



## Biological Activities of *Trifolium Pratense*: A Review

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### Abstract

*Trifolium pratense* is an important plant of the Legume family. It has drawn the attention of several researchers around the globe. This plant was traditionally used as forage or as soil improver is now seen as the plant containing vast therapeutic activities which include anti-oxidative, anti-cancer, neuroprotective, anti-hyperglycemic, anti-hyperlipidemic, osteoprotective and cardio protective properties. The therapeutic properties are shown in various *in vivo*, *in vitro* and *ex vivo* experiments. The review highlights the *Trifolium pratense* basic knowledge its extraction, components and their actions, major activities possessed by plant along with their mechanisms. *Trifolium* plant is mainly used in menopausal women to reduce the discomfort and menopausal effects such as hot flushes and increase in breast density. The plant is also majorly responsible for preventing breast cancer and other apoptosis of moderate cancer causing cells. Various strategies were applied and the plant is still under study for further development in its effects.

**Keywords:** *Trifolium Pratense*; Cancer; *Trifolium*

### Introduction

The genus *Trifolium* comprises of almost 240 species each remarkable for its agricultural and therapeutic effects. They are widely diverse but not found in Southeast Asia and Australia. They were mostly recognized as forage plants valuable herbs in the past. Some of the trifolium species are *T. repens* (white clover), *T. pratense* (red clover), *T. fragiferum* (strawberry) and *T. hybridum* (alsike clover).

The plant *Trifolium pratense* is more commonly known as red clover. The plants from *Trifolium* species have known to be used as traditional medicine in several cultures. Some *Trifolium* plants can be used as an expectorant, analgesic, antioxidant and as anti-inflammatory. They can be used from human nutrition. The *Trifolium* genus is most important genre of Leguminosae family for its agricultural and nutritional values. The genus can be found in and subtropical and temperate regions of both hemispheres.

Powder of dried flowers is given for whooping cough as antiseptic and expectorant. It is also given in asthma and bronchitis [1].

### Synonym

The synonym of *Trifolium pratense* is *T. sativum*. In English it can also be called as meadow clover, purple clover, trefoil or simply clover.

### Family

It belongs to the family *Fabeaceae leguminosae*.

### Common name

The common names are creeping clover, cow clover; peavine clover; meadow clover, purple clover and trefoil.

### Part use

The aerial parts are mostly used but roots and stalks have also found to be therapeutic.

### Habitat

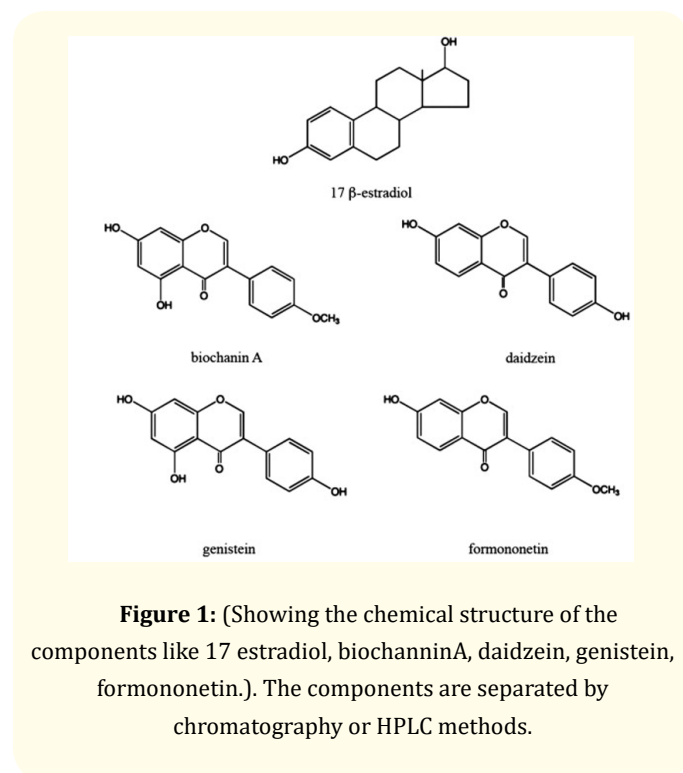
The Mediterranean and Red sea countries.

