Isoflavones, Phytohormones and Phytosterols

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Summary

A review of isoflavones, plant derived hormones and sterols in the care of the skin. The effects have been shown to include protection of the skin, the reduction of wrinkles, reduction in inflammation and a decrease in pruritis. In some commercial raw materials, there has been improvement in breast and skin firmness and reduction in sun burn cell reaction. Internally, these plants are used for reducing the incidence of hot flushes and other discomforts in menopausal women.

Riassunto

Gli isoflavoni, derivati ormonali delle piante, e gli steroli, sono frequentemente utilizzati per la cura della pelle. Di questi composti sono noti gli effetti topici quali sostanze protettive nei confronti degli inquinanti ambientali o quali sostanze attive nel ridurre il numero e la profondità delle rughe o efficaci nel mitigare i processi infiammatori e il prurito.

Dato il loro uso sempre più frequente nei disturbi della menopausa, utilizzati anche per via sistemi­ca, viene descritta e riportata la formulazione chimica, il meccanismo d’azione e i principi attivi contenuti nelle diverse tipologie di estratti vegetali già noti.
Isoflavones have the phenyl group attached to the 3-position, whereas in flavones the phenyl group is attached to the 2-position. The isoflavones are mainly found to occur within the Leguminosae (specifically in the sub-family Papilionoideae), although the literature shows many other species that contain these chemical moieties [Boland and Donnelly]. Isoflavones are also found in other botanical families such as the Compositae, the Iridaceae, the Myristicaceae, and the Rosaceae.

These isoflavones can act as steroidal mimics by filling the stereochemical space that could be occupied by oestrogenic compounds. It is this spacial chemistry that helps explain the effects of many nutritional herbal supplements and topical preparations.

Daidzein is a phyto-estrogen, but is also called a phenolic estrogen, to distinguish it from a steroidal estrogen like 17β-estradiol. The activity of phytoestrogen is much weaker than the steroidal estrogen, varying from 0.005-2% [Brand]. The estrogenic properties are insufficient in strength to replace steroidal estrogens, but they do have significant value when it comes to reducing the effects of ageing and improving the quality of the skin.

Phyto-oestrogens may also be viewed in relation to the phytochemical division of terpenoids, which comprise the largest group of natural plant products. All terpenoids are derived biogenetically from isoprene. The largest group of terpenoids are the triterpenoids, which include, amongst other divisions, the triterpenoid and steroid saponins, and, the phytosterols. The phyto-oestrogens fall into these three categories. In addition, nature has a rich portfolio of phytosterols. It is easy to understand why sterols like stigmasterol (Fig.3) and β-sitosterol (Fig.4) have an effect that is anti-inflammatory and capable of reducing swelling and erythema, when their structure is compared to corticosterone (Fig.5) and hydrocortisone (Fig.6).

The paper concludes with a look at phytohormones and compares them to synthetic hormones and explains their effects against this known background.
The most commonly occurring isoflavones are:
- Biochanin-A 5,7-dihydroxy-4'-methoxyisoflavone
- Daidzein 4',7-dihydroxyisoflavone
- (±)-Equol 4',7-isoflavandiol
- Formononetin 7-hydroxy-4'-methoxyisoflavone
- Glycitein 4',7-dihydroxy-6-methoxyisoflavone
- Genistein 4',5,7-trihydroxyisoflavone
- Genistein-4',7-dimethylether 5-hydroxy-4',7-dimethoxyisoflavone
- Prunetin 4',5-dihydroxy-7-methoxyisoflavone
with the associated glucosides
- Genistin glucosyl-7-genistein
- Glycitin 4',7-dihydroxy-6-methoxyisoflavone-7-d-glucoside
- Ononin formononetin-7-O-glucoside
- Sissotrin biochanin A-7-glucoside

A more detailed list of isoflavones is shown in Appendix I [Boland and Donnelly]. The comparison of effects and functions of plants containing the same isoflavones shows remarkable similarity.

