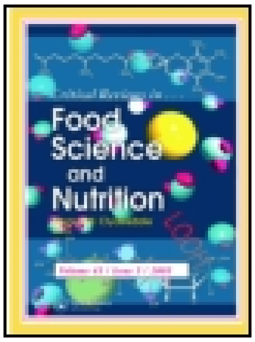

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Therapeutic potential of temperate forage legumes: A review

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Abstract

The discovery of bioactive molecules from botanical sources is an expanding field, preferentially oriented to plants having a tradition of use in medicine and providing high yields and availability. Temperate forage legumes are Fabaceae species that include worldwide-important crops. These plants possess therapeutic virtues that have been used in veterinary and folk medicine, but have also attracted the interest of official medicine. We have examined here *Medicago sativa* (alfalfa), *Trifolium pratense* and *T. repens* (clovers), *Melilotus albus* and *M. officinalis* (sweet clovers), *Lotus corniculatus* (birdsfoot trefoil), *Onobrychis viciifolia* (sainfoin), *Lespedeza capitata* (roundhead lespedeza), and *Galega officinalis* (goatôs rue). The phytochemical complexes of these species contain secondary metabolites whose pharmacological potentials deserve investigation. Major classes of compounds include alkaloids and amines, cyanogenic glycosides, flavonoids, coumarins, condensed tannins, and saponins. Some of these phytochemicals have been related to anti-hypercholesterolemia, antidiabetic, anti-menopause, anti-inflammatory, antiedema, anthelmintic, and kidney protective effects. Two widely-prescribed drugs have been developed starting from temperate forage legumes, namely the antithrombotic warfarin, inspired from sweet cloverǫs coumarin, and the antidiabetic metformin, a derivative of sainfoinǫs guanidine. Available evidence suggests that temperate forage legumes are a potentially important resource for the extraction of active principles to be used as nutraceuticals and pharmaceuticals.

Keywords

Alfalfa, clover, coumarin, food supplements, phytotherapeutics, isoflavones, sapogenins

INTRODUCTION

Forage legumes are plants belonging to the family Fabaceae, generally with well-developed underground parts, composed leaves, inflorescences of papilionaceous flowers, and podded fruits. A known feature of legumes is their ability to develop root nodules and to fix nitrogen in symbiosis with compatible rhizobium bacteria. Such a feature renders these plants suitable for the recolonization of disturbed, nitrogen deficient soils, yielding protein-rich plant material (Frame et al., 1998).

Besides their importance as fodder, forage legumes have attracted attention for non-food applications, including manure, biofuel and phytoremediation (Stoddard, 2008). However, these plants produce a complex of secondary metabolites, mostly consisting of alkaloids and amines, cyanogenic glycosides, flavonoids, coumarins and other phenolics, flavanol oligomers known as condensed tannins, triterpenoid saponins, lectin peptides, mediating binding to polysaccharides of rhizobium symbionts, and other peptides. These phytochemicals generally protect legumes from oxidative stress, competitor plants, and herbivores, while some of them account for beneficial effects to livestock (Wink, 2013). Moreover, different forage legumes have a consolidated tradition in folk medicine. Hence, given that these plants are in general widely diffused and extensively cultivated, they seem to represent an underexploited, potentially important resource for the extraction of nutraceuticals and pharmacologically active principles.

This review deals with the medicinal properties of temperate forage legumes, which are important in the sustainable productivity of ruminant feed in the developed world. These plants include some of the major forage crop cultivated in the word, like alfalfa, with more than 35 million hectares, and different clover species (Frame et al., 1998; Radovic et al., 2009). The

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herein scrutinized forage legumes have been selected by taking into account their importance as fodder crop and/or their medicinal relevance on a traditional or scientific ground. Searches were performed in Scopus, Web of Science, Google Scholar, and Medline. Available medicinal knowledge about the selected species has been covered, including folk uses based on empirical evidence, *in vitro* research on cultivated cells, preclinical studies on animal models, and clinical trials on patients.

MEDICINAL FORAGE LEGUMES

MEDICAGO SATIVA L. (ALFALFA)

General features

Alfalfa, also known as lucerne, is a perennial herb, up to 100 cm high, with a deep taproot and erect, smooth, sharply angled stems that originate from meristematic crowns at the soil surface. Leaves are alternate and trifoliate, with obovate leaflets, and can bear secondary stems at their axils. Flowers are borne in axillary racemes, and are 3-4 cm long, papilionaceous, and variable in color, from purple-blue to yellow-white. The fruit is a spiral-shaped legume.

The species is native to Asia, is the most ancient species cultivated as a fodder plant, and is currently the most cultivated legume in the world, with a production of about 400 million tons per year. It is mostly cultivated for soil improvement and animal feed, but has also been used as an ethnopharmaceutical remedy and a food flavor since ancient times.

Ethnomedicine and phytotherapy

Most common ethnomedicinal uses include memory improvement, central nervous system disorders, inflammation, cough, asthma, kidney ailments, diabetes, muscle pain, and microbial infections. A wide number of preclinical and clinical studies have confirmed some of the plant virtues exploited in empirical uses (Bora and Sharma, 2011).

