PHYTOTHERAPY RESEARCH
Phytother. Res. **23**, 439–446 (2009)
Published online 22 December 2008 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2709

REVIEW ARTICLE

Trifolium L. – A Review on its Phytochemical and Pharmacological Profile

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Plants from the genus *Trifolium* have been used in traditional medicine by many cultures. In Turkish folk medicine, for example, some *Trifolium* species are used for their expectorant, analgesic, antiseptic properties and also to treat rheumatic aches. Some species are also grown as pasture crops for animals in the Mediterranean. The high quercetin concentration and soyasaponin occurrence make the seeds of some *Trifolium* species a potential source of health beneficial phytochemicals for use in human nutrition. However, *Trifolium pratense* has also gained popularity due to research into its use for the treatment for menopausal symptoms. This paper provides an overview of the phytochemical and pharmacological profile of *Trifolium* species.

Keywords: Trifolium; phytochemistry; pharmacological profile; botany.

INTRODUCTION

The *Trifolium* (Leguminosae or Fabaceae) taxa is one of the most important genera of the Leguminosae family, both in terms of its agricultural value and the number of species (about 300) (Zohary and Heler, 1984). The genus *Trifolium* is distributed in temperate and subtropical regions of both hemispheres (Bisby *et al*., 1994). The Mediterranean region is very rich in *Trifolium* species (Zohary and Heler, 1984), especially in Turkey where it is widely spread and represented by 103 species (Zohary, 1970).

In Turkish folk medicine, some *Trifolium* species such as *T. repens* L., *T. arvense*, *T. pratense* L. are used as expectorants, analgesics, antiseptics and against rheumatism aches (Table 1) (Baytop, 1984). In addition, *T. pratense* L., *T. repens* L. (Fig. 1a), *T. resupinatum* L. (Fig. 1b), *T. incarnatum* L., *T. hybridum* L., *T. pannonicum* Jacq., *T. purpureum* Loisel. (Fig. 1c), *T. subterraneum* L. (Fig. 1d), *T. fragiferum* L., *T. ambiguum* M. Bieb, *T. nigrescens* Viv. subsp. *petrisavii* (Clementi) Holmboe and *T. medium* L. are important feeding materials for sheep and cattle in the Mediterranean region (Acikgoz, 2001; De Rijge *et al*., 2001; Oleszek and Stochmal, 2002). *T. repens* is an herbal plant that is used in the folk medicine of the Naga tribes of India as a deworming remedy (Tangpu *et al*., 2005).

T. pratense, well known as red, meadow, creeping or craw clover, is one of the most important forage plants.

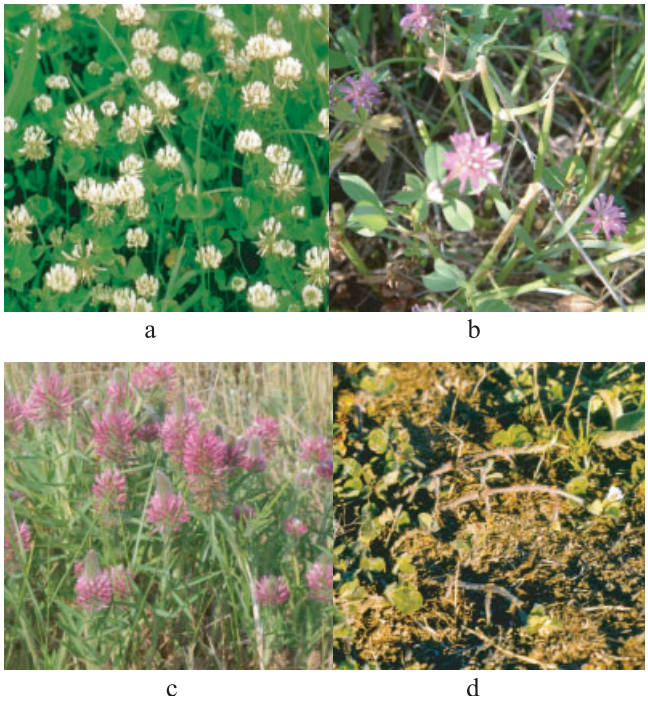

Figure 1. Some flowering species of *Trifolium:* (a) *T. repens*, (b) *T. resupinatum*, (c) *T. purpureum*, (d) *T. subterraneum* (photographs G. Savas (a, d) and N. Guler (b, c)).

