Phytopharmacology

Inforesights Publishing

Molecular and clinical role of phytoestrogens as anti-skin-ageing agents: A critical overview

Bancha Yingngam, Wandee Rungseevijitprapa*

Department of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand

*Corresponding Author: wandeeim@yahoo.com; Tel.: +66 45 353615; Fax. +66 45 353626

Received: 24rd May 2012, **Revised**: 7 June 2012, **Accepted**: 7 June 2012

Abstract

Accumulated evidence from epidemiological, clinical, and laboratory studies have supported the important role of estrogens on skin physiology. Estrogen therapy has been demonstrated as a therapy for the signs of ageing related to estrogen deficiency in menopausal women. Unlike cosmetic products, estrogens cannot be used as therapy for skin-ageing in all women due to their potencies and ability to produce undesirable side-effects. Therefore, the use of phytoestrogens as natural product remedies to alleviate skin-ageing is more common. Current literature suggests that phytoestrogens offer improvement and delay signs of skin-ageing similar to the effects of estrogens. Moreover, the lack of side-effects when using phytoestrogens points towards their safety. This review article summarizes the evidence for the potential use of phytoestrogens as an alternative method for the alleviation or prevention of the signs of skin-ageing related to estrogen loss after menopause. The classification, mechanism of action, and pharmacology by which phytoestrogens can cause changes in skin-ageing are also discussed. Additionally, gaps in information about phytoestrogens and the most promising future research are mentioned.

Keywords: Phytoestrogens; Ageing; Skin; Molecular mechanism; Benefits; Risks

Introduction

Skin is the most superficial part of the human body. Like other organs, ageing of the skin affects its structural and functional properties, and the psychology of humans. Thus, the causes of ageing and appropriate treatment and preservation approaches have attracted the interest of the already aged, young people, and cosmetic researchers. Several theories of skin ageing have been proposed. Depletion of estrogen levels (estradiol, estriol, and estrone) in blood circulation was found to be one of the major factors that influence skin ageing (Polito et al., 2012; Pontius and Smith, 2011). In normal skin, estrogens exert their effects through estrogen receptors present in the skin (Hall and Phillips, 2005). Estrogens maintain skin moisture by preserving the acid mucopolysaccharide and the hyaluronic acid (Sobel et al., 1965).

They also influence normal cellular function of keratinocytes and fibroblasts located in the epidermis and dermis layers respectively (Stevenson and Thornton, 2007). Changes in the properties of skin are more visible as humans age (Fiedler et al., 2012) and estrogen receptors in the skin decrease after menopause (Kuiper et al., 1997). These changes include increased skin dryness, followed by decreased skin elasticity, and increased skin looseness (Falla and Zhang, 2010; Sator et al., 2004; Stevenson and Thornton, 2007). In addition, Prázný et al. (2007) found that blood flow velocity to skin decreased when estrogen levels decreased in menopausal women. On the contrary, signs of skin-ageing in post-menopausal women were found to improve when using estrogen replacement therapy. This also significantly increased type III collagen as well as the number of collagen fibers at the end of the treatment period (Schmidt et al., 1996). However, the use of topical or oral estrogens for anti-skin-ageing signs must be administered by a dermatologist experienced in endocrinology to avoid their side-effects. Furthermore, applications of estrogens for anti-skin-ageing may increase the risk of breast cancer and vascular diseases (Beral et al, 2011; Rossouw et al., 2002). Pritchard (2001) estimated that estrogen therapy increased the risk of breast cancer by 1-2% per year. Such findings have led to the search for approaches involving natural product remedies that promote skin rejuvenation without the increased risk of estrogen-sensitive tumors.

Phytoestrogens are plant-derived compounds which have biological activities that mimic the effects of estrogen and are used as alternative agents in estrogen therapy (Malaivijitnond, 2012). They have been the subject of intensive investigation, as shown by Figure 1 indicating the increase in publications related to phytoestrogens in PubMed from 1990 until the end of 2011. Phytoestrogens have several other biological mechanisms of action, including anti-oxidative activity (Lee et al., 2012; Liu et al., 2011), anti-ultraviolet irradiation induced skin damage (Kitagawa et al., 2010; Widyarini, 2006), and anti-melanogenesis (Lin et al., 2011). These biological properties may be applicable in the treatment of anti-skin-ageing. Some literature demonstrated that phytoestrogens play a role in anti-skin-ageing *in vivo* (Accorsi-Neto et al., 2009; Circosta et al., 2006; Izumi et al., 2007;Moraes et al., 2009; Polito et al., 2012;Yingngam, 2011). In addition, soy isoflavonoids, a sub-group of phytoestrogens, have been reported to enhance the level of vascularization in the skin of post-menopausal women (Moraes et al., 2009).