### Chemical Components

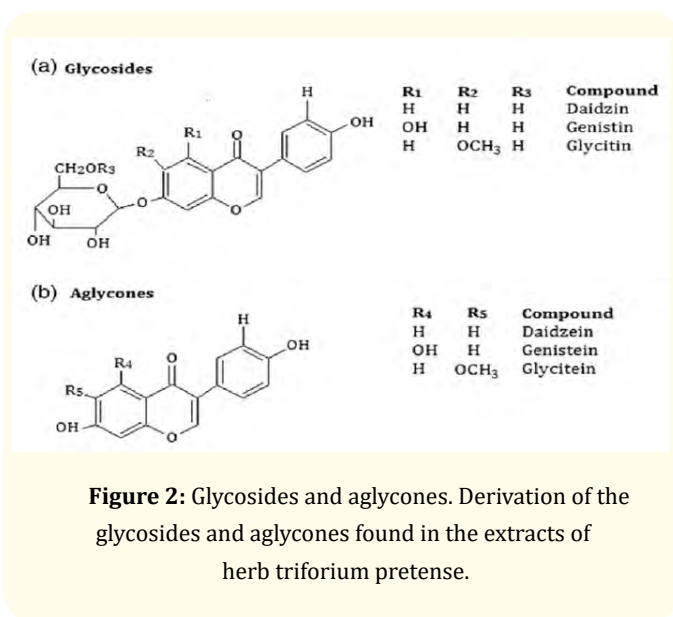
Red clover (*Trifolium pratense* L.) is a rich source of plant secondary metabolites isoflavonoids which belong to the group of phenylpropanoids. These compounds are primarily found in the Leguminosae family and can be divided into related pterocarpans and isoflavones [2]. The major chemical components found in the plants are the major compound isoflavones, being biochanin A (together with daidzein, formononetin, afrormosin, orobol, genistein, pratensein, trifoside and others), and their glycoside conjugates. Other constituents include medicagol, coumestrol, coumarin, soyasaponins, clovamides and flavonoids.

The components found abundantly in the roots are biochanin, afrormosin, daidzein, genistein, methyl orobol, irilin and irilone and components in the leaves are formononetin and biochanin A.

**Structures of components found in the plant trifolium pretense**



**Figure 1:** (Showing the chemical structure of the components like 17 estradiol, biochanninaA, daidzein, genistein, formononetin.). The components are separated by chromatography or HPLC methods.



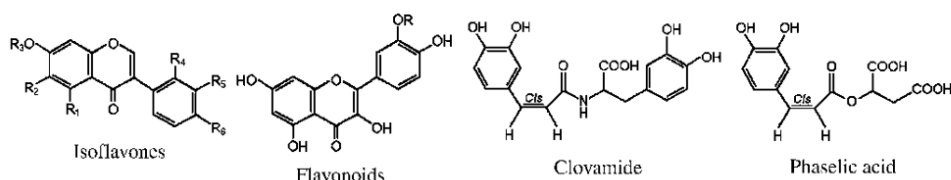
**Figure 2:** Glycosides and aglycones. Derivation of the glycosides and aglycones found in the extracts of herb trifolium pretense.