**Daidzein as an example of an isoflavone**

Daidzein is a solid substance that is virtually insoluble in water. Its molecular formula is C_{15}H_{10}O_{4}, and its molecular weight is 254.24 daltons. Daidzein is also known as 7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one and 4', 7-dihydroxyisoflavone. Daidzin, which has greater water solubility than daidzein, is the 7-beta glucoside of daidzein.

Daidzein is an isoflavone. It is also classified as a phytoestrogen since it is a plant-derived nonsteroidal compound that has estrogen-like biolo-
Fig. 16 Biochanin A.

Other sources of biochanin A are Baptisia tinctoria (Wild Indigo), Medicago sativa (Alfalfa), Sophora japonica (Japanese Pagoda Tree) and Vigna radiata (Mungbean). These two isoflavones are considerably less estrogenic in their original forms, because the stereochemistry of the methoxy groups means they are not able to bind to the estrogen receptors as efficiently.

Fig. 17 Formononetin.

However, once these molecules are ingested, bacteria in the colon are able to remove the methyl groups - biochanin A becomes genistein (Fig.18) and formononetin becomes daidzein (Fig.1 see above). Daidzein can be further metabolized to equol (Fig.19).

Other sources of formononetin are Astragalus membranaceus (Astragalus), Cimicifuga racemosa (Black Cohosh), Glycyrrhiza glabra (Licorice root), Medicago sativa (Alfalfa), Pueraria spp. (Kudzu; Pueraria), Sophora japonica (Japanese Pagoda Tree) and Vigna radiata (Mungbean).

Internally, biochanin A and formononetin are then able to be a source of considerable estrogenic activity.

Fig. 18 Genistein.

Other sources of genistein are Baptisia tinctoria (Wild Indigo), Cytisus scoparius (Scotch Broom), Glycine max (Soybean), Glycyrrhiza glabra (Licorice root), Medicago sativa (Alfalfa), Pueraria spp. (Kudzu; Pueraria), Sophora japonica (Japanese Pagoda Tree) and Vigna radiata (Mungbean).

It may well be that these mechanisms give red clover its reputation as an alternative remedy, cleansing the system yet mild enough for many children's skin problems, even eczema. A lotion of red clover can be used externally to give relief from itching in skin disorders. Specific for acne, boils and similar eruptions, including eczema and skin problems especially where irritation is a factor [Evans].

Fig. 19 Equol.

Historically, the flower tea has been used as an antispasmodic, expectorant and mild sedative. It is also recommended for athlete's foot, sores, burns, and ulcers. [Leung & Foster] and has been used in the herbal treatment of cancer, especially of the breast or ovaries [Mills]. Red Clover is also a very popular remedy as the
alternative for hormone replacement therapy and is sold extensively for this purpose.

**Sweet Yellow Melilot (Melilotus officinalis)**

Melilot is soothing, lenitive, astringent, refreshing and anti-irritant and has similar properties to the red clover described above. It is also described as possibly having the additional properties of being anti-inflammatory, anti-oedema, a venous astringent (haemorrhoids) and anaesthetic [Council of Europe]. However, it is perhaps not the isoflavones at force here, but maybe the β-sitosterol or coumarin the roots contain.

Melilotus officinalis L. extract, containing 0.25% coumarin (Fig 20) was studied on acute inflammation induced with oil of turpentine in male rabbits. M. officinalis had anti-inflammatory effects because it reduced the activation of circulating phagocytes and lowered citrulline production.

![Fig. 20 coumarin.](image)

These properties were similar to those of hydrocortisone sodium hemisuccinate and coumarin. [Plesca-Manea et al]

**PHYTOSTEROLS AND RELATED COMPOUNDS**

The benefits of these phytosterols may be seen in the common herbal materials indicated for arthritis, such as Frankincense (Boswellia serrata). The boswellic acid present inhibits two inflammatory enzymes, 5-lipoxygenase (which produces leukotrienes) and human leukocyte elastase HLE (which degrades elastase).