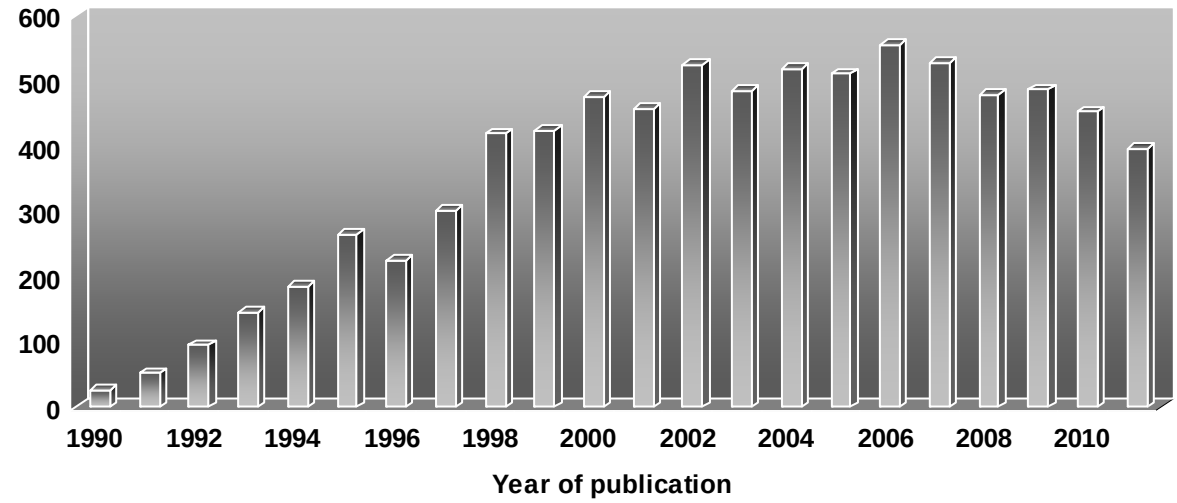

Figure 1. Annual number of PubMed-listed publications for phytoestrogens as a function of year

Table 1. Some botanical sources of phytoestrogens.

Botanical name	Family	Phytoestrogen	Reference
Anaxagorea luzonensis A. Gray	Annonaceae	8-isopentenynaringenin	Kitaoka et al., 1998
Asparagus racemosus Wild	Asparagaceae	Kaempferol	Saxena et al., 2010
Avena sativa Linn.	Poaceae	Secoisolariciresinol	Smeds et al., 2009
Brassica oleracea Linn.	Brassicaceae	Secoisolariciresinol	Milder et al., 2005
Curcuma comosa Roxb.	Zingiberacease	Diaryheptanoids	Su et al., 2011
Glycine max (L.) Merr.	Leguminosae	Genistein daidzein, formononetin, bioclanin A, glycitein	Yingngm et al., 2011
Glycyrrhiza glabra Linn.	Leguminosae	Glycyrrhizin	Dong et al., 2007
Humulus lupulus Linn.	Cannabaceae	8-Prenylnaringenin	Milligan et al., 2000
Linum usitatissimum Linn.	Linaceae	Secoisolariciresinol	Yingngam et al., 2011
Pueraria candollei Grah. ex Benth. var. *mirifica*	Leguminosae	Miroestrol, deoxymiroestrol, isomiroestrol, coumestrol, genistein, daidzein, puerarin	Boonsnongcheep et al., 2010
Pueraria lobata (Willd.) Ohwi	Leguminosae	Flavonoids, isoflavonoids	Cherdshewasart and Sutjit, 2008
Sophora japonica Linn.	Leguminosae	Genistein, genistin, rutin, kaempferol, quercetin	Chu et al., 2005
Trifolium pretense Linn.	Leguminosae	Isoflavones (genistein, daidzein, biochanin A, formononetin)	Occhiuto et al., 2007
Vitis vinifera Linn.	Vitaceae	Stilbenes (resveratrol)	Patisaul and Jefferson, 2010

Although phytoestrogen-based cosmetic products are largely believed to be beneficial in the maintenance of the youthful appearance of aged skin, there is a lack of supportive scientific data. This review article focuses on the roles of phytoestrogens that can alleviate the changes due to ageing that occur in menopausal women's skin. Overviews of phytoestrogens, the molecular mechanism of action, potential benefits and risks of phytoestrogens in skin-ageing therapy, and safety are presented. Additionally, gaps that exist in information about phytoestrogens and the most promising future research are mentioned.

Phytoestrogens

Phytoestrogens are a sub-class of polyphenols derived from plants. Their chemical structures are similar to estrogens in that they have a pair of hydroxyl group and a phenolic ring. This property is required for binding to human estrogen receptors and enables them to exhibit both estrogenic and anti-estrogenic effects on metabolism (De-Eknamkul et al., 2011). To the present, a variety of phyto-constituents responsible for estrogenic activities have been identified from various plants (Table 1). Based on their chemical structures, they can be classified into six distinct categories: chromenes, coumestans, diarylheptanoids, flavonoids, lignans, and stilbenes. The chemical structures of 17β-estradiol and some phytoestrogens are shown in Figure 2.

Chromenes are found in the roots of *Pueraria candollei* Grah. ex Benth. which belongs to the family Fabaceae. These plants consist of two varieties, viz. *P. candollei* var. *candollei* and *P. candollei* var. *mirifica*. The tuber of these plants has been traditionally used for rejuvenation in aged women and men (Malaivijitnond, 2012). Three types of chromenes have