Experimental and clinical studies

Effects on hypercholesterolemia

In various studies on animal models (e.g. rats, prairie dogs, and monkeys), as well as on human volunteers, plant seeds, root and flowering tops have displayed anti-hypercholesterolemic properties (Bora and Sharma, 2011). This kind of effects include total and LDL plasma cholesterol decrease, generally without variations in HDL cholesterol levels, reduced intestinal cholesterol absorption, and increased cholesterol excretion (Cheeke, 1973; Cohen et al., 1990; Molgaard et al., 1987).

The plant contains glycosylated triterpenoid saponins, mainly derivatives of medicagenic acid, zanhic acid, lucernic acid, hederagenin, bayogenin, and soyasapogenols (Bialy et al., 1999; Massiot et al., 1988). These compounds are thought to play a role in plasma cholesterol lowering activities (Malinow, 1984; Oleszek, 1996). A saponin-enriched leaf extract has been found to modulate the expression of genes involved in hepatic cholesterol metabolism in the rat, providing some hint about the mechanism of action of saponins, and suggesting their potential usefulness in the treatment of hyperlipidemia.(Shi et al., 2014).

Antimicrobial activity

Saponins have displayed antimicrobial activities against Gram-positive bacteria (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis*) and fungi (*Saccharomyces cerevisiae*), allegedly related to the presence of medicagenic acid (Avato et al., 2006). The monodesmosidic glycoside, medicagenic acid 3-O-ϐ-D-glucopyranoside, has been found to inhibit *in vitro* the growth of the dermatophyte fungi *Microsporum gypseum*, *Trichophyton interdigitale* and *T. tonsurans*, suggesting its possible use against skin mycoses (Houghton et al., 2006). The antifungal action of this compound, observed in the yeast *Saccharomyces cerevisiae*, consists of membrane ergosterol deprivation, possibly due to the formation of stable complexes, which is thought to cause massive ion leakage out of fungal cells (Polacheck et al., 1991).

Antidiabetic effect

Dietary alfalfa has been reported to reduce hyperglycemia in streptozotocin-induced diabetic mice (Swanston-Flatt et al., 1990). An *in vitro* study using aqueous and methanol alfalfa extracts, conducted on the BRIN-BD11 pancreatic beta cell line, has suggested possible mechanisms for the antidiabetic effect, involving the induction of glucose uptake, glycogen synthesis and insulin secretion (Mohamed et al., 2006).

Effects on menopause symptoms

The plant is known to possess estrogenic activities and the ability of relieving menopause symptoms in women. Accordingly, it has been shown to contain various phytoestrogens, including the coumestan coumestrol, and the flavonoids apigenin, luteolin, quercetin, and medicarpin (Seguin and Zheng, 2006). The estrogenic activity of a plant extract has been determined by the estrogen-dependent, MCF-7 breast cancer cell proliferation assay, with an induction of cell proliferation rating above estradiol levels. The estrogen antagonist fulvestrant (ICI 182,780) has suppressed such proliferative effect, suggesting an estrogen receptor-related mechanism (Boue et al., 2003).

Anticancer effects

Due to their estrogenic activity, coumestrol, apigenin and quercetin are thought to have potential also for use in the treatment of hormone-related cancers (Huyghe et al., 2007). However, antiproliferative activities have been found also for other cancer types. A bioguided fractionation of a leaf toluene extract has led to the detection of terpenoids and flavonoids, among which medicarpin, melilotocarpan E, millepurpan, tricin, and chrysoeriol, having cytotoxic effects on the leukaemia P388 cell line and on its doxorubicin-resistant counterpart P388/DOX (Gatouillat et al., 2014).

Antioxidant activity

Beneficial effects of the plant are likely to depend, at least in part, on antioxidant processes. Compounds with radical scavenging attitude have been isolated from fresh and dehydrated samples, viz. the carotenoids lutein, violaxanthin, cryptoxanthin, zeaxanthin, and neoxanthin (Bickoff et al., 1954).

Various plant compounds seem also able to stimulate the antioxidant defense system. A lyophilized aqueous extract has exerted hepatoprotective and anti-oxidative stress activities on carbon tetrachloride-induced liver injury in rats (Al-Dosari, 2012). Alfalfa polysaccharides, given as dietary supplement to mice, have improved superoxide dismutase and glutathione peroxidase activity, while concomitantly having reduced malondialdehyde formation. (Zhu et al., 2014). In addition, polysaccharides have been found to stimulate the proliferation of peripheral blood and splenic lymphocyte in broilers and mice (Li et al., 2013; Zhang et al., 2010).

Noxious side effects

Alfalfa has been proposed in human nutrition as a dietary supplement for both healthy and patient subjects (Gawel, 2012; Lodha et al., 2009). However, dosages should be carefully

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evaluated due to the occurrence of antifeedant saponins (Oleszek, 1996), and of the nonprotein amino acid canavanine, which is toxic to animal species. This latter constituent is reputed to be responsible for an alfalfa-induced systemic lupus erythematosus-like syndrome in monkeys (Malinow et al., 1982), and for SLE exacerbation in humans (Roberts and Hayashi, 1983).