**Extraction of plant materials**

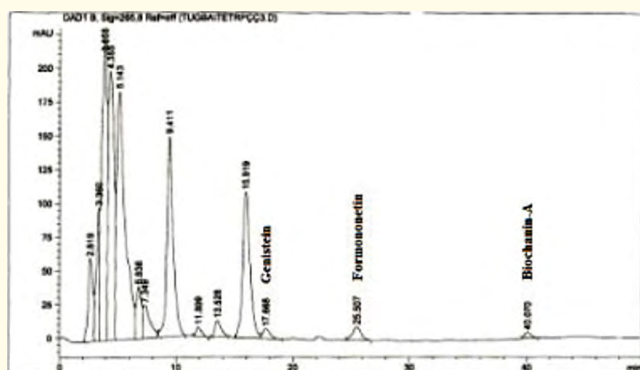
The extraction of the plant was done by the triturating the dries plant and mixing with sufficient quantity of methanol and hydrochloric acid. Butyl hydroquinone was added as an antioxidant. Sonicate the sample for 30 minutes and after 24 hours HPLC method was applied on the mixture at -25 temperature. HPLC reveled the concentration of biochanin A, isoflavones, daidzein, genistein, coumarin and other cardiac glycosides [3].

Structures of main phenolics found in red clover leaves (modified from He et al., 1996; Foo et al., 2000; Tazaki et al., 2002; Klejdus et al., 2001, 2003; Oleszek et al., 2007).

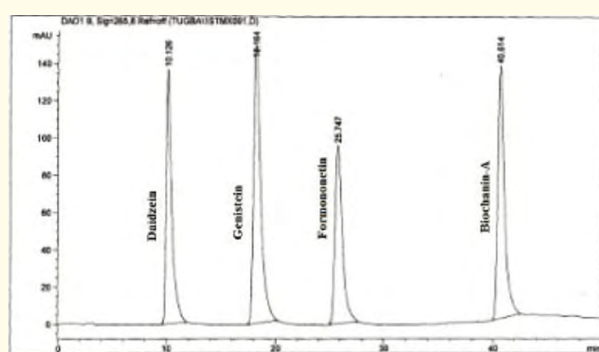
Isoflavones	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>
Afrormosin	H	OCH <sub>3</sub>	H	H	H	OCH <sub>3</sub>
Biochanin A	OH	H	H	H	H	OCH <sub>3</sub>
Calycosin	H	H	H	H	OH	OCH <sub>3</sub>
Daidzein	H	H	H	H	H	OH
Formononetin	H	H	H	H	H	OCH <sub>3</sub>
Genistein	OH	H	H	H	H	OH
Irilin B	OH	OCH <sub>3</sub>	H	OH	H	H
Irilone	OH	-O-	-CH <sub>2</sub> -	H	H	OH
Methylorobol	OH	H	H	H	OCH <sub>3</sub>	OH
Pratensein	OH	H	OH	H	OH	OCH <sub>3</sub>
Prunetin	OH	H	CH <sub>3</sub>	H	H	OH
Pseudobaptigenin	H	H	H	H	-O-	-OCH <sub>2</sub> -
Texasin	H	OH	H	H	H	OCH <sub>3</sub>
Flavonoids	R					
Kaempferol	H					
Quercetin	OH					



**Table 1:** Structures of phenolics in red clover leaves.



**Figure 3:** HPLC chromatogram of tetraploid *Trifolium pratense* flower.



**Figure 4:** Chromatogram of mixture of genistein, daidzein, biochanin A and formononetin at 254 nm by modified HPLC method (Tripathi, A. *et al.*, 2014)

### Assay

Loss on drying: Dry 1g for 2 hours at 105C. it loses not >12%.

Foreign organic matter: not >2%.

Total ash: not >10%.

Acid insoluble ash: not > 2 % [4].

### Pharmacological uses

#### External uses

It helps reduce skin aging, accelerate wound healing, and helps in healing of burn wounds. It also relieves from eczema, psoriasis and skin cancers.

