menstrual cycle lengths and menstrual phase lengths, of soy in premenopausal women and reported that lowered risk of breast cancer is often associated with a longer menstrual cycle, reduced estrogens, increased sex-hormone binding globulin and increased excretion of 2 to 16 α-hydroxy estrogens. Time exposure has also been proposed to explain the benefits of soy. Limited evidence exists to suggest that soy consumption in adult life is protective against breast cancer, however, high levels of consumption (Peeters et al., 2003) or consumption throughout life (Adlercreutz, 2002) could potentially reduce the risk of breast cancer.

A detailed discussion of soy for women who have survived breast cancer has been presented (Messina and Loprinzi, 2001). The avoidance of soy for breast cancer patients has been reported in one study that showed increased breast nipple aspirate fluid (NAF) secretion and breast cell hyperplasia in postmenopausal women on ERT who consumed daily 80 mg of soy isoflavones over 5 months (Petakis et al., 1996). A drawback of this study is the lack of a control group and fluid secretion continued to increase in the patients even after they ceased their consumption of soy. Presently soy
isoflavones have not been reported to show significant effects on endometrial tissue (Vincent and Fitzpatrick, 2000; Balk et al., 2002).

Several studies have reviewed the use of soy for HRT (Burke et al., 2000; Kronenberg and Fugh-Berman, 2002). A double-blind parallel multi-center randomized placebo controlled trial of 104 postmenopausal women who took 60 g of isolated soy protein daily (76 mg of isoflavones) versus placebo reported that the mean number of daily hot flushes were significantly reduced in the group consuming soy ($p < 0.01$) (Albertazzi et al., 1998). Other studies have also shown positive effects with soy extracts (Scambia et al., 2000; Faure et al., 2002; Han et al., 2002). However, negative and mixed results of soy for menopausal symptoms have also been reported (Quella et al., 2000; St. Germain et al., 2001). These studies often differ by soy product, dose, length of study and subjects therefore making it difficult to compare the results. More studies are needed to elucidate the effects and safety of soy on women with a history of breast cancer and osteoporosis.

**Black cohosh**

Black cohosh (*Actaea racemosa, Cimicifuga racemosa*) is a North American plant in the buttercup family (*Ranunculaceae*) that grows in Eastern North America, from southern Maine to Georgia (Ramsey, 1997). Black cohosh, also known as bugbane, black snakeroot, bugbane and rattlesnake is used for various women's health conditions. Native Americans used the roots and rhizomes for a variety of indications such as stimulation of menstrual flow, dysmenorrhea, suppression of cough, treatment of diarrhea, childbirth and rheumatism (Foster, 1999). The 19th century American Eclectic physicians recognized black cohosh to be 'very efficacious in maladies of the female reproductive organs' (King and Newton, 1852).

Knowledge of this useful herb soon reached Europe, where it has been a popular herbal medicine for menopausal symptoms for over 50 years. A standardized black cohosh extract (Remifemin™) was developed in Germany, and this product has been studied both in animals and short-term clinical trials of menopausal women. According to these studies the extract appears to provide relief for hot flashes (Jarry et al., 1985; Stoll, 1987).

A historical study using the Allen-Doisy bioassay did not detect estrogenic activity in black cohosh, which was part of the Lydia E. Pinkham mixture (Costello and Lynn, 1950). More recent studies reported that formononetin was detected in a methanol extract of a black cohosh sample that previously bound to an ER (Jarry and Hamischfeger, 1985; Jarry et al., 1985), although this is surprising based on chemotaxonomy. Others have reported detecting additional flavonoids, such as kaempferol (Schmitz, 1993) and biochanin A-4-methyl ether (McCoy and Kelly, 1996). The authors speculated that black cohosh may work through estrogen-like compounds (Jarry et al., 1985), but to date this has not been established. Others have looked for and not found formononetin in black cohosh extracts (Struck et al., 1997; Kennelly et al., 2002).