Future perspectives

The accumulation of experimental evidence on the biological properties of the plant provides a scientific basis for empirical and traditional medicinal uses. Pharmacological characterizations have been achieved for some saponins, phytoestrogens, terpenoids, flavonoids, and polysaccharides. Yet, the plant contains fair amounts of various other compounds that have been almost unexplored. Considering the available biomass of alfalfa crops, the plant represents a potentially important phytopharmacological source.

TRIFOLIUM PRATENSE **L. (RED CLOVER)**

General features

Perennial herb, 15-40 cm high, with rhizomes and erect stems. Leaves are trifoliate and inflorescences form ovoid-rounded heads, 1.0-2.5 mm in diameter, consisting of fleshy red flowers. The species is indigenous to Europe, central-Western Asia and North Africa, and have become naturalized in many temperate regions of the world. It is widely cultivated and prevalently used as pasture, manure, and fodder crop legume. Popular medicinal and culinary uses are also known (Sabudak and Guler, 2009).

Ethnomedicine and phytotherapy

Red clover is used internally for cough, whooping cough and respiratory conditions, as an expectorant and antispasmodic, and for menopause syndrome. It is also commonly used

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externally in the treatment of chronic skin conditions, such as eczema and psoriasis (Gruenwald et al., 2007). Red clover extracts are marketed as dietary supplements (Burdette and Marcus, 2013).

Experimental and clinical studies

Effects on menopause symptoms

A main pharmacological trait of red clover is the presence of estrogenic isoflavones, such as biochanin A, daidzein, formononetin, and genistein (Coon et al., 2007). These compounds have been reputed to confer the plant healing properties for menopausal symptoms (Ehsanpour et al., 2012; Fugh-Berman and Kronenberg, 2001), and have been recommended as an alternative to estrogen replacement therapy (Beck et al., 2005). Phase-I and -II clinical trials have been conducted using oral treatments with major red clover isoflavones. In most cases, these studies have shown no significant differences with respect to placebo, thus providing no clear demonstrable benefit for menopausal symptoms (Fugh-Berman and Kronenberg, 2001; Geller et al., 2009). Conversely, a clinical trial on 60 postmenopausal women, randomly and equally divided into a placebo group and a group treated with 54 mg/day genistein, has reported an increase of flow-mediated, endothelium-dependent vasodilation of the brachial artery induced by genistein, coupled to an increased ratio between plasma nitric oxide breakdown products and endothelin-1 (Squadrito et al., 2002).

Despite controversial results, no evidence of noxious side effects has been found in short-term applications of red clover isoflavones. Further study is nevertheless needed for the evaluation of long-term safety (Kolodziejczyk-Czepas, 2012).

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Little is known about the mechanism of action of red clover isoflavones, although genistein is considered the most active compound (Dornstauder et al., 2001). The estrogenic properties of genistein have been widely investigated, establishing its ability of binding to both α and ɓ estrogen receptors and of regulating estrogen-dependent signaling pathways (Saha et al., 2014). In addition, a clinically tested isoflavone extract has been found to bind *in vitro* to the ε- and ŭ-opiate receptor. This could help to explain isoflavone beneficial effects on menopausal symptoms, since the opioid system regulates temperature, mood, and hormonal levels (Nissan et al., 2007).

Effects on metabolic syndrome

In vitro transactivation of the plasma lipid regulator PPAR-γ receptor by red clover isoflavones argues for possible positive effects in the therapy of the metabolic syndrome. Support to this hypothesis has been brought by clinical data. Two mixtures of red clover isoflavones, enriched in either the genistein precursor biochanin A or the daidzein precursos formononetin, have been used in a randomised, placebo-controlled, double-blind trial concerning baseline LDL-cholesterol, conducted on a total of 46 middle-aged men and 34 postmenopausal women, at doses of 40 mg/day for 6 weeks. Data have shown an LDL-cholesterol lowering effect of biochanin confined to men, whereas women have failed to respond significantly to the treatment (Nestel et al., 2004). In another study, a red clover extract has shown non-antinflammatory, PPARα-independent, antisteatotic effects on mice with experimentally-induced nonalcoholic steatosis (Chen et al., 2014).

Anticancer effects

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Genistein has been found to inhibit ATP-dependent enzymes, like protein tyrosine kinases and topoisomerase II, thus attracting interest for its possible anticancerogenic potential (Polkowski and Mazurek, 2000). An *in vitro* study has reported antiproliferative effects of red clover isoflavones on a total of 11 human cancer cell lines, representing cancers of the colon, prostate, breast, cervix, liver, pancreas, stomach and ovaries, with genistein showing stronger activity (Reiter et al., 2011).

Epidemiologic evidence suggests a relationship between legume consumption and a lower incidence of benign prostatic hyperplasia among Asian men (Katz, 2002). Aimed at finding pharmacological effects supporting this medicinal use, major red clover isoflavones have been examined on isolated rat prostate gland. Genistein, formononetin, and biochanin A have inhibited prostatic smooth muscle contractions, but such an effect has been observed at concentrations above doses achievable in clinical settings (Brandli et al., 2010).