It has been used by the Oriental and the European cultures, and more recently also by the Americans, as a medicinal herb for the treatment of eczema and psoriasis (Klejdus *et al*., 2001, Figueiredo *et al*., 2007). Native Americans traditionally valued red clover for the treatment of external skin problems and lung, nervous and

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Received 1 September 2008
Accepted 2 September 2008

Table 1. The uses of *Trifolium* species in the traditional medicine and cultivation, medicine and distribution

Species	Local name	English name	Uses in traditional medicine and cultivation	Uses in medicine	Origin	Distribution
T. pratense	Cayır ucgulu	Red clover, meadow clover, creeping clover, craw clover	Expectorant, analgesic, antiseptic, fodder plant	Cancer, mastitis, joint disorders, jaundice, sedative, menopausal symp., bronchitis, asthma, spasmodic coughing	Turkey	N. temperate zone
T. pratense			Psoriasis, eczema,		America	
T. arvense	Ucgul		Constipation		Turkey	Europe, Cyprus, Turkey, W. Syria, Caucasia, N. Iran
T. repens	Akucgul	White clover	Rheumatism aches, fodder plant		Turkey	Temperate Eurasia
T. repens			Deworming remedy		India	
T. alexandrinum	Iskenderiye ucgulu	Berseem clover	Antidiabetic, fodder plant		Egypt	E. Mediterranean
T. resupinatum	Iran ucgulu	Persian clover	Fodder plant		Turkey and Mediterranean	Turkey, W. Syria, N. Iraq, N. Iran, Egypt
T. incarnatum	Kırmızı ucgul	Crimson clover	Fodder plant		Turkey and Mediterranean	S. and W. Europe, Turkey
T. hybridum	Melez ucgul	Alsike clover	Fodder plant		Turkey and Mediterranean	S. Europe to Caucasia
T. pannonicum	Ucgul		Fodder plant		Turkey and Mediterranean	S.E. Europe, Turkey
T. subterraneum	Yeraltı ucgulu	Subterranean clover	Fodder plant		Turkey and Mediterranean	W. & S. Europe, S. Russia, Crimea, Georgia, Turkey, Iran, W. Syria, N.W. Africa
T. fragiferum	Cilek ucgulu	Strawberry clover	Fodder plant		Turkey and Mediterranean	Eurasia, N. Africa
T. medium	Ucgul		Fodder plant		Turkey and Mediterranean	Europe, Turkey
T. nigrescens	Anadolu ucgulu		Fodder plant		Turkey and Mediterranean	S.E. Europe, Turkey, W. Syria

reproductive system ailments. The isoflavone constituents in red clover have estrogenic properties. They are thought to have positive effects on menopausal disorders such as osteoporosis, cardiac risk factors or breast cancer (Beck *et al*., 2005; Fugh-Berman and Kronenberg, 2001). Extracts of red clover are commercially available as dietary supplements on the US and European markets (Polasek *et al*., 2007).

T. alexandrinum L. is an annual plant cultivated in Egypt (Tackholm, 1974; Muschler, 1970). Its aerial part is used as a cattle feed and the seeds are used as an antidiabetic treatment (Khaled *et al*., 2000). *T. repens* is a dominant pasture legume in a number of temperate pasture ecosystem communities, including New Zealand swards, where it performs vital functions due to its high feed value and as the major nitrogen fixing plant species (Hofmann *et al*., 2000).