In vitro data on black cohosh in breast cancer cell lines is limited and the case is being made in both the scientific and popular press that the biological activity of black cohosh is not estrogenic, but rather, through some SERM or non-steroidal compound that has clinical effects similar to some of the activities of estrogen. Inconsistent results are reported for *in vitro* studies using black cohosh extracts and estrogen receptor binding activity and stimulation of breast cancer cells (Jarry and Hamischfeger, 1985; Jarry et al., 1985; Dükert et al., 1991; Nesselhut et al., 1993; Hamischfeger and Cillien, 1996; Zava et al., 1998; Dixon-Shanies and Shaikh, 1999). Fukinolic acid, a black cohosh constituent, showed increased cell proliferation of an estrogen dependent MCF-7 cell system with reference to estradiol (Kruse et al., 1999). Another study tested estrogenic and anti-estrogenic effects of black cohosh extracts on proliferation of MCF-7 cells and on gene expression using ethanol and isopropanol extracts and concluded that black cohosh contains antiestrogenic compounds because the extract antagonized estradiol induced activities in the different experiments (Zierau et al., 2002). Another recent investigation found that black cohosh extract did not display significant competitive binding to ERα nor ERβ (Liu et al., 2001a). Furthermore, in this same study black cohosh did not show estrogen activity, as indicated by alkaline phosphatase (AP) activity with cultured Ishikawa (endometrial) cells. In addition, black cohosh extracts do not have any estrogenic activity in a number of assays where ER-positive breast cancer cells were used (personal communication, Dr Ruth Lupu).

Kronenberg and Fugh-Berman (2002) reviewed randomized, controlled trials of Remifemin™ for menopausal symptoms conducted in Germany and the United States. Three of the four trials report black cohosh helpful for hot flashes, although more research is needed to examine long-term effects. A recent study reported beneficial effects of combined administration of tamoxifen and black cohosh extract (CR BNO 1055) for hot flushes in women surviving breast cancer (Hernández Muñoz and Pluchino, 2003). A double-blind placebo-controlled study with postmenopausal women demonstrated that CR BNO 1055 was comparable to conjugated estrogens in treating climacteric complaints and maintaining bone metabolism, and did not increase endometrial thickness (Wuttke et al., 2003c).

Investigators propose that black cohosh contains unidentified compounds that produce estrogenic effects (Kronenberg and Fugh-Berman, 2002; Wuttke et al., 2003c). An additional hypothesis is that the compounds in black cohosh interact with a third estrogen receptor, ERγ yet to be identified in humans but recently reported in teleost fish (Jarry et al., 2003). Metabolic conversion of precursor molecules, serotonin receptor blocking activity and dopaminergic compounds have also been suggested as potential mechanisms of action for black cohosh (Jarry et al., 2003). Currently, black cohosh is in a clinical trial at Columbia University and the University of Heidelberg for the treatment of menopausal hot flashes, as well as assessment of cognitive function and bone metabolism.

Most studies have reported that black cohosh extract is free of significant side effects. However, the length of these studies is not sufficient to insure safety with respect to uterine tissue and function. Further studies are needed to elucidate the mechanism of action of black cohosh. In addition, studies are needed to determine...
whether users of black cohosh may benefit from decreased risk of fractures and cardioprotective effects, as purported of traditional ERT, as well as to clarify whether black cohosh extract stimulates breast cancer cells in vivo, in vitro and in women.

Red clover

Red clover (Trifolium pratense) in the Fabaceae family is a herb that is indigenous to Europe and parts of the Middle East and has naturalized to North America (Mabberley, 1997). It is well known as animal fodder. Humans have rarely consumed red clover, although it has been used medicinally. In the beginning of the twentieth century a ‘Trifolium compound’ that included red clover blossoms along with other botanicals was marketed by pharmaceutical companies for venereal disease, although there was little evidence to support this use (Foster and Tyler, 1999). Traditionally it has been reported by Native American Iroquis as a gynecological aid for ‘the change of life’ (Herrick, 1995). The Celts and Romans employed red clover as a sedative. It has also been used to purify blood, treat skin conditions and for bronchial asthma because it reduces muscle spasm and is a decongestant (Keville, 1999).