![Fig. 21 Boswellic acid.](image)

Committee on Toxicity of chemicals in food, consumer products and the environment. Working group on phytoestrogens Cellular & molecular mechanisms of phytoestrogen activity The Department of Biochemical Pharmacology, Imperial College School of Medicine prepared a paper for discussion: “Assessment of the estrogenic potency of phyto-compounds”. This reviewed the available information on cellular and molecular mechanisms and phytoestrogen estrogenic potencies.

Out of the 28 points (statements for comment really) the following stood out:

Taking all estrogen receptor binding assays into account the review proposed the following rank order of phytoestrogen potency: estradiol >> coumestrol > 8-prenylnaringenin > equol >= genistein > biochanin A > daidzein > genistein glucuronide* > daidzein glucuronide* > formononetin (the activity of those compounds marked * may be due to the presence of activating enzymes present in the receptor preparation). Phytoestrogens stimulated in vitro cell proliferation at concentrations of 0.1 - 10mM (3 - 4-fold less than estradiol). They did not induce the maximal proliferative effect of estradiol as
higher concentrations inhibited proliferation. The majority of endogenous estrogens (> 90%) were not freely available but bound to plasma proteins. Phytoestrogens bound at 1/100th to 1/1000th the affinity of estradiol. The availability of phytoestrogens in plasma relative to estradiol will be greater.

Coumestrol, 8-prenylnaringenin and equol were > 1000-fold less potent than estradiol and the isoflavones > 10 000-fold less potent.

**The Wild Yam (Dioscorea villosa)**

The Wild Yam (Dioscorea villosa) was the source of diosgenin (Fig. 21), a steroidal saponin used as the starting point for the commercial source of pregnanolone (Fig. 22) and progesterone (Fig.23) used as the first birth control pills. The root of Dioscorea is used for numerous purposes, but the major use is for the suppression of menopausal symptoms like hot flushes [Watson].

**Fig. 22 diosgenin.**

There are many other sources of diosgenin

During pregnancy, small frequent doses will help allay nausea [Lust; Grieve]. It is antispasmodic. It is valuable neuralgic affections, spasmodic hiccup and spasmodic asthma [Grieve].

**Fig. 23 pregnanolone.**

It is spasmolytic, a mild diaphoretic. It has potential in skin care and body care being anti-inflammatory and anti-rheumatic. It is also cited for dysmenorrhoea, ovarian and uterine pain [British Herbal Pharmacopoeia; Hoffman], perhaps showing the power of this herbal root.
It is interesting to note that Vitex agnus-castus is a source of natural progesterone. Proprietary preparations containing this material have been available in Germany since the 1950s and many documented studies have investigated the use of these products to treat various gynaecological disorders [Newall]. The fruit of Vitex contains essential oils, iridoid glycosides, and flavonoids. Essential oils include limonene, 1,8 cineole, and sabinene. The primary flavonoids include casticin, orientin, and isovitexin. The two iridoidglycosides isolated are agnuside and aucubin. Agnuside serves as a reference material for quality control in the manufacture of Vitex extracts. One other report demonstrated delta-3-ketosteroids in the flowers and leaves of Vitex that probably contained progesterone and 17-hydroxyprogesterone [Brown]. The active constituents have been determined as 17-α-hydroxyprogesterone (leaf), 17-hydroxyprogesterone (leaf), androstenedione (leaf), δ-3-ketosteroids (leaf), epitestosterone (flower), progesterone (leaf), testosterone (flower and leaf) [Phytochemical and Ethnobotanical Databases]. It is highly unlikely that the diosgenin in the plant could ever be synthesised on the topical application to the skin to form a corticosteroid or hormonal derivative. However, it does seem likely that this material (being the precursor to these estrogenic molecules) will to some extent mimic the function of those pharmaceutical active materials and benefit the skin [Dweck, 2002].