MORPHOLOGICAL CHARACTERS AND CULTIVATION

Trifolium is a large genus including annual and perennial species. The leaves are trifoliate or rarely digitate with 5–9, usually dentate, leaflets; stipules conspicuous. The flowers are in sessile or pedunculate heads or short racemes, rarely solitary, bracteate or bractless. The calyx is variable, sometimes accrescent, indurated or inflated, throat open or closed by a 2-lipped callosity; teeth equal or unequal. The corolla is pink, red to purple, white or yellow, usually persistent. The stamens are diadelphous (9 + 1). The legume is usually included in the calyx, often indehiscent, 1–2 (10)-seeded. The flowering time is from March to September (Zohary, 1970).

Numerous *Trifolium* species were originally native to the middle and south of Europe, North Africa and in the area ranging from the Asia Minor to China. *Trifolium* species are found in a wide variety of moist habitats throughout those areas. Today they spread over many other parts of the world where they have accidentally been introduced. However, many *Trifolium* species are cultivated extensively as fodder plants. *Trifolium* is sown alone or in a mixture with ryegrass (*Lolium* sp.). There are several reasons for *Trifolium* cultivation: it grows freely, constitutes a good grazing plant in meadows; can be used for green and dried fodder production and soil improvement due to its ability of free nitrogen fixation; grows in a great range of soil types and climates (Zohary, 1970; Acikgoz, 2001; Keskin, 2001; Acar and Erac, 1999; Basak and Savas, 2001; Elci, 2005).

The most widely cultivated perennial *Trifolium* species as fodder plants are *T. repens* (white clover) and *T. pretense* (red clover). The other cultivated perennial species are *T. hybridum* (alsike clover), *T. fragiferum* (strawberry clover) and *T. ambiguum* (Kura clover). There are also agriculturally important annual species including: *T. resupinatum* (Persian clover), *T. nigrescens* subsp. *petrisavii* (small white or Anatolian clover), *T. incarnatum* (crimson clover), *T. alexandrinum* (Egyptian or berseem clover) and *T. subterraneum* (subterranean clover). Although cultivated fields of the latter are limited compared with perennial species, they are being cultivated for several purposes, i.e. for grazing in short-term sown grassland, production of green and dried fodder and for soil improvement with green fertilization. Among these, the most important cultivated species for Turkey are *T. repens, T. pratense* and *T. resupinatum*. *T. repens* is also very important as a park, garden and green place plant. *T. subterraneum* and *T. repens* are the most represented species in temperate and cool grassland, while *T. resupinatum, T. incarnatum* and *T. alexandrinum* are important species of temperate and moist grassland and *T. hybridum* and *T. fragiferum* are found in wet and moist meadows (Acıkgoz, 2001; Acar and Erac, 1999; Elci, 2005).

Trifolium species are generally adapted to cool and moist climate conditions. *T. ambiguum* is the most resistant species to frost and dryness. Watering is needed in areas where the rainfall is limited if one wants to obtain a good amount of crop from other species. Both *T. resupinatum* and *T. alexandrinum* are the most sensitive species to cold. *T. hybridum* is the species most resistant to lower pH levels. *T. alexandrinum* grows well in slightly sandy places, whereas the other species prefer places with good water retention properties. *T. fragiferum*, *T. hybridum* and partly *T. nigrescens* subsp. *petrisavii* are tolerant to very moist soils which resulted from the ground water. *T. fragiferum* also has tolerance for soil saltiness (Acıkgoz, 2001; Acar and Erac, 1999; Elci, 2005).

PHYTOCHEMISTRY

Flavonoids, isoflavonoids, chalcones and coumarins

Flavonoids and isoflavonoids play an important role in human nutrition as health promoting natural chemicals (Pare, 2000). Flavonoids are found throughout the plant kingdom, whereas isoflavonoids are more restricted. Isoflavonoids are particularly prevalent in the Papilonoideae subfamily of the Leguminosae (Dixon and Summer, 2003). The three main flavonoid components of *T. repens* flower extracts, quercetin-3-*O*-galactoside, its 6″-*O*-acetyl derivative and myricetin-3-*O*-galactoside were isolated and identified by spectroscopic means (Schittko *et al*., 1999). In this study, the larvae of the butterfly *Polyommatus icarus* Rott., were reared on inflorescences of *T. repens*. Individual extracts from larvae, pupae and imagines as well as extracts from exuviae, larval faeces and the host plant were analysed by HPLC.