The estrogenic activity of red clover was initially recorded in 1950 (Legg, 1950). Later the estrogenic compounds were identified as formononetin, biochanin A, daidzein and genistein (Mazur, 1998). Coumestrol has been reported in clover but the species was not specified (Franke et al., 1995). Although in vitro data on red clover are limited, studies do support the plant’s estrogenic activity. Red clover extracts tested in four in vitro assays showed consistent estrogenic effects with the highest binding potency for both ERs compared with other plant extracts tested (Liu et al., 2001b). Genistein appeared as the most active component in AP induction ability and up-regulation of the progesterone receptor (PR) expression in Ishikawa cells and there was no evidence of antiestrogenic activity. This study supports the results of earlier work using the radioreceptor assay that showed red clover extracts bound to the ER of MCF-7 cells and to the PR of T47-D cell lines (Zava et al., 1998). Two preparations of Menoflavon®, a red clover extract, showed a higher binding affinity to ERβ than ERα in a transactivation assay (Dornstauder et al., 2001).

The in vivo estrogenic and antiestrogenic effects of red clover extract have been studied in the uterus, vaginal cells and mammary glands of ovariectomized Sprague-Dawley rats (Burdette et al., 2002). Uterine weight and thickness were increased with the extract, although less than increases observed with E2 and there was no evidence of antagonizing effects on E2. Red clover extract did induce partial cornification of vaginal cells at high doses after 2 weeks. Lastly, no estrogenic or antiestrogenic effects of red clover extract were observed on the mammary gland (Burdette et al., 2002).

Several human studies have evaluated red clover for a variety of health conditions such as breast cancer, cardiovascular disease and menopausal symptoms. Rimostil™, a red clover extract, has significantly increased HDL cholesterol in serum at different doses (28.5, 57 and 85.5 mg/day) and lowered levels of HDL cholesterol in serum after stopping the treatment; however, there was no control group in this study (Clifton-Bligh et al., 2001). A double-blind placebo-controlled trial with postmenopausal women consuming isoflavones daily derived from red clover reported improved arterial compliance, although the study design has been questioned as well as the high dropout rate (Nestel et al., 1999). On the other hand, a randomized double-blind study using purified isoflavones, predominately formononetin and biochanin A from red clover, did not significantly change the total plasma cholesterol, LDL cholesterol, HDL cholesterol or plasma triglyceride levels in postmenopausal women with slightly elevated plasma cholesterol levels (Howes et al., 2000).

The effects of red clover on endometrial cancer have also been studied. A double-blind randomized, controlled study using purified isoflavones (50 mg daily) from red clover did not show reduced cell proliferation in the endometrium of 30 women ranging in age from 45 to 50 based on the detection of a Ki-67 antigen in endometrial biopsy specimens (Hale et al., 2001). Small sample size, the type of isoflavone treatment and the limited timing of examination in the menstrual cycle may have affected the results.

Red clover has been reviewed for menopausal symptoms (Fugh-Berman and Kronenberg, 2001; Kronenberg and Fugh-Berman, 2002). A red clover extract, Promensil™, contains isoflavones in the aglycone form and was used in a randomized, double-blind, placebo-controlled trial with 30 menopausal women. Significant differences between control and placebo group were observed at week 8 and 12 with the control group showing reduced hot flush count (Van der Weijer and Barentsen, 2002).

One note of caution about red clover is the potential presence of coumarins that have been reported in some clover species, often in damaged plant tissue. These compounds act as anticoagulants and therefore should be evaluated in red clover extracts prior to consumption (Fugh-Berman and Kronenberg, 2001). Additional studies are needed to further elucidate the benefits or adverse effects of red clover as a source of phytoestrogens.