Depigmenting effect

Biochanin A has shown melanogenesis inhibition, both *in vitro* on melanoma cells, and *in vivo* in zebrafish and mice, suggesting its possible use as a whitening agent in the treatment of skin hyperpigmentation (Lin et al., 2011).

Anti-inflammatory effects

A red clover extract has been found to inhibit the activation and proliferation of mouse lymphocytes and the secretion of nitric oxide by mouse macrophages, suggesting possible soothing of inflammation (Yang et al., 2008). Lotions containing genistein and its metabolites equol, isoequol, and dehydroequol have been shown to reduce inflammatory edema induced by simulated solar UV radiation in the skin of hairless mice. Equol has also protected from immunosuppression induced by the UV-photoproduct cis-urocanic acid, indicating a profitable use of this compound in solar skin products (Widyarini et al., 2001).

TRIFOLIUM REPENS **L. (WHITE CLOVER)**

Widely cultivated, temperate clover similar to *T. pratense*, but with creeping stems and white or pink-tinged flowers. It has been much less investigated than red clover as a medicinal plant, and its curative applications are supported essentially by traditional medicine, while clinical data are lacking (Kolodziejczyk-Czepas, 2012). It is locally used as an expectorant, antirheumatic, and antihelmintic (Baytop, 1984).

Like other clover species, white clover contains fair levels of isoflavones, suggesting that its therapeutic properties might be similar to those of red clover (Hanganu et al., 2010). An Indian folk use of white clover as a deworming remedy has inspired a study on its anticestodal activity using *Hymenolepis diminuta* infections in rats. This study has shown reductions in fecal egg counts and worm recovery rate similar to those obtained with the standard drug praziquantel (Tangpu et al., 2005).

In a study on phenolic compounds of flowers and leaves, strong antioxidant effects against DPPH have been observed, especially for the flavonoids hyperoside, myricetin 3-O-ß-galactopyranoside, and quercetin (Kicel and Wolbis, 2012).

MELILOTUS OFFICINALIS **(L.) PALL. (YELLOW SWEET CLOVER)**

General features

Biennial herb with erect growth habit, strong taproot, and a root crown from which new shoots emerge. Leaves are alternate, elongated and trifoliate, with finely toothed leaflets. Flowers are stalked, white and arranged on the top of an elongated stem.

The genus name is due to the plantôs sweet smell, which derives from the presence of coumarin. The species is native to Eurasia, and naturalized in North America, Africa and Australia. It grows preferentially in open environments, including roadsides, abandoned fields, pastures, riparian areas and prairie. The plant develops a root system during the first season, and produces flowers and seeds during the second season. Second-year plants may appear bushy and reach a height of 1.5--2 m.

Ethnomedicine and phytotherapy

Yellow sweet clover is the major medicinal plant of the genus. Its therapeutic use can be traced back in the work of Plinius and Hippokrates. The plant is mainly known for its antinflammatory, antioedematous, phlebotonic, spasmolytic, diuretic and sedative properties. It has been empirically used through the centuries for several problems, including septic ulcers, venous disturbances, haemorrhoids, thrombosis, oedema, rheumatism, bladder disorders, stomach ache, headache, skin rash, and as an antispasmodic, diuretic, hepatoprotector, espectorant, and balsamic (Burlando et al., 2010).

Experimental and clinical studies

Antiedematous effects

Various studies have been conducted on the medicinal properties of yellow sweet clover. Extracts enriched in coumarin have been clinically tested for their effects against edema and lymphedema, particularly for the treatment of postoperative circulatory problems (Casley-Smith, 1992; Consoli, 2003). The therapeutic activity of a coumarinic extract of *M. officinalis* (CEMO) was evaluated in women with chronic lymphedema of the upper arm caused by lymphadenectomy for breast cancer. A group of 14 patients received a daily dose of 400 mg of CEMO, containing 8 mg of coumarin, for 6 months, resulting in an improvement of symptoms in 11 patients (Pastura et al., 1999).

In another double-blind clinical study, 46 patients undergoing augmentation rhinoplasty and double-eyelid blepharoplasty were checked for postoperative edema and ecchymosis in the upper eyelid, lower eyelid, and paranasal area along 7 postoperative days. A group of 16 patients received *M. officinalis* extract tablets containing 1% coumarin 3 times a day, a second group of 16 patients received oral dexamethasone in tapering daily doses from 6 to 1.5 mg, and a control group of 14 patients received neither agents. Although the melilotus group did not show significant difference in eyelid edema reduction when compared with the control group, paranasal edema was much more reduced at postoperative days 4 and 7 (Xu et al., 2008).

The ability of the plant to improve edema has been attributed to a stimulatory effect of coumarin on macrophage proteolysis, which would remove osmotic pressure and allow tissue draining. Coumarin also reduces epinephrin catabolism, thus improving vessel contractility (Hoult and Paya, 1996; Piller, 1980). Moreover, the plantøs flavonoids are believed to support coumarin antiedematous effect by strengthening blood vessel walls. The improvement of tissue draining can be exploited in the treatment of cellulite and varicose veins (Preisich, 1963; Vettorello et al., 1996).