Ponce *et al*. (2004) isolated and characterized the flavonoids in *T. repens* by chromatographic and spectrophotometric methods. This plant was grown either in the presence or absence of the arbuscular mycorrhizal fungus *Glomus intraradices* Schench & Smith. Flavones, 4′,5,6,7,8-pentahydroxy-3-methoxyflavone and 5,6,7,8-tetrahydroxy-3-methoxyflavone, as well as two flavones 3,7-dihydroxy-4′-methoxyflavone and 5,6,7,8-tetrahydroxy-4′-methoxyflavone never previously reported in plants, were isolated from *T. repens*. The known flavonoids 3,5,6,7,8-pentehydroxy-4′-methoxyflavone, 6-hydroxykaempferol, 4′,5,6,7,8-pentahydroxyflavone and 3,4′-dimethoxykaempferol were also obtained.

In another study, the flavonoid composition and concentration were determined by TLC and HPLC in the seeds of 57 species of the genus *Trifolium*. The majority of species contained quercetin as a sole flavonoid or

DOI: 10.1002/ptr

in the mixture with a number of unidentified flavonoid components. The concentration of quercetin was in the range 0.05–3 mg/g in the *Trifolium* species. The authors suggested that the seeds of some *Trifolium* species may provide a potential source of benefical phytochemicals in the human diet, due to their high concentration of quercetin (Oleszek and Stochmal, 2002).

Hofmann *et al*. (2000) identified flavonoids involved in the response of nine populations of white clover (*T. repens*) to ultraviolet-B radiation (UV-B). Methanol-water extractable flavonoids were quantified using HPLC. The HPLC analysis demonstrated that the major flavonoids present in the *T. repens* leaf samples were derivatives of the flavonols quercetin and kaempferol. The structures of the compounds involved in the response to UV-B were identified by ^{1}H and ^{13}C NMR spectroscopy to be the flavonols quercetin-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-galactopyranoside and kaempferol-3-*O*-*β*-D-xylopyranosyl-(1→ 2)-*β*-D-galactopyranoside.

Pope *et al*. (1953) isolated biochanin A from red clover (*T. pratense*) extracts. In 1965, Schultz showed that biochanin A and formononetin are present as glycosides in red clover. More recently, isoflavones, their glycosides, their glycosides malonates and their acetyl glycosides were determined in red clover extracts using chromatographic and spectrometric methods (De Rijge *et al*., 2001; Klejdus *et al*., 2001; Gu and Gu, 2001; Krenn *et al*., 2002).

Flavonoids, isoflavonoids and their glycosides have also been isolated from *T. striatum*, *T. alexandrinum*, *T. subterraneum*, *T. echinatum*, *T. polyphyllum* C. A. Meyer, *T. resupinatum* L. var. *microcephalum* Zoh., *T. pratense* and *T. repens* (Tarr, 1993; Shehata *et al*., 1982; Wang *et al*., 1998; Shalashvili, 1993; Lukyanchikov and Kazakov, 1982; Isik *et al*., 2007; Toebes *et al*., 2005; Foo *et al*., 2000).

The chalcone (2′,3′,4′,5′,6′-pentahydroxy-chalcone) and chalcanol glucosides (trifochalcanoloside I, trifochalcanoloside II and trifochalcanoloside III) were isolated from the roots of *T. repens* (Oleszek and Stochmal, 2002) and seeds of *T. alexandrinum* (Mohamed *et al*., 2000).

Two new biocoumarins, named repensin A and repensin B, were isolated from *T. repens*. On the basis of spectral data, the structures of repensin A and repensin B were established as 7-methoxy-7′,8′-dihydroxy-8,6′-biocoumarinyl and 7,5′-dihydroxy-3,6′-biocoumarinyl, respectively (Zhan *et al*., 2003).