Flax

Flax (Linum usitatissimum) in the Linaceae family is an herb that is considered one of the oldest continuously cultivated plants. The Latin name means ‘most useful’ (Haggerty, 1999). As a fiber, it is well-known in both tropical and temperate regions and was found in the Egyptian tombs. Several European Pharmacopoeias have included it as a medicinal plant (Grieve, 1985).

The seeds, also known as linseed, are medicinal and have been used to make flour; they produce mucilage when infused or boiled in water. Flax has been used for colds, coughs and fevers, to reduce inflammation and as a demulcent. Native American Cherokee used flax for fevers and ‘violent colds, coughs and diseases of lungs’ (Hamel and Chiltoskey, 1975) and the oil has been used as a laxative (Haggerty, 1999). In the Middle Ages the flowers were used for protection against sorcery (Grieve, 1985). Flax has been attractive as an oil seed because it contains polyunsaturated fatty acids such
as α-linolenic acid, which may lower cholesterol and have antioxidant effects for health.

Flaxseed is considered one of the richest sources of lignan phytoestrogens (Thompson et al., 1991), although a recent study has reported Norway spruce as another rich source (Saarinen et al., 2000). Several studies have developed techniques to identify and quantify phytoestrogens in flaxseed (Obermeyer et al., 1995; Muir and Westcott, 2000; Charlet et al., 2002), yet limited clinical studies have been conducted on the phytoestrogens in flax and their role in human health.

In rats flaxseed consumption has resulted in development of reproductive changes (Tou et al., 1998), changes in estrous cycle (Orcheson et al., 1998) and menstrual cycle (Phipps et al., 1993). One in vivo study with nude mice fed a diet supplemented with 10% flaxseed showed a decrease in tumor growth rate and metastasis as well as lowered extracellular levels of vascular endothelial growth factor (Dabrosin et al., 2002). Another study testing spermatogenesis and testis structure in Sprague-Dawley rats showed no effect on maternal ingestion of flaxseed during lactation and gestation followed by postnatal consumption of the same diet as the mother (Sprando et al., 2000).

In a randomized, crossover trial of two 3-week periods with defatted flaxseed lignans there was a reduction in LDL cholesterol, total cholesterol, and apolipoprotein B and A-1 and no significant effect on HDL cholesterol. However, consumption of flaxseed did reduce protein thiols compared with the controls, which may suggest increased oxidative activity (Jenkins et al., 1999). A 12 week study of 145 women with climacteric complaints showed a reduction in menopausal symptoms (including hot flush and vaginal dryness) with the consumption of a diet rich in flaxseed (Hamel and Chiltoskey, 1975). Menstrual disturbances were frequently reported as lowered extracellular levels of vascular endothelial growth factor (Dabrosin et al., 2002). Another compound in licorice root to double activity in skeletal and cardiovascular tissue (Tamir et al., 1998), yet limited clinical studies have been conducted on the phytoestrogens in flax and their role in human health.

Licorice

The licorice (Glycyrrhiza glabra) plant is a perennial, belonging to the Fabaceae family and indigenous to Eurasia. In Greek Glycyrrhiza means ‘sweet root’ (Wang and Nixon, 2001). The sweet yellow wood of the licorice root has been consumed for thousands of years in China for its health benefits and detoxification effects as well as its use as a flavoring and sweetening agent (Foster and Tyler, 1999; Wang and Nixon, 2001). The earliest recorded medicinal use is most likely around 2100 BC (Gibson, 1978). Medicinally it has been used as a demulcent and expectorant and has been shown to have antioxidant and antimicrobial activity. In the United States, licorice is added to tobacco as well as candies, toothpaste and beverages (Wang and Nixon, 2001). Other medicinal uses are as a tonic, to quench thirst, for asthma, fever and externally for burns and sores (Li, 1973; Gibson, 1978). Although it is not indigenous to North America, reports indicate that it was purchased by Native Americans and used for female troubles (Smith, 1928) as well as for coughs, asthma and as an expectorant (Hamel and Chiltoskey, 1975). The main components of licorice are glycyrrhizin (glycyrrhizic acid), which is sweeter than sugar, and glycyrrhetinic acid. Both have been clinically used in the treatment of hyperlipidemia, allergic inflammation, atopic dermatitis and atherosclerosis (Tamir et al., 2001).