Anti-inflammatory and wound healing effects

Anti-inflammatory effects observed in rats and rabbits have been ascribed to coumarin (Foldi-Borcsok et al., 1971; Plesca-Manea et al., 2002), while an improvement of the immune system is allegedly induced by polysaccharides (Podkolzin et al., 1996).

Different medicinal products have been developed from yellow sweet clover extracts. A drug developed in Iran under the trade name of AngiparsÊ (Semelil) has been clinically evaluated on chronic wounds, such as diabetic foot ulcer and pressure ulcer. In a double blind clinical trial on diabetic peripheral neuropathy, 25 diabetes patients received oral AngiparsÊ 100 mg twice a day for 12 weeks, while 24 of them received placebo. The results have shown limited evidence of efficacy of the drug in diabetic neuropathy treatment, indicating the need of more studies with larger samples and longer duration times (Bakhshayeshi et al., 2011). However, the drug has shown anti-ischemic and anti-inflammatory effects in male rats (Asadi-Shekaari et al., 2010).

Positive results have also been obtained with an electromagnetically-processed yellow sweet clover extract, used as a wound dressing in diabetic mice (Farzamfar et al., 2008). Due to its phlebological and anti-inflammatory properties, yellow sweet clover is used on the skin as a decongestant, astringent and soothener.

Anticoagulant effects

Coumarin is a naturally occurring fragrance, widely used in consumer products such as cosmetics, soaps and perfumes. This compound has received much attention among sweet clover active principles, as it has been involved in a cattle hemorrhage syndrome known as ñsweet clover diseaseò (Chae and Cho, 2003; Estevez-Braun and Gonzales, 1997). By the end of 1930s, the biochemist K.P. Link and his colleagues found that the disease was caused by dicoumarol (3,3'-methylenebis 4-hydroxycoumarin). This compound is an anti-vitamin K anticoagulant that

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originates from coumarin through fermentation operated by *Aspergillus* molds under unproper sweet clover hay storage. Dicoumarol has inspired the synthesis of its analogue Warfarin, widely used as a rodenticide and anticoagulant drug (Kresge et al., 2005). Coumarin itself has been associated to hepatic intoxication (Casley-Smith, 1995).

Besides coumarin, other yellow sweet clover constituents that could be allegedly involved in the plantõs curative virtues include the coumarin derivatives scopoletin, umbelliferone and melilotin, flavonoids, e.g. kaempferol and quercetin glycosides, phytosterols, triterpene sapogenins, and steroidal glycosides (Yang et al., 2014). Components of essential oil seem to vary considerably in plants from different regions (Gudzenko and Vinogradov, 2014; Quijano-Celis et al., 2010).

***MELILOTUS ALBUS* MEDIK. (WHITE SWEET CLOVER)**

Biennial herb quite similar to *M. officinalis* but with white flowers. It shares different compounds with its congener, such as coumarins and sapogenins (Chindriк, 1987; Krzakowa and Grzywacz, 2010), but studies on medicinal applications are lacking, with the possible exception of antibacterial activity (Acamovic-Djokovic et al., 2002). However, some literature information on folk remedies is available, including an ointment from Serbia used for external ulcer and as an anticoagulant (Saric, 1989), another ointment for ulcer, acne and sunburn, and an anti-typhoid fever tea from North American natives (Foster, 2002), an antimalarial decoction from Peru (Bussmann and Glenn, 2010), and an emollient and anti-diarrheic preparation from Pakistan (Ullah et al., 2014).

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LOTUS CORNICULATUS L. (BIRDSFOOT TREFOIL)

General features

Cosmopolitan, perennial herb native to Eurasia and North Africa. The root system is extensive, stems are solid, almost square, creeping or ascending, and reach a height of about 50 cm. Leaves are alternate, pentafoliate, with lanceolate-ovate leaflets. Flowers are yellow or gold, and develop in axillary, pedunculated groups of 3-7. Pods are pea-like, linear to cylindrical, 20-25 mm long, and contain many ovate seeds.

The plant grows preferentially in moist and alkaline soils, but is soil tolerant and drought resistant. It can be found spontaneous on hillsides, grasslands, crop ýelds, and riverbanks, and is used as forage for pasture, hay, and silage (Seaney and Henson, 1970).

Ethnomedicine and phytotherapy

Biological properties of birdsfoot trefoil have been mainly ascribed to the presence of condensed tannins, containing catechin and epicatechin residues as major elements, coumarins, and flavonoid and phenolic glycosides (Girardi et al., 2014). Condensed tannins are of interest for cattle rearing, since they form stable complexes with dietary protein, thereby increasing the proportion of undegraded rumen protein (Hedqvist et al., 2000).