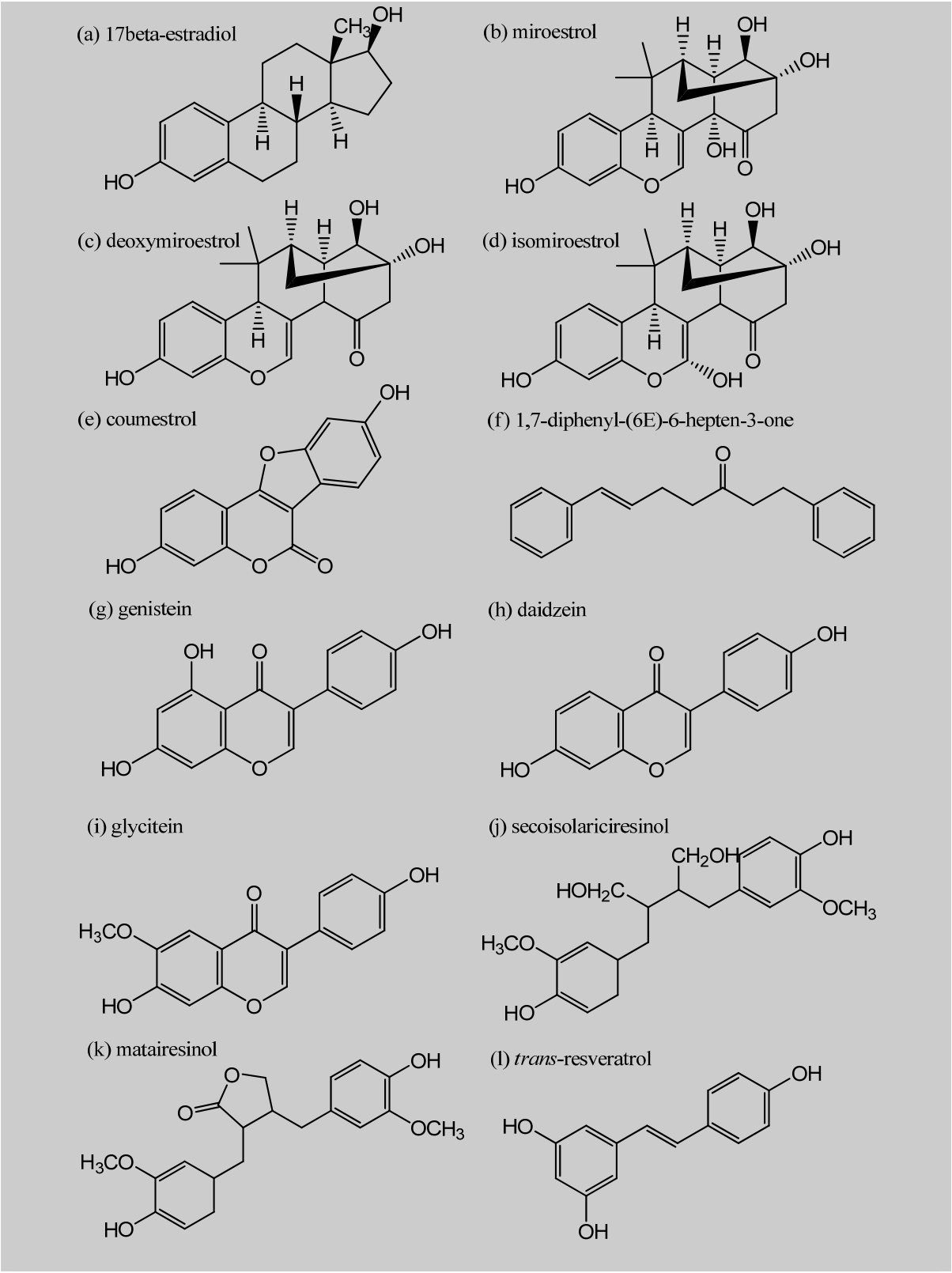

Figure 2. Chemical structures of (a) 17β-estradiol, (b) miroestrol, (c) deoxymiroestrol, (d) isomiroestrol, (e) coumestrol, (f) 1,7-diphenyl-(6*E*)-6-hepten-3-one, (g) genistein, (h) daidzein, (i) glycitein, (j) secoisolariciresinol, (k) matairesinol, and (l) *trans*-resveratrol

been identified in these plants, miroestrol, deoxymiroestrol, and isomiroestrol. The deoxymiroestrol can be converted to miroestrol and isomiroesterol when it is exposed to the oxygen in the air during extraction and storage (Chansakaow, 2000). Thus, both miroestrol and isomiroestrol are in fact artifacts. A previous study reported that the contents of chrome-nes in such plants were very low (Yusakul et al., 2011), but the estrogenic activity of miroestrol and its derivatives was potent, much more so than that of other isoflavonoids (Matsum-ura et al., 2005).

The main source of coumestans is legumes such as *Vigna radiata* sprouts, *Trifolium pratense* sprouts, *Mesicago sativa* sprouts, *P. candollei* var. *mirifica* tuber*, and P. candollei* var. *candollei* tuber (Malaivijitnond, 2012; Yingngam, 2011). Coumestrol has been shown to have high potency in estrogenic activity, exhibiting more potent activators for estrogen receptors signaling pathway than that of genistein and daidzein (Matsumura et al., 2005).

Diarylheptanoids consist of two aromatic rings linked by a linear seven carbon aliphatic chain. Su et al. (2011) stated that a high content of diaryheptanoids can be found in a rhizome of *Curcuma comosa* Roxb. This herb belongs to family Zingiberaceae and has long been used as folk medicine in Thailand for the treatment of symptoms related to estrogen deficiency in menopausal women, such as pre-menopausal bleeding. The haxanic extract of *C. comosa* rhizome has been scientifically reported to have estrogen-like effects (Piyachaturawat et al., 1995; Winuthayanon et al., 2009b). The major compounds of this herb which exert estrogenic effects are diarylheptanoids (3*S*)-1,7-diphenyl-(6*E*)-6-hepten-3-ol, 1,7-diph-enyl-(6*E*)-6-hepten-3-one, and (3*R*-) 1,7-diphenyl-(4*E*,6*E*)-4,6-heptadien-3-ol (Su et al., 2011; Suksamrarn et al., 2008). Winuthayanon et al. (2009a) reported that these diarylheptanoids induced transcription through a ligand-dependent human estrogen receptor α-estrog-en receptor element-driven pathway.

Flavonoids are the largest group of the investigated phytoestrogens. Based on the number of published papers, the sub-group 'isoflavonoids' is of most interest to cosmetic researchers. The major types of isoflavonoids are found in glucoside conjugates which are biological inactive. The plant which contains high content of these isoflavonoids is *Glycine max* (L.) Merr. Four types of isoflavonoids have been widely studied, biochanin A, daidzein, genistein, and glycitein. Setchell (2001) found that these isoflavonoids showed short half life in the human body and were excreted from the body within 24 hours after ingestion. As a result, administration of them via a dermal route may be an alternative for the provision of sufficient phytoestrogens in the skin. Other identified phytoestrogens are the substances belonging to flavonols (quercetin, kaempferol, myricetin), flavanones (catechin), and flavo-nes (apigenin, luteolin).