The estrogenic activity of licorice was first published in 1950 (Costello and Lynn, 1950). Licorice has shown antiprogestin activity, which may be mediated by other compounds because purified glycyrrhizin did not bind to ER or PR (Zava et al., 1998). However, in another study licorice extracts showed weak binding affinity to both the ERs as well as weak stimulation of PR expression (20 µg/mL) (Liu et al., 2001b). Glabrene, glabridin, and isoliquiritigenin, 2',4',4'-three hydroxyl chalcone (ILC) found in the ethanol extract of licorice root show estrogenic activity (Tamir et al., 2001). Glabrene, an isoflavone, has been reported as a new class of phytoestrogens and glabridin is a novel phytoestrogen that is lipophilic and structurally similar to E2 (Tamir et al., 2001). It has been isolated from licorice extract and appears to have ER mediated estrogen effects. Glabridin binds to human ER and in vitro showed biphasic effects on estrogen-dependent human breast cancer cells and increasing concentrations showed biphasic effects on the growth of anchorage-independent growth MCF-7 cells (Tamir et al., 2000). Another compound in licorice, licochalcone-A, has shown estrogenic activity with ERs and induced apoptosis in MCF-7 and HL-60 cell lines (Rafii et al., 2000).

Additional in vivo results demonstrated the ability of licorice root to double activity in skeletal and cardiovascular tissue (Tamir et al., 2000) and its effect on reducing testosterone production in rats (Moyad, 2002). Daily consumption of 7 g of a licorice dietary supplement (0.5 g of glycyrrhizic acid) in seven men significantly reduced serum testosterone within 4 days (Armanini et al., 1999). Health risks such as hypermineralocorticoidism involving potassium loss and sodium retention, edema and increased blood pressure have been associated with the human consumption of glycyrrhizinic acid and need to be considered when administering licorice (Stormer et al., 1993; Wang and Nixon, 2001). More research is needed to evaluate the benefits and adverse effects of licorice as a source of phytoestrogens.

Hops

Hops (Humulus lupulus), a perennial climbing vine in the Cannabaceae family, has been extensively cultivated for its bitter properties found in the female flowers used in beer and medicine. Medicinally hops have been valued as a sedative, for inflammation and as a tonic (Foster and Tyler, 1999). The Native Americans and the Cherokee used the plant for inflamed kidneys, as a sedative, for pain relief and for breast and female complaints where the womb was debilitated (Hamel and Chiltoskey, 1975). Menstrual disturbances were frequently observed in women hops pickers and their estrogenic activity was associated with the plant (Verzile, 1986). The following compounds have been identified as estrogenic: 8-prenyltaxilin, 6-prenyltaxilin,
xanthohumol and isoxanthohumol. The female flowers of hops are considered estrogenic. The estrogenic activity of hops is variable between 0 and 300 μg E2 equivalents/g, which may be due to the physiological state of the plant or environmental effects (Verdeal and Ryan, 1979). This difference should be kept in mind when evaluating studies based on plant extracts versus isolated, purified compounds. The most potent phytoestrogen in hops is 8-prenylaragenin, which is found in beer in low quantities (18 ng/mL) (Milligan et al., 1999). Isoflavones have also been reported in beer, most notably formononetin, although they are thought to be from barley seeds before brewing (Lapcík et al., 1998). Other studies have tested the presence of hops in dietary supplements used for breast enhancement (Coldham and Sauer, 2001). Further testing is needed in vivo and in humans to evaluate adequately the potential estrogenic activity of these hop-based dietary supplements.