Lipids and fatty acids

The antioxidant activity and contents of fatty acids and lipids of five *Trifolium* species, *T. balansae* Boiss., *T. stellatum* L., *T. nigrescens* subs. *petrisavii*, *T. constantinopolitanum* Ser. and *T. resupinatum* var. *resupinatum* have been investigated using GC-MS. The oil of the five *Trifolium* species showed a similar fatty acid profile. The major fatty acids were linolenic acid (16.6–31.1%), linoleic acid (5–11.3%) and palmitic acid (11.1–18.3%). In this study, eicosane was the most abundant hydrocarbon in *T. nigrescens* subs. *petrisavii* and phytol was found to be at the highest concentration in *T. resupinatum* var. *resupinatum* oil (Sabudak *et al*., 2008c).

Linolenic acid was found to be the predominant free fatty acid and triacontanol was determined as the principal free fatty alcohol in *T. repens*. The hydrocarbons of C_{29} and C_{31} were observed in the largest amounts in *T. repens* (Body, 1974; Maffei, 1996). Also, linolenic acid (61.5%) was the major component in *T. resupinatum* (Nazir and Shah, 1990).

The lipids of red clover leaves have been fractionated by chromatography on DEAE-cellulose columns to give a preliminary separation of lipid mixtures. The approximate composition of the fraction was waxes (23%), galactolipids (25%) and phospholipids (52%) (Weenink, 1964). The acetone-soluble lipids of *T. pratense* leaves have been shown to contain minor amounts of sterol esters, triglycerides, diglycerides, free sterols and hydrocarbons. These lipids are present to the extent of 0.57%, 1.50%, 0.60%, 0.80% and 0.38% of the total extract respectively (Weenink, 1962).

Sabudak *et al*. (2006, 2007) determined the lipid constituents of *T. resupinatum* var. *microcephalum* isolating two new compounds (3-methyl-1-nonene-3-ol and 2′,3′-dihydroxypropylpentadecanoate) and four known compounds (*β*-sytosterol, lupeol, coumarin, phytyl-1-hexanoate).

Volatile compounds

There is relatively little information available on the volatile compounds produced by *Trifolium* species. The essential oils of *T. repens* and *T. pratense* obtained by steam distillation were analysed by Kami (1978) who isolated about 80 compounds consisting acids, phenol, aldehydes, ketones, alcohols, esters and hydrocarbons. Srinivas (1988) identified 210 volatile constituents in CH_2Cl_2 extracts from *T. pratense*.

In another study, 25 compounds in the head space of the leaves, flowers and seed pod of *T. pratense* were determined using Tenax trapping and GLC-MS analysis. The major volatile components identified were, for the leaves, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol and (*E*)- and (*Z*)-*β*-ocimenes, for the flowers, acetophenone, methyl cinnamate and 1-phenylethanol and, for the seed pods, (*E*)- and (*Z*)-*β*-ocimenes, an unidentified sesquiterpene hydrocarbon and longifolene (Buttery *et al*., 1984).

Figueiredo *et al*. (2007) investigated the volatile profile of three red clover forages, fresh plant, hay, silage using GC and GC/MS. The most abundant identified compounds in the green sample of red clover were 3-octanol, 6,10,14-trimethyl-2-pentadecanone, benzaldehyde, (*Z*)-*β*-caryophyllene, *β*-farnesene, 3-methyl-1-butanol and 3-octanone. In the case of hay, phenylethyl alcohol, (*Z*)-*β*-caryophyllene, *β*-farnesene and 6,10,14-trimethyl-pentadecanone, are among the most abundant identified compounds. In red clover silage, the most abundant compounds were ethyl hexanoate, phenylethyl alcohol, 3-methylbutyl butanoate, 3-methyl-1-butanol, 3-methylbutanoic acid and ethyl-2-methylpentanoate.