In medicinal applications, birdsfoot trefoil has been used empirically as an anti-inflammatory, antispasmodic, cardiotonic, carminative, febrifuge, hypoglycaemic, restorative, sedative, tonic, and vermifuge (Chiej, 1984; Duke and Ayensu, 1985). The plant has been reported in ethnomedicinal surveys for skeleto-muscular problems, genital and sexual diseases, snake bites, abdominal and stomach pain, and as a sedative and diuretic (Akhtar et al., 2013; Alamgeer et al., 2013; Altundaga and Ozturk, 2011; Megersa et al., 2013).

Experimental and clinical studies

Antimicrobial activity

Some empirical uses of the plant seem to find confirmation in experimental studies. Antibacterial activities against Gram + and ī strains have been reported for extracts obtained from aerial parts and their constituents. A hexane fraction is active against *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *S. epidermidis*, *Acinetobacter calcoaceticus*, and *Providencia alcalifaciens*. An ethylacetate fraction has shown strong activity on *Bacillus cereus*, *E. faecalis*, and *A. calcoaceticus*. Oleanolic acid isolated from the hexane fraction has been effective on methycillin-resistant *S. aureus*, *L. monocytogenes*, and *B. cereus*. Kaempferitrin isolated from the ethylacetate fraction has displayed antibacterial activity on *Shighella flexinerii*, *Salmonella typhimurium*, *A. calcoaceticus*, *E. faecalis*, and *B. cereus* (Dalmarco et al., 2010).

Anti-inflammatory effects

The above fractions and compounds, with the possible inclusion of β-sitosterol, have shown anti-inflammatory activity on a mouse model of pleurisy. These findings have been related to the inhibition of various agents and processes, including leukocyte proinflammatory activities, the enzymes adenosine deaminase and myeloperoxidase, and the release of interleukin-1β and nitric oxide (Koelzer et al., 2009; Pereira et al., 2011).

Anticancer effects

A galactose-specific lectin has been found to exert antiproliferative and anti-locomotion activities towards human leukemic cancer cells (THP-1), lung cancer cells (HOP62), and colorectal carcinoma (HCT116), and to induce apoptosis features in THP-1 cells (Rafiq et al., 2013).

Noxious side effects

Different cultivars may be toxic for the presence of enzymes that break down cyanogenic glycosides, giving rise to hydrocyanic acid. This knowledge is essential for selecting non-toxic cultivars to be cultivated as forage (Waller et al., 2001).

ONOBRYCHIS VICIIFOLIA **SCOP. (SAINFOIN)**

General features

Perennial herb with tap-roots and stems arising from a branched root crown, reaching a height of 80 cm. Leaves are pinnate with 5-6 pairs of obovate leaflets. Flowers are pink, seldom white, and arranged in conical erect racemes. Fruits are spike-bearing, single-seeded pods.

Sainfoin is native to arid regions of Eurasia. It has been used as forage in warm-temperate regions of Europe, Asia and North America until the 1950s, then replaced by high yielding alfalfa and clover, and eventually reconsidered for pasture, hay or silage (Carbonero et al., 2011).

Experimental and clinical studies

Antimicrobial activity

Medicinal interest has been focused on phenolic compounds. Extraction in aqueous acetone of aerial parts has revealed the presence of a wide phenolic complex, dominated by arbutin, rutin, catechin, kaempferol, quercetin, and afzelin. Fair amounts of condensed tannins are also present, consisting of procyanidin and prodelphinidin units (Marais et al., 2000; Regos et al., 2009). Condensed tannins precipitate proteins and could account for sainfoinǿs activity against *Escherichia coli*, possibly through the induction of outer membrane alteration and cell aggregation (Liu et al., 2013).

Anthelmintic properties

Condensed tannins from the plant have been shown to act against the parasitic nematodes *Teladorsagia circumcincta*, *Haemonchlus contortus,* and *Trichostrongylus colubriformis*, suggesting possible pharmaceutical application as an anthelmintic (Barrau et al., 2005; Paolini et al., 2004).

LESPEDEZA CAPITATA MICHX. (ROUNDHEAD LESPEDEZA)

General features

Perennial shrub with a deep taproot, superficial branched roots, and erect or ascending stems, growing up to 1.5 m. Leaves are alternate and pinnate with three leaflets. They are densely covered with hairs, giving the plant a silvery aspect. Flowers are white or purple, forming terminal capitate heads, and fruits are short, single-seeded pods. The plant is native to eastern North America, and is common in meadows and sparse woods. It is used as forage for livestock, while seeds are used as food for aviary birds.

Ethnomedicine and phytotherapy

Native Americans used aerial portions as a moxa for rheumatism and neuralgia, leaf tea as a diuretic, and roots as an antidote against poisoning (Moerman, 1998). The plant is rich in flavonoids and tannins, which are thought to account for most of the plantôs therapeutic virtues (Glyzin et al., 1973; Linard et al., 1978).

Experimental and clinical studies

Effects on the excretory system

Roundhead lespedeza is prevalently known for its kidney protecting virtues (Yarnell, 2002). An *in vitro* study conducted on the LLC-PK1 proximal tubule cell line has discriminated between flavonoids exerting protective effects on cells and others being inactive, reaching the conclusion that the number and position of phenolic hydroxyl groups play a decisive role in the cytoprotective ability of these compounds (Yokozawa et al., 1999).