Lignans are substances that possess a 2,3-dibenzylbutane structure. They are found as minor constituents of some plants, such as whole grains, legumes, vegetables, and seeds (Velentzis et al., 2009). The seeds of *Linum usitatissimum* Linn. are the richest identified source of lignans, for example malairesinol and seco-isolariciresinol (Sacco et al., 2011).

Finally, stilbenes are chemicals that are synthesized in plants in response to stress or microbial infection. The best-known stilbene is resveratrol, present in two genometric isomers, *cis*- and *trans*-forms. It has been reported that a rich source of resveratrol is *Polygonum*

cuspidatum Sieb. & Zucc. root (Family Polygonaceae) (Kimura and Okuda, 2001) and *Vitis vinefera* Linn. seed (Family Vitidaceae). Other stilbens are piceatannol which was first isolated from seeds of *Euphorbia lagascae* (Ashikawa et al., 2002).

Possible mechanisms of action of phytoestrogens on skin

The pharmacological activities by which phytoestrogens can rejuvenate aged skin include genomic estrogen receptor pathway, non-genomic estrogen receptor pathway, and other mechanisms. More information is needed to clarify how these mechanisms work.

1) Genomic estrogen receptor pathway

In this pathway, the mechanism of action of phytoestrogens involves interaction with estrogen receptors. The complex reaction between phytoestrogens and estrogen receptors can recruit co-activators to form a transcription complex and protein that results in a change in cell function. Studies have reported that several parts of skin contain estrogen receptors (ER-α and ER-β). These parts include keratinocytes, fibroblasts, sebaceous glands, hair follicles, and blood vessels (Schmidt et al., 1990). Both estrogen receptor sub-types are different in the C-terminal domain and in the N-terminal transactivation domain (Morito et al., 2001). The estrogen receptors also depend on an anatomical site that presents a high density of estrogen receptors, such as facial skin, pelvic skin, and breast skin (Schmidt et al., 1990). Due to their low molecular weights and structural similarity to 17β-estradiol, phytoestrogens can pass through cell membranes to bind to both sub-types of estrogen receptors. Phytoestrogens have key structural elements enabling them to bind with estrogen receptors. These include (i) the phenolic ring that is indispensable for binding to estrogen receptors, (ii) the optimal hydroxylation pattern, and (iii) a distance between two hydroxyl groups at the isoflavone structure similar to that occurring in estradiol (Setchell, 1998).

Genistein is a typical example of estrogen receptor modulator. At molecular level, genistein is involved in hydrogen bonding with Arg394, Glu353 and His524 of ER-α, at a distance of 2.899 °A, 2.570 °A, and 2.531 °A (Figure 3). Polar surface area is present at both terminal ends of the estrogen receptor alpha, which is attributed to the presence of electrostatic nature of polar amino acid side chains (Figure 4). Apart from terminal ends, the central core of estrogen receptor is comparatively hydrophobic and non polar in nature, which favors the estrogen like steroidal molecules and aromatic rings.

Kuiper at al (1998) suggested that the estrogenic activity of phytoestrogens depend on their affinity for estrogen receptors. This modulates the transcription of target genes in a variety of organs. Some phytoestrogens appear to preferentially bind to ER-β more than ER-α. For example, Kuiper et al. (1998) found that the relative binding affinities of genistein and daidzein to 17β-estradiol for ER-α were 0.7 and 0.2%. On the contrary, affinities for ER-β were reported at approximately 13% and 1% for genistein and daidzein. Genistein and daidzein also showed estrogenic properties in breast cancer cells (an ER positive cells) at low concentrations (<10 μmol/L) while its inhibitory effect was found at higher concentrations (> 10 μmol/L) (Yingngam, 2011). It should be noted that most phytoestrogens show weak estrogenic activity compared with 17β-estradiol because 17β-estradiol has a lipophilic property thought to influence receptor-binding while genistein and daizein have lower lipophilic