Saponins and their glycosides and megastigmane glycosides

Triterpene saponins have been reported in *T. pratense* roots (Oleszek and Jurzysta, 1986), seeds of *T. incarnatum* (Jurzysta *et al*., 1989), *T. repens* (Sakamoto *et al*., 1992), seeds of *T. alexandrinum* (Mohamed *et al*., 1995), seeds

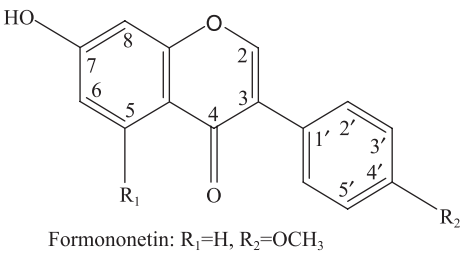
DOI: 10.1002/ptr

of *T. resupinatum* (Simonet *et al*., 1999) and seeds of 57 species of the genus *Trifolium* (Oleszek and Stochmal, 2002). It was suggested that soyasapogenol B (Fig. 2) glycosides, 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-22-*O*-β-D-glucopyranosyl, could be recognized as the chemotaxonomic character of Leguminosae (Fabaceae) family (Oleszek and Stochmal, 2002).

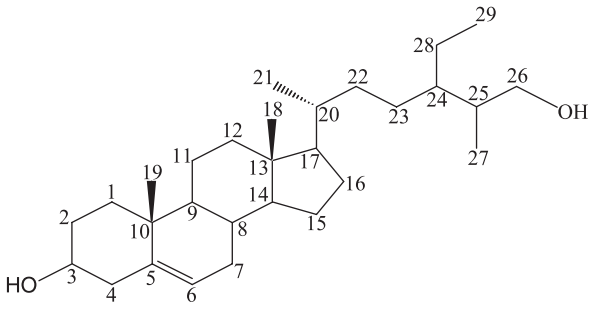
Khaled *et al*. (1999) determined five megastigmane glycosides from the seeds of *T. alexandrinum*, of which two were known compounds, while three were new compounds, trifostigmanoside I, trifostigmanoside II, trifostigmanoside III, showing the presence of apio-furanosyl-(1→2)-glucopyranosyl residue as a sugar moiety.

The other compounds

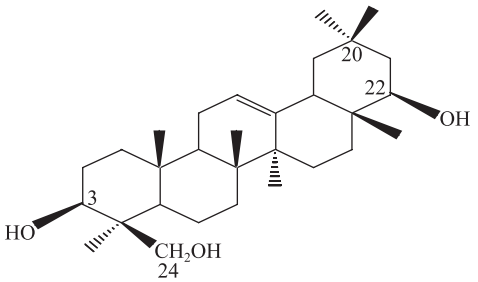
Foo *et al*. (2000) have reported the chemical nature of the proanthocyanidins and other low molecular phenolic compounds present in the flowers of *T. repens* (white clover). The phenolics (*cis*- and *trans*-p-coumaric acid-4-*O*-β-glucopyranoside), gallocatechin, epigallocatechin, gallocatechin-(4α-8)-epigallocatechin and their corresponding prodelphinidin polymers were isolated in this study.



Formononetin: R_1=H, R_2=OCH_3
Daidzein: R_1=H, R_2=OH
Genistein: R_1=OH, R_2=OH
Biochanin A: R_1=OH, R_2=OCH_3



Stigmast-5-ene-3β,26-diol



Soyasapogenol B

Figure 2. Some of the compounds isolated from *Trifolium* species.

Sabudak *et al*. (2008b) has reported the isolation and structure determination of an alkyl glucoside (1-undecene-1-*O*-β-2′,3′,4′,6′-tetra acetylglucoside) and an alkenol (4,15-dimethyl-2-(1,2-dihydroxyethyl)-hexadecene) from *T. resupinatum* var. *microcephalum*.

In another study, three steroids (stigmast-5-ene-3β,26-diol, stigmast-5-ene-3-ol (Fig. 2) and campestrol) were isolated from *T. balansae* (Sabudak *et al*., 2006).