#### Internal uses

#### Historical uses

Cherokee Indians used a tea of aboveground parts or the flowers to treat fevers, Bright's disease<sup>^</sup> (nephritis), and leukorrhoea. The Iroquois referred to red clover as a Blood medicine. The Nevada Ute and Utah used a decoction as an abortifacient. Red clover tea or tincture (ethanolic extract) was used as an antispasmodic in,

measles, whooping cough laryngitis, bronchitis and tuberculosis in the 19<sup>th</sup> and 20<sup>th</sup> centuries. Red clover cigarettes were considered a treatment for asthma, according to an early edition of the National Formulary.

### Cardiovascular effect

Red clover prevents incidence of coronary heart disease, increase HDL, act as antioxidant, stabilize atheroma plaque and scavenges free radicals.

A number of studies has shown that the consumption of polyphenols limits the incidence of coronary heart disease [5].

### Central nervous system effect

#### Mechanism

The isoflavones and the biochanin A are majorly responsible for the inhibition of activation of the microglia cells that leads to neuro degeneration, neuro inflammation. They are also responsible for the prevention of degeneration of the dopaminergic neurons thus controlling the transmission of the dopamine. They also inhibit the IL6 and TNF, the major causes of Parkinson's disease [6].

### Anti-diabetic effect

Impairment in glucose metabolism leads to physiological imbalance with the onset of the hyperglycemia and subsequently diabetes mellitus. Studies have shown that several physiological parameters of the body get altered in the diabetic conditions [7].

Red clover has shown hypoglycemic activity. The components have shown to prevent neuropathy and retinopathy. In some studies the insulin sensitivity of patients was also seen to be controlled at certain rate.

### Mechanism of action

Diabetes and obesity are somewhat related to each other. Being overweight places extra stress on physiological functioning including increase in glucose level.

Edible flowers from *Trifolium pratense* were evaluated for their chemical composition hypoglycaemic potential, antioxidant activity by inhibition of  $\alpha$ -glucosidase,  $\alpha$ -amylase and pancreatic lipase inhibitory properties [8].

### Ageing

Several researches suggest that the combination of anti-inflammatory/antioxidant polyphenolic compounds found in vegetables and fruits may show efficacy as anti-ageing compounds [8].

### Bone health

Isoflavones and genistein target the bone cells and prevent bone loss. It is majorly helpful in postmenopausal women. It doesn't show any hormonal activation to estrogen when given at specific dose or bone loss prevention. Reduce chance of bone fracture. Stabilizes bone mass in both pre and post-menopausal women [9].

### Cancer

Effect of polyphenols on human cancer cell lines, is most often protective and induced a reduction of the number of tumors or of their growth [10]. These effects have been observed at various sites, including stomach, mouth, duodenum, colon, liver, lung, skin or mammary gland. Many polyphenols, such as quercetin, catechins, lignans, isoflavones flavanones have been tested; all of them showed protective effects in some models although their mechanisms of action were found to be different [11].

### Breast cancer

Breast cancer prevention is especially important for postmenopausal women, because breast cancer risk increases with age. Post-menopausal women have a higher risk than premenopausal. Red clover (*Trifolium pratense*) contain estrogenic bioactive isoflavones, genistein and daidzein. In addition to their estrogenic properties, some epidemiological data suggest that these isoflavones might be contributing to the lower incidence of breast cancer [12].

### Mechanism

Isoflavones in red clover binds to the estrogen receptors ER1 and ER2 and prevents proliferation. Genistein is also responsible for anti inflammatory effect by reducing chemical carcinogenic inflammation of the breasts. Isoflavones also mediate the estrogen metabolizing enzymes thus helping in reducing the incidence of the breast cancer. They also regulate the IL-6 and tumor necrosis factor thus controlling or even reducing the incidence of cancer [13].

### Prostate cancer

Red clover studies have revealed its positive effects in lower the induction of prostate cancer if not then halt the progression of prostate cancer by apoptosis in low to moderate type tumor cells.

### Mechanism of cancer prevention

The administration of different doses i.e. 40mg\day or 80mg\day have shown to be helpful in prevention of mammographic breast density and also in the cases of family history of breast cancer (Garcia Lafuente., et al. 2009).