Hops extract in vitro did show significant binding affinities to both ERα and ERβ, estrogenic activity with AP induction, and up-regulated PR expression in Ishikawa cells, however, it showed strong cytotoxicity (Liu et al., 2001b). Hops extract has also acted as a growth inhibitor and showed significant antiproliferation effects in serum stimulated T-47D cells at 0.1% and 0.01% concentration (Dixon-Shanies and Shaikh, 1999) and stimulated T-47D and MCF-7 cells in depleted serum at 0.2% (Zava et al., 1998). In various bioassays, 8-prenylaragenin has shown estrogenic potency (Milligan et al., 2000; Coldham and Sauer, 2001). However, hops showed little uterotrophic activity in prepubertal and adult ox mouse bioassays (Coldham and Sauer, 2001). Presently, there is limited scientific evidence concerning the estrogenic activity and health benefits of hops.

**Dong quai**

Dong quai (*Angelica sinensis*) is an herb that belongs to the Apiaceae family and has been used extensively in traditional Chinese medicine for many years (Mei et al., 1991). Dong quai has been referred to as the ‘female ginseng’ and is used for a variety of conditions such as a blood tonic and decongestant for body organs (Hardy, 2000). The root is used for women as a tonic often in combination with other herbs. Other women’s conditions treated with dong quai are dysmenorrhea, irregular menstruation, anemia, constipation and abdominal pain (Zhu, 1987).

In vitro dong quai has acted as a growth inhibitor with breast cancer cell lines (Zava et al., 1998; Dixon-Shanies and Shaikh, 1999) but has also been observed to stimulate the growth of MCF-7 cells (Amato et al., 2002). Dong quai has shown weak binding affinity for ERα and ERβ and weak stimulation of PR expression (Liu et al., 2001b).

A randomized, controlled trial using a combination of 60 mg soy isoflavones, 100 mg dong quai, 50 mg black cohosh taken by women who suffered from menstrual migraines reduced the frequency of attacks, and their severity after 1 month of initiation (Burke et al., 2002). A double-blind randomized placebo controlled clinical trial with dong quai did not significantly reduce hot flushes or endometrium thickening in postmenopausal women over a 24-week period (Hirata et al., 1997).

Further studies are needed to evaluate the active compounds and estrogenic effects of dong quai. Polysaccharides identified in dong quai may cause photosensitization and therefore health risks should also be considered (Hann et al., 1991).

**Other botanical sources**

Other botanical sources tested for their estrogenic effects and potential health benefits are: evening primrose oil (*Oenothera biennis*, Onagraceae); chasteberry (*Vitex agnus-castus*, Verbenaceae); alfalfa (*Medicago sativa*, Fabaceae); and ginseng (*Panax spp.*, *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., Araliaceae) (Glazier and Bowman, 2001; Liu et al., 2001b; Amato et al., 2002; Kronenberg and Fugh-Berman, 2002). Coffee (*Coffea arabica L.*, Rubiaceae) has also shown weak estrogenic activity (Kitts, 1987). Other plants that have shown ER binding activity and activity in estrogen dependent breast cancer cells are presented in Table 1 using phylogenetic relationships based on Daly and coworkers, 2001.

Evening primrose is a common herb in North America that has been used medicinally by the Native Americans (Grieve, 1985). A randomized controlled trial of oral gamolenic acid from evening primrose oil on hot flushes showed no significant benefits over the placebo group (Chenoy et al., 1994). For centuries the dried ripe fruit of chasteberry tree has been used medicinally for its beneficial effects on female reproduction and to decrease sexual desire, specifically of monks, hence the name (Hardy, 2000). Chasteberry extract has shown growth inhibition with T-47D breast cells (Dixon-Shanies and Shaikh, 1999) and estrogenic activity based on in vitro assays (Liu et al., 2001b). Several other studies have evaluated chasteberry for premenstrual syndrome with beneficial effects (Berger et al., 2000; Loch et al., 2000).