PHARMACOLOGY

The effect of red clover isoflavones on menopausal symptoms

Herbalists have employed red clover (*T. pratense*) extracts as a blood cleaner, expectorant, alterative and sedative (Booth and Piersen, 2005). Red clover, a medicinal herb traditionally used in the treatment of chronic skin diseases, contains at least four estrogenic isoflavones: formononetin, biochanin A, daidzein and genistein (Coon *et al.*, 2007). Its extracts are becoming increasingly popular, primarily for the treatment of menopausal symptoms (Carusi, 2000; Arjmandi, 2001; Netsel *et al*., 1999; Rassi *et al*., 2002; Van de Wewijer and Barentsen, 2002). Red clover has also been promoted as a phytoestrogen source (Fugh-Berman and Kronenberg, 2001).

Besides their estrogenic activity phytoestrogens also display nonhormonal actions such as antioxidant effects and may have a possible role in cancer protection (Adlercreutz, 1995). Genistein (Fig. 2) is anticarcinogenic *in vitro*, probably due to its inhibitory effect on protein tyrosinase kinase (Reddy *et al*., 1992) and angiogenesis (Fotsis *et al*., 1993), perhaps due to its antioxidative properties. Genistein and other flavonoids have been shown to be antiproliferative with regard to breast cancer cells (Peterson and Barnes, 1991). The three main categories of phytoestrogens are isoflavones, lignans and coumestans. Red clover contains the isoflavones genistein, daidzein (Fig. 2), biochanin A (4′-methoxy-5,7-hydroxy isoflavone) (Fig. 2) and formononetin (4′-methoxy-7-hydroxy isoflavone) (Fig. 2) (Beck *et al*., 2005). Red clover includes small amounts of coumestanes (Lindner, 1976), which do not contribute to a large extent to its estrogenic effects. Genistein is the most active of the isoflavones contained in red clover (Dornstauder *et al*., 2001).

Squadrito *et al*. (2002) investigated whether genistein alters the balance between the nitric oxide products and endothelin-1 and influences endothelium-dependent vasodilation in postmenopausal women. Genistein therapy improves flow-mediated endothelium dependent vasodilation in healthy postmenopausal women. This improvement may be mediated by a direct effect of genistein on the vascular function and could be the result of an increased ratio of nitric oxide to endothelin.

However, further studies are necessary to evaluate the risks of a long-term use of phytoestrogens (Booth *et al*., 2006a). The effects of red clover extracts on menopause, bone health, cognition, cardiovascular health and breast health are discussed in several studies. Safety data on red clover extracts are also discussed (Piersen *et al*., 2004; Booth *et al*., 2005; Booth *et al*., 2006b).

DOI: 10.1002/ptr

Antiinflammatory and antioxidant activity

A study on an alcohol extract of *T. resupinatum* var. *microcephalum* revealed antiinflammatory and antioxidant activities in arthritic rats. The extract (1.35 and 13.5 mg/kg, i.p.) significantly reduced the paw edema induced by the complete Freund's adjuvant. Furthermore, it also inhibited lipid peroxidation (Sabudak *et al*., 2008a).

In an antioxidant study, the antioxidant potential of hexane extracts of five *Trifolium* species, *T. balansae*, *T. stellatum*, *T. nigrescens* subsp. *petrisavii*, *T. constantinopolitanum* and *T. resupinatum* var. *resupinatum*, was investigated by the β-carotene bleaching method, DPPH free radical scavenging activity, metal chelating activity and total phenolic content. The highest activity was observed for *T. nigrescens* and *T. constantinopolitanum* in a β-carotene-linoleic acid system. All five *Trifolium* extracts were found to be only effective in the β-carotene–linoleic acid system which indicates their antioxidant potential. This finding was parallel with the unsaturated fatty acid content in the *Trifolium* extracts (Sabudak *et al*., 2008c).