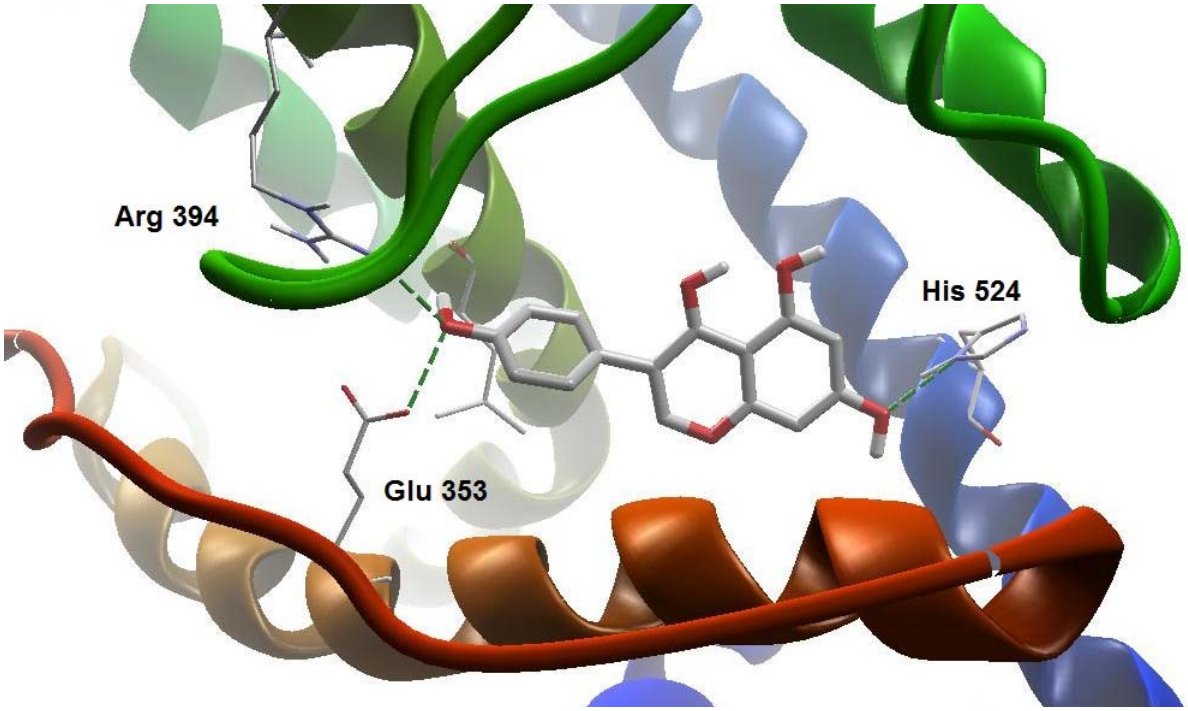

Figure 3. Molecular binding mode of Genistein inside active site of estrogen receptor alpha. Green dotted lines represent hydrogen bonds between the receptor and ligand.

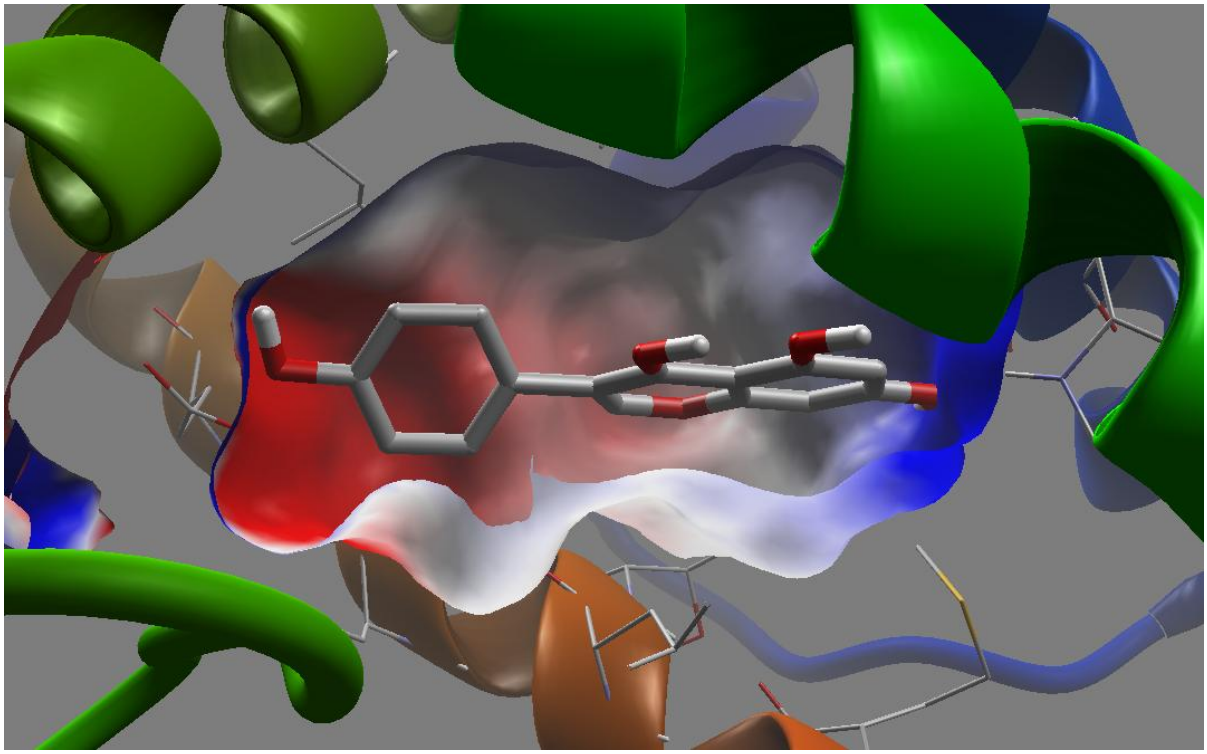

Figure 4. Molecular interactions of Genistein inside active site of estrogen receptor alpha. Red, blue and gree represents aggregated negatively charged surface and aggregated positively charged surface area respectively. White color represents hydrophobic surface area of the receptor.

property. However, genistein and daizein may exert estrogenic effect greater than 17β-estradiol when they are added into cosmetic formulations at high concentrations. However, high concentration of phytoestrogens such as genistein induced down-regulation of ER-α mRNA and protein level in human breast cancer cells (Maggiolini et al., 2001). Furthermore, phytoestrogens exhibit estrogenic activities which differ in their pharmacological activities due to different structures. As a result, the pharmacological activities of phytoestrogens on skin-ageing are influenced by several factors. These include type and concentration of phytoestrogens, dosage form of administration, target tissue, number and type of estrogen receptors, and presence or absence of endogenous estrogen level.

2) Non-genomic estrogen receptor pathway

Some phytoestrogens have demonstrated that they exert their estrogenic activity via estrogen receptors associated with the cell surface membrane (Jeng et al., 2009). The interaction between phytoestrogens and estrogen receptors at the cell surface membrane can be coupled to cytosolic signals transduction proteins and the signals are able to initiate different signaling cascades via secondary messengers (Jeng et al., 2009). This mechanism is called a 'non-genomic estrogen receptor pathway' which Levin (2002) found to have much faster effects than the genomic estrogen receptor pathway. For example, coumestrol which has been shown to have high relative estrogenic potency for nuclear actions can initiate signaling through a membrane such as activation of extracellular regulated kinase, prolactin release, and changes in Ca^{2+} fluxes (Bulayeva and Watson, 2004; Wozniak et al., 2005). However, the sub-cellular localization and the role of phytoestrogens via a non-genomic estrogen receptor pathway are not fully understood.