Alfalfa is a perennial herb that has a limited history of medicinal use although it is a common forage plant. A homeopathic physician Alexander L. Blackwood made the observation that if alfalfa fattened cows it might work in the same way with humans and it was purported by homeopathic and eclectic physicians to ‘increase the appetite and the flesh’ (Foster and Tyler, 1999). Coumestrol was the first phytoestrogen identified in alfalfa (Bickoff et al., 1957) and is considered one of the richest food sources for this phytoestrogen (Kurzer and Xu, 1997). Alfalfa did not show affinity to ER or PR binding (Zava et al., 1998) and therefore may act through a different mechanism. More studies are needed on serum levels of phytoestrogens after alfalfa consumption to elucidate the effects of metabolism on estrogenic activity (Hwang et al., 2001).

Ginseng has a long history of medicinal use in Asia as a tonic and stimulant. There are several different plants that are referred to as ginseng and all are in the Araliaceae family: *Panax ginseng* C.A. Mey. (Chinese or Korean ginseng), *P. quinquefolium* L. (American ginseng) and *Eleutherococcus senticosus* (Siberian ginseng). Case studies have shown postmenopausal vaginal bleeding with *Panax* sp. intake, which may suggest weak estrogenic effects (Punnonen and Lukola, 1980), although a randomized, double-blind, placebo-controlled
multicenter trial of postmenopausal women consuming Ginsana® (100 mg of standardized ginseng extract from Panax ginseng) did not show significant differences in menopausal symptoms between groups (Wiklund et al., 1999).

Historically, Bradbury and White as well as Farnsworth and coworkers compiled extensive data about plant species with estrogenic activity and compounds. Some of these studies have been further confirmed with more advanced laboratory techniques. As techniques continue to be refined, they will provide researchers with higher resolution to detect phytoestrogenic activity and compounds. These methods coupled with phylogenetic analysis can offer effective ways to search for new sources of phytoestrogens in the plant kingdom.

ADVERSE EFFECTS OF PHYTOESTROGENS

The mechanisms and potencies of phytoestrogens are not completely clarified and they may be considered potential endocrine disrupters, and therefore caution should be exercised when taking them. Some concerns have been discussed about the risks associated with phytoestrogens such as increased plasma concentration of isoflavones in babies that ingest soymilk, the ability of non-hormonal secondary plant metabolites to modify sex steroid metabolism, and the effects of phytoestrogens on thyroid (Ibarreta et al., 1999). In addition, the genetic toxicity potential of phytoestrogens has recently been reviewed (Kulling et al., 2002). As discussed earlier, sheep consuming large amounts of clover showed infertility and reproductive disorders (Bennetts et al., 1946; Adams, 1995). Cheetahs in captivity also had reduced fertility rates when consuming a feline diet composed of a soybean product, which was reversed when it was removed from the diet (Setchell et al., 1987). Toxicities associated with herbal medicines that include phytoestrogens have also been presented in the literature (Sheehan, 1998).

Several articles have discussed the potential risks involved with soy-based infant formulas (Murphy et al., 1997; Fitzpatrick, 1998; Setchell et al., 1998; Zung et al., 2001). Some negative effects of phytoestrogens may be the result of receiving high levels of isoflavones during perinatal development (Setchell et al., 1998). Studies by Setchell et al. (1997) showed that infants fed soy-based formula have high concentrations of daidzein and genistein in their plasma, 13 000 to 22 000 times higher than E$_2$ in early life and proportionately higher than normal adult intake of isoflavones (Setchell et al., 1997). This has raised concern about the health benefits and long-term effects that phytoestrogens may have on developing and mature neuronal function and the interaction of phytoestrogens with E$_2$ during perinatal development of the brain (Belcher and Zsarnovszky, 2001). Postnatal rats did show estrogenic activity, including induced permanent estrus with a 40 mg/kg dose of isoflavones, while no effects were observed at the 4 mg/kg dose which is predicted of infants fed soy formula (Lewis et al., 2003). Some propose that health protection may be provided for infants that consume soy-based formulas (Murphy et al., 1997). Zung and collaborators (2001) reviewed studies on soy-based formulas in relation to growth rate and concluded that there is no significant effect on growth, although with premature infants there are conflicting data. More studies conducted on infants who consume soy-based formulas are needed to further understand the beneficial and adverse effects of phytoestrogens.