Enzyme activity

The compounds isolated from *T. balansae*, phytyl-1-hexanoate, stigmast-5-ene-3β,26-diol, stigmast-5-ene-3-ol and campestrol, pentacosanol, were tested for their enzyme tyrosinase activity. Stigmast-5-ene-3β,26-diol exhibited highly potent (IC_{50} = 2.39 μM) inhibition against the enzyme tyrosinase, when compared with the standard tyrosinase inhibitors kojic acid (KA, IC_{50} = 16.67 μM) and L-mimosine (LM, IC_{50} = 3.68 μM) (Sabudak *et al*., 2006). Tyrosinase inhibitors can be useful clinically for the treatment of some dermatological disorders associated with melanin hyperpigmentation. They are also used in cosmetics for whitening and depigmentation after sunburn (Shiino *et al*., 2001).

In another study, the effect of biochanin A on the gene regulation and enzyme activity of aromatase was investigated. By assaying MCF-7 cells stably transfected with enzyme aromatase (CYP 19), biochanin A inhibited aromatase activity and hampered cell growth attributed to the enzyme activity (Wang *et al*., 2007).

OTHER BIOLOGICAL ACTIVITIES

Tangpu *et al.* (2005) investigated the anticestodal activity of *T. repens* extract. Doses of *T. repens* aerial shoots extract, 200 and 500 mg/kg, reduced the mean fecal egg counts of *Hymenolepis diminuta* by 47.72% and 54.59% and worm recovery rate by 60.0% and 40.0%, respectively. The results of the study showed that the aerial shoots of *T. repens* bear anticestodal properties and supports its use in the traditional medicine system.

Feeding deterrent activity against the redlegged earth mite *Halotydeus destructor* was demonstrated for the compounds coumarin and β-ionone, and detected in the volatiles of *T. stirictum* and *T. glanduliferum* (Wang *et al*., 1999).

DISCUSSION AND CONCLUSIONS

Trifolium species are a potent natural source of isoflavonoids and are used in traditional medicine to treat a variety of disorders. Traditional application in humans of *Trifolium* species is supported by pharmacological investigation (Markovits *et al*., 1989; Ogawara *et al*., 1989; Reddy *et al*., 1992; Fotsis *et al*., 1993; Fugh-Berman and Kronenberg, 2001; Clifton-Bligh, 2001; Atkinson *et al*., 2004; Polkowski *et al*., 2004; Beck *et al*., 2005; Hidalgo *et al*., 2005).

The concentration of soyasapogenol glycosides in the seeds of *Trifolium* species is similar to the concentration in other leguminous plants. The high concentration of quercetin and the presence of soyasapogenol B glycosides make the seeds of some *Trifolium* species a promising plant material to be used in human nutrition as nutraceuticals or food additives (Burda and Oleszek, 2001).

Some *Trifolium* species exhibited biologically activities including antiinflammatory activity, antioxidant activity, anticestodal activity, cytostatic activity, cytotoxic activity and estrogenic activity and are used as a chemoprotective agent against cancers and cardiovascular diseases in some traditional medicinal applications (Sabudak *et al*., 2008a, 2008c, 2006; Wang *et al*., 2007; Tangpu *et al*., 2005; Polkowski *et al*., 2004; Atkinson *et al*., 2004; Clifton-Bligh *et al*., 2001; Hidalga *et al*., 2005; Fugh-Berman and Kronenberg, 2001; Beck *et al*., 2005).

T. pratense is one of the most important sources of phytoestrogens in nature. The estrogenic activity of red clover is mainly due to isoflavones and to a smaller extent due to coumestans (Beck *et al*., 2005). Genistein isolated from *Trifolium* species is a molecule of great interest as a potentially chemotherapeutic agent or as a lead compound in anticancer drug design (Polkowski *et al*., 2004). Stigmast-5-ene-3β-26-diol isolated from *T. balansae*, may be a potential candidate for the treatment of melanin biosynthesis related skin diseases, such as hyper- and hypo-pigmentation of humans as well as animals (Sabudak *et al*., 2006). However, the other biological activity studies also support the use of *Trifolium* species used in the traditional medicine system.

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