3) Other pathways

As reported elsewhere, mechanisms of action of phytoestrogens to reverse skin-ageing is not only dependent on genomic estrogen pathway and non-genomic pathway. Many other mechanisms have been reported including antioxidant activities and inhibitory effects on several enzymes of the estrogen receptor-independent signal pathway. In this case, it is well known that the matrix-degrading metalloproteinase can degrade collagen, elastin, and other proteins in the connective tissue. To support this mechanism of action, Kim et al. (2004) published the anti-photo-ageing effects of soy isoflavonoid administrated by oral route in hairless mouse induced by ultraviolet. The female hairless mice were subjected to soy isoflavonoid extract and irradiated with ultraviolet-light for 4 weeks. The researchers reported that isoflavonoid showed anti-ageing effects by the suppression of the ultraviolet-induced metaloproteinase-1 expression. Moreover, the collagen content was higher in the mice treated with isoflavonoids compared with the control group. It was suggested that these effects were attributed to isoflavonoid inhibiting the transcription of genes for metalloproteinase (transcription factor activation protein 1 and the nuclear factor-kappa B). Furthermore, dermal application of resveratrol at a concentration of 25 μmole/0.2 ml acetone/mouse to SKH-1 hairless mice resulted in significant inhibition of ultraviolet B–induced skin edema and cyclooxygenase activity (Afag et al., 2003). The study of Polito et al (2012) reported that treatment of the ovariectomized rats with 1 mg/kg genistein via a subcutaneous route significantly increased the thickness of collagen and increased the transforming growth factor-β1, vascular endothelial growth factor, matrix metalloproteinase-2, matrix metalloproteinase-9, tissue inhibitor of

metalloproteinase-1, and 2. In addition, free radicals in skin cells may create abnormal crosslikes of collagens and result in to sags and wrinkles. These effects also cause the skin to be stiff and lose the ability to retain water (Sander et al., 2002). The isoflavonoids have been reported to have the ability to protect oxidative stress induced skin-ageing (Kang et al., 2003). This may be attributed to the presence of hydroxyl groups at position 4' and 5' and the presence of the aromatic ring to scavenge free radicals.

Benefits of phytoestrogens on skin-ageing

Phytoestrogens and skin-ageing have become the focus of much study over the last few years. The efficacies of phytoestrogen-based anti-skin-ageing products are still contriversial due to most products not being tested in controlled clinical trials. A few studies have shown that phytoestrogens displayed the ability to improve and restore skin function and appearance both in animals and human volunteers through improved skin hydration and elasticity, improved skin coloration, and anti-inflammatory qualities. Some of the phytoestrogenic extracts with potential anti-skin-ageing properties are enlisted in Table 2.

Table 1. Summary of phytoestrogens and their effects on skin-ageing oestrogens.

Phytoestrogen treatment (Length of treatment)	Dosage (sample size)	Results (References)	Side-effect	Limitations
Human studies				
Concentrated *G. max* extract (6 months)	100 mg/day of concentrated, isoflavones-rich soy extract, dietary (N = 30, post-menopausal women)	Significant increases in epithelial thickness, number of elastic and collagen fibers, and blood vessels compared with before treatment. (Accorsi-Neto et al., 2009)	Not reported in the study	Small sample size, did not measure biomarkers, and no control group. The goal of the study was to evaluate efficacy and there were no data reported on side-effects of the treatment
Concentrated *G. max* extract (24 weeks)	Gel containing concentrated soy extract (4% Genistein, N = 40 post-menopausal women, isoflavone group 20, estrogen group 20)	Significant increases in epidermal thickness (20%) and dermal capillary vessel (36%), no significant gain in number of dermal papillae and dermal fibroblasts. (Moraes et al., 2009)	One subject in isoflavone group was excluded from analysis because of a cutaneous rash. No increase in the Frost index of vaginal cytology during all visits. No change in hormonal vaginal cytologies at 3 months and at the end of 6 months in comparison to the baseline.	Small sample size, at the 24 weeks follow-up the study had N = 18 in isoflavone group and N = 18 in estrogen group.
Animal studies				
Genistein (12 weeks)	1 and 10 mg·kg^{-1} s.c. daily	Significant increases in TGF-β1, VEGF,	Not reported in the study	-

	(N = 12, ovariectomized rats)	MMP-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP-1) and TIMP-2 in the ovariectomized rats (Polito et al., 2012)		
Concentrated *P. candollei* var. *mirifica* extract (4 weeks)	2mg/cm^2/rat/twice a day of cream containing 1%w/v *P. candollei* var. *mirifica* extract (N = 5, ovariectomized rats)	Significant increases in skin hydration and skin elasticity (Yingngam, 2011)	Systemic adverse effect was observed in the study by gain of the uterine weight and changes of vaginal cornification	Did not measure biomarkers
Concentrated *Aspergillus oryzae*-fermented *G. max* extract (12 weeks)	2 mg/cm^2/rat once a day of Gel containing 1%w/v concentrated *A. oryzae*-fermented *G. max* extract ((N = 5, ovariectomized rats)	Significant increases in skin hydration and skin elasticity (Yingngam, 2011)	No adverse effects	Did not measure biomarkers
Isolflavonoid extract (14% isoflavonoids) from *T. pretense* (14 weeks)	20 and 40 mg of total isoflavonoids, oral treatment (N = 5, ovariectomized rats)	Improved thickness and keratinization of epidermis, number of gland and vascularity, collagen bundles and elastic fibers (Circosta et al., 2006)	Not reported in the study	Did not measure biomarkers

1) Skin hydration and elasticity

Regarding the improvement of skin hydration, lipids in stratum corneum and dermal glycosaminoglycans are the major factors that play an important role in the retention of water in the skin. Dry skin is commonly observed as women age. The results obtained from the authors' studies found that dermal administration of phytoestrogenic molecules derived from 1 %w/v *P. candollei* var. *mirifica* extract and 1 %w/v *Aspergillus oryzae* fermented-*G max* extract at a concentration of 200 μg/9 cm^2 increased the dermal water retention capacity in ovariectomized rats. In addition, the skin elasticity of the ovariectomized rats also improved following treatment by these phytoestrogenic extracts as measured by a Cutometer® at 4 weeks and 12 weeks respectively (Yingngam, 2011).