### Anti platelet effect

Triforium pretense is found to have an anti platelet effect as it activates the antiplatelet factor nitric oxide synthesis in the cells

and various systematic pathways. Furthermore the plants also increase 17 beta estradiol activation and endogenous nitric oxide activity expression [5].

### Toxin elimination

The components of Triforium pratensis have shown to metabolize the toxic xenobiotics or any harmful foreign particle. They help in the elimination of toxins or even the toxin metabolites by regulating the hormones.

### As a dietary supplement in menopause

*Trifolium pratense* is majorly used as a dietary supplement in the menopausal women. Different administrations of the plant give women the relief in menopause [14]. It has a potential use in treating the symptoms in menopausal women, bone health and cardiovascular activity. The plant has also shown positive effects in the reduction in breast cancer in such women and also the prevention in increase of breast density and the endometrial thickness.

Dose	Breast and endometrial effects
40 mg	No change in breast density especially in women that are at high risk to breast cancer
40\80mg	Significant decrease in breast pain
50mg	No change in antigen level when measured in the endometrial proliferation
85.5mg	No change in endometrial thickness
86mg	Non significant decrease in insulin growth factors but in premenopausal women only. In post menopausal women it showed reduction in breast pain and reduction in endometrial lining thickness.

**Table 2:** Different doses of trifolium pretense extracts showing effect to various diseases [9].

Studies have shown no effect on the endometrial thickness of trifolium pretense when taken during late follicular stage neither were any changes seen in progesteron or plasma estradiol atleast not when the plant dosage was taken for a shorter time period. The effects of several doses can be seen in table above [12].

Dose	Lipid parameters
40mg	No change in plasma lipids of women but 40% reduction in LDL of men
40\80	No change in plasma lipid of menopausal women
50	No change in plasma lipids of premenopausal women
82	Decrease in triglycerides in both pre and post menopausal women
86	Increase in HDL in post menopausal women only

**Table 3:** dose to lipid parameters.

The effect of several doses of red clover were given for six months and each group of different showed different results that can be seen in table above [4].

Dose	Menopausal symptoms
40mg	Significant decrease in hot flushes
43.5mg	No change in hot flushes
80mg	44% decrease in hot flushes

Table 4: Dose to menopausal symptoms relief.

Studies that have administered semi purified red clover preparations to women to relieve menopausal hot flashes (Table 3) have generally been of short duration (12 weeks) and any positive results [9].

### Antibacterial activity

The activity against triforium pretense against bacteria was not observed but due to the Flavonoids, biochanin and isophenolic contents present in plants the studies of microbial assay against several bacteria showed positive result to the therapy following table showing activity against a few bacteria;

Drug	Disc diffusion assay against bacteria				
	<i>Pseudomonas auregenosa</i>	<i>Escherishia coli</i>	<i>Enterobacter aerogenes</i>	<i>Enterobacter faecalis</i>	<i>Staphylococcus aureus</i>
Triforium pretense	Absence of inhibition zone	13.4+/-0.25	12.5+/-0.68	8.6+/-1.3	7.4+/-0.64

Table 5: Disc diffusion assay against bacteria.

The results show antibacterial activity against few of the gram positive and few gram negative bacteria.

### Mechanism of bacterial inhibition

Antimicrobial activity of the tasted extracts is the due to the phenolic content found in them are partially hydrophobic and are considered to interact with lipopolysaccharide and the bacterial cell wall interfaces by decreasing membrane stability [15].

### Antifungal activity

During the studies most of the red clover extracts were found to show antifungal properties especially against *D. teres*. However the extracts obtained from the roots showed more promising results. Root extracts of red clover also showed activity against low temperature fungus *M. nivale*. The studies showed that the roots not only gather carbohydrates from the environment for the survival in winter but also show defense against phytopathogenic fungi.