However, the production of wild yam was unable to sustain the demand for diosgenin as the starting precursor, for the production of birth control materials, which by this stage was dominated by estrone (Fig. 24 estrone).

**Fenugreek (Trigonella foenum graecum)**

The world turned its attention to Fenugreek (Trigonella foenum graecum) for its source of diosgenin. Fenugreek or Foenugreek seeds are emollient and accelerate the healing of suppurations and inflammations. Externally cooked with water into a porridge and used as hot compresses on boils and abscesses in a similar manner to the usage of linseed [Fluck]. Decoctions of whole plant are used as a bath for uterus infections. The seeds are tonic, restorative, aphrodisiac and galactagogue. Their emollient properties are useful for the itch. A cataplasm obtained by boiling the flour of the seeds with vinegar and saltpetre is used for swelling of the spleen [Bonlos]. Extracts of the seeds are incorporated into several cosmetics claimed to have effect on premature hair loss, and as a skin cleanser [Iwu], and it is also reported in Java in hair tonics and to cure baldness [Leung]. Many of the herbal materials found to have an effect on hair growth have a hormonal or hormonalmimetic basis. Likewise there are a number of references to fenugreek having galactagogue (increase milk in
nursing mothers) activity [Bunney; Burkill; Mills], which again is indicative of an estrogennlike activity. The plant should be used with caution as Fenugreek is reputed to be oxytocic and in vitro uterine stimulant activity has been documented [Newall et al], so the use of fenugreek during pregnancy and lactation in doses greatly exceeding those normally encountered in foods is not advisable.

**Pomegranate (Punica granatum)**

Pomegranate is one of the many plants that contain substances with hormone-type action. The seeds of Pomegranate, that ancient symbol of fertility, were found to contain an estrone identical with the genuine hormone. Punica granatum seeds are the best source of plant estrone to date [Weiss].

The antioxidant and eicosanoid enzyme inhibition properties of pomegranate (Punica granatum) fermented juice and seed oil flavonoids were studied, which showed strong antioxidant activity (determined by measuring the coupled oxidation of carotene and linoleic acid) close to that of butylated hydroxyanisole (BHA) and green tea, and significantly greater than that of red wine. [Schubert et al].

This is clearly a fruit worthy of further exploration, especially as most of the information to date relates to the use of the bark, seeds and the roots as a taenicide (expelling worms). The rind is used as an astringent [Lust]. The leaf has antibacterial properties and is applied externally to sores [Stuart].

**Other plants that contain estrone**

Dukes data base [Phytochemical and Ethnobotanical Databases] shows the following plants as containing estrone.

Punica granatum L. Pomegranate (Seed) 17ppm, Malus domestica BORKH. Apple (Seed), Zea mays L. Corn (Seed Oil), Humulus lupulus L. Hops (Fruit), Olea europaea L. Olive (Seed), Panax quinquefolius L. American Ginseng (Plant), Phaseolus vulgaris L. Anasazi Bean (Flower), Phoenix dactylifera L. - Date Palm (Seed), Prunus armeniaca L. - Apricot (Seed).

**Date Palm (Phoenix dactylifera)**

Body hormones play a central role in skin appearance and are implicated in skin aging. Studies have shown that the decrease of these hormones plays an important role in skin endogenous aging, reduced skin thickness, and the disturbance of normal collagen turnover which, in turn, results in a decrease in collagen I and III synthesis. Date Palm has seven compounds with regenerative, anti-oxidizing, firming, and soothing properties, extracted from the kernel: phytosterols, phytosteroids, ursolic acid, isoflavones, policosonols, pro-vitamin A and vitamin E.

Some studies suggest that DHEA administration would have a beneficial effect against signs of aging. DHEA is known for its capacity to promote keratinization of the epidermis or to reinforce the barrier function of the skin.