Antidiabetic effect

Kaempferitrin is insulinomimetic, thus correcting hyperglycemia in diabetic rats, and promoting glucose uptake by the skeletal muscle of healthy animals (Jorge et al., 2004).

Effects on cardiovascular diseases

The presence in the plant of the flavonoid glycoside homorientin is thought to account for hypoazotemic and hypocholesterolemic effects, preventing atherosclerotic degeneration (Burlando et al., 2010). In addition, a dimeric procyanidin is known to act as a hypothensive agent via ACE inhibition and prevention of angiotensin II formation (Elbl et al., 1990; Wagner and Elbl, 1992).

Antioxidant and antiedematous effects

The free-radical scavenging activity of flavonoids can be exploited in skin antiaging products to protect dermal collagen degradation, while their tissue draining activity could be profitable to combat cellulite (Pauly and Pauly, 1997).

GALEGA OFFICINALIS **L. (GOAT'S RUE)**

General features

Perennial herb with hollow, erect, stems arising from buds located on the underground parts and reaching a height of about 1 m. Leaves are alternate, imparipennate, with many lanceolate leaflets. Flowers are white, blue, or purple, and form axillary racemes. Fruits are cylindrical pods, 20-50 mm long, irregularly striated and spirally dehiscent, containing 3-5 seeds.

The species is native to the Middle East, and is naturalized in many temperate world areas. It has been extensively cultivated as forage and manure, but its agricultural use has declined due to the toxicity of underground portions and flowering tips (Rasekh et al., 2008).

Ethnomedicine and phytotherapy

The plant was in use in medieval Europe as a galactogogue, diaphoretic, and in the treatment of plague, fevers, infectious diseases and digestive problems. In addition, it was historically used to treat symptoms ascribable to type 2 diabetes (Chiej, 1984).

Experimental and clinical studies

Antidiabetic effect

The plant has lactogenic effect that is thought to have clinical relevance (Heiss, 1968). However, most scientific interest has been focused on the ability of reducing blood glucose. Such a property has been related to the presence of guanidine, a compound that decreases insulin resistance but is unsuitable for clinical use due to high toxicity (Goetz and Le Jeune, 2008).

The less toxic galegine (isoamylene guanidine), has been also isolated from the plant and found to reduce blood sugar levels (Petricic and Kalodera, 1982). This compound was used as an antidiabetic agent in the 1920s, but its beneficial effects have not been confirmed in clinical trials on diabetic patients. However, guanidine and its derivatives eventually inspired pharmacochemical work that led to the synthesis of metformin, a widely prescribed antidiabetic drug (Bailey and Day, 2004).

Plant extracts have shown hypoglycaemic and body weight-reducing properties in streptozotocin-induced diabetic rats, while body weight reduction has also been observed in normal mice (Khokhla et al., 2010; Palit et al., 1999; Shojaee et al., 2015). A possible

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explanation of the plant hypoglycemic mechanism has been suggested by data of glucose transport inhibition in monolayers of human intestinal Caco-2 cells (Neef et al., 1996). In a study on diabetes mellitus type 1 in a rat model, a nonalkaloid-containing fraction has been found to inhibit leukocyte apoptosis (Khokhla et al., 2013).

Antithrombotic effect

Different studies indicate that goatös rue has the ability of inhibiting platelet aggregation, suggesting possible antithrombotic application (Atanasov and Tchorbanov, 2003).

Depigmenting effect

The alkaloid fraction of the plant has been claimed to exert an inhibitory effect on melanogenesis (Lee et al., 2012; Ohara, 2005).

CONCLUDING REMARKS

Warfarin and metformin are two of the most widely prescribed drugs. The events that led to their discovery teach us a lesson about forage legume potential in drug discovery and development. These medicines represent the tip of an iceberg consisting of numerous folk remedies and pharmaceutical preparations that have been obtained along centuries from these plants.

In modern times, drug discovery from botanical sources is an expanding field of interest (Heinrich and Gibbons, 2001; Katiyar et al., 2012). A preliminary step of this approach involves an accurate search for suitable plant species. These latter should arguably satisfy two kinds of requirements, i.e. having a certain tradition of use in folk and/or standard medicine, and in addition providing high yields and availability. Forage legumes seem to be ideal plants since on one side, they possess various interesting therapeutic properties (Table 1), and on the other side their use is expected to intensify on a world scale. Some of these species, besides fixing atmospheric nitrogen, are drought tolerant, invasive plants, whose cultivation could help in the recovery and conversion of degraded and desertified lands, particularly in southern temperate areas (Graham and Vance, 2003). Part of current forage legume crops, or even new crops from rehabilitated soils, could therefore be destined to extraction processes for fractionation, screening, and identification of high-value phytochemicals, to be used in pharmaceuticals, nutraceuticals and cosmeceuticals. By this way, high amounts of bioactive-rich biomass could be easily obtained at quite sustainable rates.