As potential endocrine disrupters, phytoestrogens may act as antiestrogens and harm the reproductive health of males (Sharpe and Skakkebaek, 1993; Santti et al., 1998). Reduced sperm quality, undescended testes and urogenital tract abnormalities were increased in the sons of mothers taking DES compared with those who did not take the miscarriage preventative drug (Sheehan, 1998). Animal studies conducted with DES resulted in male genital abnormalities during development, including cysts, testicular lesions and lack of growth of the seminal vesicles and therefore concern has been raised about the effects of phytoestrogens on male development (Santti et al., 1998). High doses of genistein are shown to alter pituitary responsiveness and basal luteinizing hormone (LH) secretion in castrated postpubertal rats. Coumestrol consumption by rat pups suppressed testicular testosterone concentrations and resulted in abnormal sexual behavior in adulthood (Whitten and Naftolin, 1992). Studies in cultured human lymphoblastoid cells reported that coumestrol was mutagenic and clastogenic (Domon et al., 2001). Recent work has shown that prenatal exposure to phytoestrogens may interact with platelet-derived growth factor during testis development (Thullier et al., 2003). However, Kurzer (2002) reviewed studies on adult men that showed no adverse effects on sperm quality with the consumption of soy isoflavones. A recent study evaluating the long-term reproductive effects of genistein (0–10 mg/kg per day) during gestation and lactation in mice showed no significant effect on sperm count, the number of motile sperm, or sperm mobility nor was there any effect on testicular gene expression (Fielden et al., 2003). In adolescent boys, isoflavone supplementation showed no significant effect on bone turnover and growth (Jones et al., 2003). Additional studies are needed to further evaluate the risks of phytoestrogen consumption in male development.

Concern has been associated with thymic weight and effects on the immune system. Ovariectomized adult mice injected with genistein produced dose-responsive decreases in thymic weight of up to 80% (Yellayi et al., 2002). Other concerns related to phytoestrogens are their effect on thyroid hormone, insulin and glucagon (Ohno et al., 1993). Hypothyroid cases were associated with infants fed soybean diets (Fort et al., 1990). Thyroid hormone concentrations were observed in ovariectomized ewes fed red clover silage which showed changes in the area of the thyroid follicles and significantly higher concentrations of total T$_4$ and free T$_3$ in plasma, however, no differences were observed between the total T$_4$ and free T$_3$. These results suggest that men and women with thyroid disorders should use caution when consuming phytoestrogens (Madej et al., 2002).

In the United States, regulation is limited on standardization, preparation and extraction methods of the phytoestrogen products being sold and marketed as nutritional supplements. As nutritional supplements these products are not supported by clinical trials and therefore should be administered and taken with this in mind (This et al., 2001).
CONCLUSIONS

Research in phytoestrogens has increased dramatically in the past several years as seen by the numerous publications. However, many questions remain. Research is still needed to evaluate the safety of phytoestrogens on human systems, beneficial and harmful doses, gender differences in response to phytoestrogens, differences in the chemical classes of phytoestrogens and the effects phytoestrogens may have with other drugs or dietary products. Due to the functional and structural differences of phytoestrogens, their biological activities are also highly variable and there may be other effects that have not yet been studied.

Phytoestrogens are common in the human diet and able to exert many biological effects that have been observed in cell, animal and human systems. The majority of these effects are seen as beneficial to our health, although contradictory effects have been shown and further research is needed to confirm these findings. Estrogenic compounds and their activities are complex and often species specific. The complexity of phytoestrogens suggests that interpretations need to be made with caution.

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