Circosta et al. (2006) examined the effects of isoflavonoids from *T. pratense* on skin changes induced by the loss of estrogen in ovariectomized rats. These researchers reported that the thickness of the skin and the keratinization of the epidermis were reduced following ovariectomy. On the contrary, treatment of the ovariectomized rats with 20 mg and 40 mg isoflavones daily via an oral route for 14 weeks restored the epidermis to uniform thickness. The phytoestrogens also significantly increased collagen and elastic fibers, and vascularity was improved. This evidence supports the potential benefit of phytoestrogens in the reduction of skin-ageing. Although the molecular mechanism of action of phytoestrogens was not investigated by Circosta et al. (2006), it may be suggested that molecular mechanisms are involved in the production of transforming growth factor-β1 that plays a role in the increase of the collagen synthesis similar to the effect of 17β-estradiol (Creidi et al., 1994). A few researchers studied the influence of phytoestrogens on human skin cells and reported that

phytoestrogens derived from *G. max* extract induced a level of type I pro-collagen production and a number of fibroblast cells (Varani et al., 2004).

An evaluation of Novadiol® (a cosmetic cream containing isoflavonoids) was conducted using a controlled open European multicenter design. The evaluation involved 234 women who were assigned to apply this cream containing 0.0075% isoflavonoids in the morning and a concentration of 0.015% isoflavonoids in the evening on their face, neck, and one upper arm for 12 weeks. The other arm served as a control. The evaluation found that skin dryness and roughness of treated skin significantly improved by 32.9% and 21.9% compared to untreated skin. Moreover, facial wrinkles and skin looseness were reduced by 22% and 23.7% respectively (Bayerl and Keil, 2002). These effects were believed to be the result of isoflavonoids in this formulation improving a proliferation of epidermis and improve collagen synthesis.

In the study of Accorsi-Neto et al. (2009), the oral intake of isoflavonoid extract derived from *G. max* seed in post-menopausal women (N = 30) for 6 months improved the thickness of the epidermis by 9.46% compared to before treatment. Furthermore, the content of collagen, elastic fibers, and dermal blood vessels significantly improved. The mechanisms of action that phytoestrogens played an important role in were probably related to a complex array of cellular factors independent of the genomic estrogen receptor pathway. The results of this study were in agreement with those reported by Moraes et al (2009) that the topical application of gel containing isoflavonoid extract (4% genistein) from *G. max* seeds improved the epidermal thickness by 20% in post-menopausal women (N = 18). However, Moraes et al. (2009) showed contrasting results compared with Accorsi-Neto et al. (2009) that showed no significant changes in collagen fibers and dermal blood vessels. This may have been attributed to the fact that the topical application of isoflavonoids in conventional gel was not sufficient to induce a strong estrogenic action.

A double-blind, placebo-controlled study by Izumi et al. (2007) investigated the effects of dietary soy isoflavones in middle-aged women. The volunteers received 40 mg isoflavones per day for 12 weeks (n = 13). The test group showed statistically significant improvement in their wrinkles and malar skin elasticity without adverse symptoms during the study period. The limitation of this study was that the researchers did not elucidate the mechanism of action of isoflavones. However, possible mechanism should be a role of isoflavones through the improvement of hyaluronic acid synthesis.

2) Skin coloration effect

The regulation of pigmentation synthesis of the skin is partly influenced by estrogens. These hormones were shown to modulate skin proliferation and melanogenesis in normal human skin culture (McLeod et al., 1994). The production of melanin in ovariectomized guinea pigs increased following treatment with estrogens (Snell and Bischitz, 1960). In the case of phytoestrogens, their effects have been investigated in aged menopausal cynomolgus monkeys. The results showed that the oral administration of phytoestrogens derived from *P. candollei* var. *mirifica* powder exhibited estrogenic action by an increase in reddish sexual skin coloration within 24 hours. The researchers suggested these effects occurred through the binding to estrogen receptors. However, the reddish skin returned to a pale color in the seco-

Table 3. Some anti-skin-ageing products containing phytoestrogenic extract currently on the market.

Product	Active ingredient	Manufacturer
Aveeno® cream	*G. max* extract	Johnson & Johnson consumer company
Emerita-phytoestrogen® cream	*T. pratense* extract, *Cimicifuga racemosa* extract, *V. vinifera* extract and *Panax ginseng* extract	Emerita company
L'Oreal hip® cream	*L. usitatissimum* extract	L'Oréal company
Rojukiss protox day cream SPF 50 PA+++	*Pueraria lobata* extract	Aisance Co., Ltd
Joyce® breast cream	*P. candollie* var. *mirifica* extract	Loxley Public Com., Ltd

nd half of the study period. This may be attributed to a high concentration of phytoestrogens in blood circulation leading to an apparent down-regulation of estrogen receptors and reduced responsiveness (Trisomboon et al., 2006). In addition, it was shown that some phytoestrogens such as resveratrol could inhibit melanin synthesis in B16F10 mouse melanoma cells. This result suggested that resveratrol should be used as a new skin lightening product.

3) Anti-inflammatory effect

Apart from skin-ageing caused by estrogen deficiency, the topical application of genistein could protect human skin from erythema caused by ultraviolet radiation (Huang et al., 2008). The mechanism of action of this extract was partly due to the inhibition of the metalloproteinase expression (Kim et al., 2004).