The author compared on ex vivo skin, the effects of Date Palm kernel extract with those of DHEA in reference to DHEA as an anti-aging molecule. There was a decrease of wrinkles within only
five weeks of Date Palm kernel extract application and also improved the skin structure in a way superior to that of DHEA [Dal Farra]. The seed and the pollen have both been shown to contain estrone and this may further explain the reasons for this activity [Morton; Duke].

**Plants with a future for topical application**

In view of the benefits seen with those plants containing genistein and daidzein, we looked at other plants that might have potential as topical materials and also looked to see if they contained phytosterols and/or phyto-hormones. The results were promising.

**Calabar Bean** *(Physostigma venenosum)*

![Stigmasterol](image)


**Suma or Brazilian Ginseng (Pfaffia paniculata)**

![β-ecdysterone](image)


**Cherimoya (Annona cherimoya)**

![β-sitosterol](image)


**Lima Bean or Butter Bean (Phaseolus lunatus)**

![Fig. 30 Estradiol.](image)


**Hops (Humulus lupulus)**

The hop contains β-sitosterol, estradiol, stigmasterol and estrone. In addition it contains many other materials that are known for their sedative and relaxing attributes.

Regular doses of the herb can help regulate the menstrual cycle [Keville]. It was the girls and women picking hops who first discovered that hops have an effect on genital organs. Before machines were introduced, hop pickers used to spend several weeks at this work, and it had always been known that menstrual periods would come early in young girls while they were doing this work. The reason is that hops contain plant hormones, particularly when very fresh, and these are similar to oestrogens. Considerable amounts have been found, 30,000 to 300,000 i.u. of oestrogen in 100g of hops. This also explains why hops will suppress sexual excitement in men. It has been shown that there are substances called anti-androgens that are able to cancel the effects of the male hormone (androgen) [Weiss].

It was found that hop extract not only recovered the proliferation of hair follicle derived keratinocyte (HFKs) suppressed by androgen but also stimulated the proliferation of HFKs. Furthermore, the effects of hop were evaluated using both animal tests and human volunteers in vivo. It was demonstrated that hop showed a potent acceleration on hair growth. [Okano et al]

**Sarsaparilla (Smilax ornate)**

It is used in concoctions with other plants as a tonic or aphrodisiac [Seaforth].

Sarsaparilla was formerly used in the treatment
of syphilis [Carrington], gonorrhoea [Honychurch], rheumatism and certain skin diseases. Used in soft drinks, the genins are also used in the partial synthesis of cortisone and other steroids [Evans]. As part of a wider treatment for chronic rheumatism it should be considered as it is especially useful for rheumatoid arthritis. It has been shown that Sarsaparilla contains chemicals with properties that aid testosterone activity in the body [Hoffman].

Sarsaparilla contains saponis, sarsapomin and parallin, which yield isomeric sapogenins, sarsapogenin and smilogenin. It also contains sitosterol and stigmasterol in the free form and as glucosides. It is antirheumatic, antiseptic, antipruritic and is indicated for psoriasis, and other cutaneous conditions. Like other steroidal plants it is indicated for chronic rheumatism and rheumatoid arthritis. It is specifically used in cases of psoriasis especially where there is desquamation [British Herbal Pharmacopoeia].
### Appendix

#### Isoflavones

<table>
<thead>
<tr>
<th>Isoflavone</th>
<th>Plant Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>2'-Hydroxy-5-methoxy-6,7-methylenedioxy-8-C-methylflavone</td>
<td>Calotis impetula</td>
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<td>2'-Hydroxy-5,7-dimethoxy-8-C-methylflavone</td>
<td>Calotis impetula</td>
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<td>Ficlusin B</td>
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#### Glycosides

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</table>
References

30) Phytochemical and Ethnobotanical Databases. www.ars-grin.gov/duke/

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