The herein examined forage legumes have in all cases some medicinal or veterinary use, either in the form of popular remedies, or as pharmaceutical and herbal products. Yet, the exploitation of these botanical sources is definitely below their potentials in terms of phytochemical production. Herein-provided evidence strongly suggests that the exploitation profile of these crops should be reconsidered, with an extension from agricultural and livestock production to modern drug farming. Such a reconversion will hopefully boost the value of these crop materials, with a predictable benefit to the economies of areas devoted to farming.

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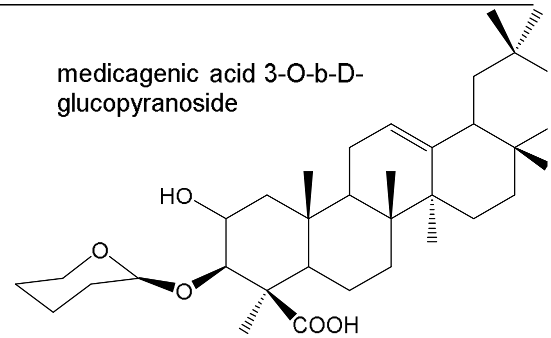
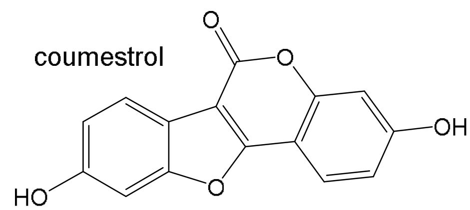
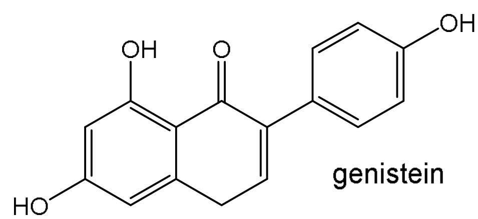
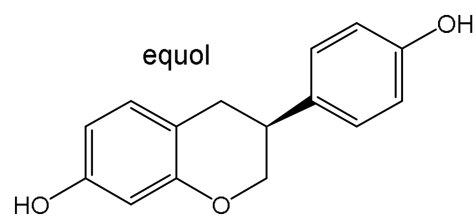
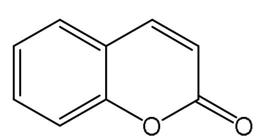
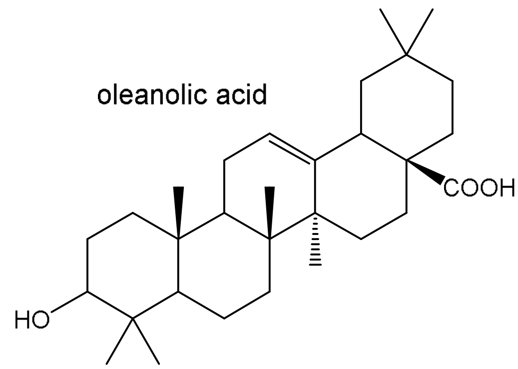
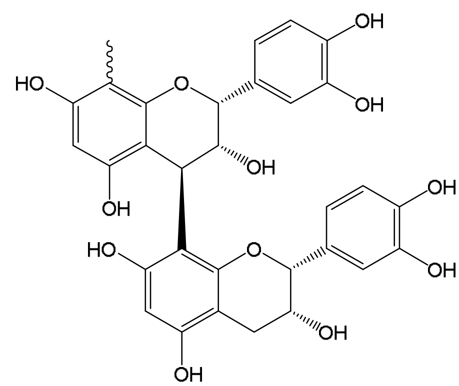
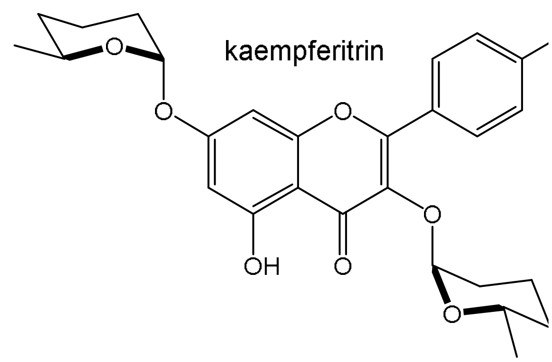
Table 1. Main properties and active principles of temperate forage legumes

Species	**Therapeutic property**	**Body system**	**Active principles**	**Molecular structure**
Medicago sativa (Alfalfa)	anti-hypercholesterolemia	blood	sapogenins	medicagenic acid 3-O-b-D-glucopyranoside 
	anti-menopause	reproductive system	coumestans	coumestrol 
Trifolium pratense (Red clover)	anti-menopause	reproductive system	isoflavones	 genistein
	anti-inflammatory	various	isoflavones	equol 
Melilotus officinalis (Yellow sweet clover)	antiedema	circulatory system	coumarin	coumarin 

Lotus corniculatus (Birdsfoot trefoil)	anti-inflammatory	various	oleanolic acid	
Onobrychis viciifolia (Sainfoin)	anthelmintic	digestive system	condensed tannins	
Lespedeza capitata (Roundhead lespedeza)	kidney protection	excretory system	flavonoids	
Galega officinalis (Goatôs rue)	anti-hyperglycemia	blood	alkaloids