Example of phytoestrogens used for skin-ageing

There has been a recent development in cosmetics and nutraceuticals. Anti-skin-ageing products containing phytoestrogens are promising because of the perceived safety of their natural origins to consumers. The most popular phytoestrogen-based anti-skin-ageing products are listed in Table 3. The extracts obtained from *G. max* seed, *P. candollei* var. *mirifica* tuber, and *P. lobata* tuber seem to more popular compositions for a wide variety of skin care products. It is noted that plant extracts are more popular in the cosmetic industry than pure phytoestrogenic compounds because they contain several phyto-constituents which may exert synergistic action of activities as well as being lower in costs of production compared to pure phytoestrogens. Applications of phytoestrogens to the skin has some advantages, including delivering phytoestrogens continuously, fewer adverse effects, and avoiding hepatic first pass effect. However, these phytoestrogens should be formulated appr-opriately by improving their penetration of viable epidermis and dermis, but with reduced systemic absorption. Yingngam (2011) investigated the effects of formulation kinds on efficacies of *P. candollei* var. *mirifica* extract and found that the delivery of 1%w/v of such plant extract in niosomes could improve skin penetration of phytoestrogenic markers in porcine skin *in vitro* more than that of conventional dosage form. Moreover, the skin elasticity of the ovariectomized rats improved rapidly within four weeks.

Potential risks of phytoestrogens

The above mentioned data showed that phytoestrogens have potential benefits regarding skin-ageing. However, some studies suggested that these agents could promote the growth of stimulatory effects in human breast adenocarcinoma cells and the growth is concentration dependent (Seo et al., 2006; van der Woude et al., 2005; Yingngam et al., 2011). Their estrogenic effects were observed at low concentrations of phytoestrogens while higher concentrations seemed to have anti-estrogenic effects. These results suggested that consumers who had a history of breast cancer should avoid using anti-skin-ageing products containing phytoestrogens due to the possibility that these products may stimulate the growth of existing tumors. Only a few studies suggested that phytoestrogens may increase the risk of hormone-dependent cancers (Zheng et al., 1999). In the authors; unpublished study, the dermal application of *P. candollei* var. *mirifica* extract (a potent Thai phytoestrogenic plant) showed a systemic effect when tested in the ovariectomized rats. The ovariectomized rats treated with 1%w/v *P. candollei* var. *mirifica* extract at a dose of 200 μg/rat showed a gain in relative weight of uterine and a change in the induction of vaginal epithelial cornification. Thus, the optimal doses of this plant extract to obtain anti-skin-ageing effects should be further investigated to avoid undesired side-effects before use.

However, epidermiologic data suggested that women with high phytoestrogen consumption had a decreased risk of breast cancer (Duffy et al., 2007). For example, Sakamoto et al. (2010) suggested that resveratrol correlated with a reduced risk of breast cancer due to its anti-tumor activity. Moreover, a recent meta-analysis study conducted in randomized controled trials reported that the use of phytoestrogens in women was not associated with the rates of hormone-related adverse effects such as breast and endometrial cancers, and the rates of vaginal bleeding or endometrial hyperplasia did not increase among phytoestrogen-users (Tempfer et al., 2009). Thus, the risks and benefits of the application of phytoestrogens as active ingredients in anti-skin-ageing products remain controversial. More epidermiological studies are required about the benefits and risks of phytoestrogens in carcinogenesis.

For skin irritation, a study involving women using a 24-hour patch test demonstrated that isoflavonoids (genistein and daidzein derived from *G. max*) caused negligible skin irritation in human volunteers (Huang et al., 2008). In the authors' unpublished data, gel containing 1%w/v *Aspergillus oryzae* TISTR 3018-fermented *G. max* extract did not cause any irritation in New Zealand White rabbits. This result was in agreement with data obtained from the effect of cream containing 1%w/v *P. candollei* var. *mirifica* extract. However, Moraes et al (2009) reported that one subject had a cutaneous rash following the application of gel containing concentrated *G. max* extract among 18 post-menopausal women. The limitation of this study was that the cutaneous rash that occurred was not identified as a result of the use of phytoestrogens or gel components. Based on the available evidence, phytoestrogens seem to be safe with respect to skin irritation.

Conclusions and perspectives

Phytoestrogens are plant-derived molecules which structurally resemble estrogens. Six main classes of phytoestrogens are identified, chromenes, coumestans, diarylheptanoids, lignans, flavonoids, and stilbenes. The beneficial effects of phytoestrogens on skin are curre-

ntly understudied and years of research may be required to establish their effects. Some animal studies and a smaller number of clinical trials in humans showed the positive effects of dermal and oral administrations of phytoestogens appear to be an effective therapy for skin-ageing but with lower efficacy than estrogens. These effects included the enhancement of skin hydration, improvement of vascularity function, and maintenance of skin elasticity. Mechanically, it was suggested that at least three pathways are involved, genomic, non-genomic, and other pathways. Phytoestrogens can exert both estrogenic and anti-estrogenic effects depending on circulating levels of endogenous estrogens. The combination of multiple phytoestrogens in plant extracts may offer the potentially synergistic action in treating skin-ageing. Overall, current evidence suggests the potential benefits of phytoestrogens for anti-skin-ageing with few risks. However, it should be kept in mind that phytoestrogens may promote the growth of estrogen-dependent tumors. Thus, the anti-skin-ageing products containing phytoestrogens should be avoided when there is a risk of cancer. Prevention and improvement of signs of skin-ageing via topical application of cosmetic products or nutraceuticals containing phytoestrogens may provide an important alternative therapy. More research is required to establish the risks, benefits, and mechanism of the actions of phytoestrogens.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

Acknowledgments

The authors thank Bob Tremayne of the Division of International Relations at Ubon Ratchathani University for proof-reading the manuscript.

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