### Anti inflammatory activity

Excessive inflammation is considered to be a critical factor in many human diseases, including cancer, cardiovascular diseases, obesity, type II diabetes, or inflammatory bowel disease. The reported anti-inflammatory properties of natural products such as flavonoids may be a crucial factor in using these substances for the treatment of such diseases.

### Uses from literature

Red clover grows well in various environmental conditions across wide geographical areas and therefore may be an inexpensive and valuable resource for many applications [3].

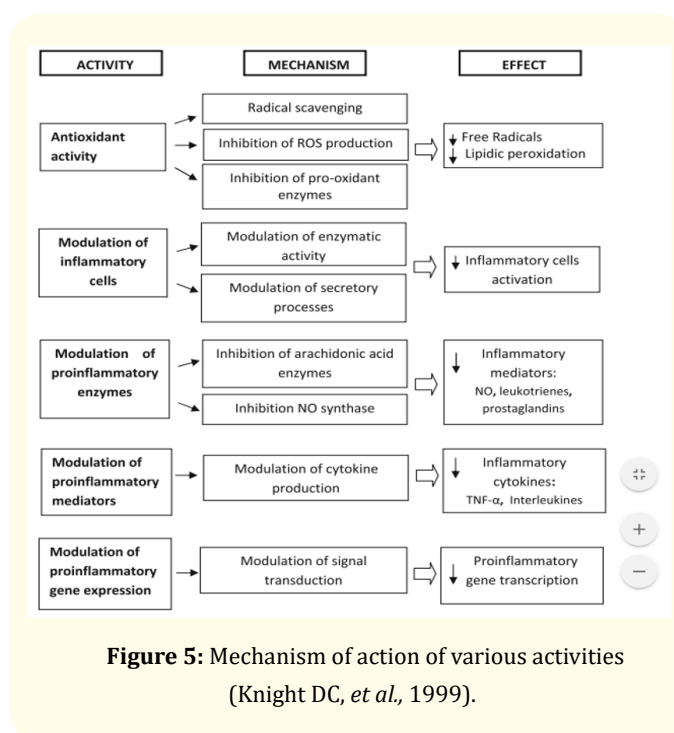


Figure 5: Mechanism of action of various activities (Knight DC, et al., 1999).

Various activity are showed in following list
Anticarcinogenic = + +
Antioxidant = ++
Cytotoxic\antitumor = +
Anti-inflammatory = +
Immune stimulant = + +
Antimicrobial = +
Choleretic = +

Table 6: Various red clover activities + = some evidence ++ = strong evidence (C. Tamayo. et al., 2000).



### Adverse effects

Only a few of the toxic effects of the herb red clover have been found during the studies. Liver toxification from clover was only found in horses. The first clinical signs are photosensitivity along with the formation of lesions on the body. The lesions can progress in form of edema, erythema and finally scab forming the surface of mucous membranes. Lesions were also seen on the cornea [2].

The condition was first thought as allergic reaction but now recognized as hepatogenous photosensitivity. As the disease progresses several symptoms have been seen such as laziness, slow gait, loss of appetite, weight loss and unpatterned hair balding [16].

### Commercial products

Promensil, Rimostil, Novogen, Trifolii rubri flos, Trifolii pratensis herba are the common products [2,11,17].

### Discussion

*Trifolium pratense* is a herb belonging to the Leguminosae family. It was first thought as a forage plant but now several properties and actions such as anti-bacterial action, anti-fungal activity, help in breast cancer, removing hot flushes in menopausal women, osteoprotective and anti-aging activities have been shown by several studies. Further clinical trials must be done on the drug to further increase its therapeutic value. Some of the effects and mechanism of the drug are not clear and some activities show effect only when the drug was taken for a longer time period so the clinical trials on this drug will help in long run [18-20].

### Conclusion

Red clover is a herb found in meadows and temperate zones. A herb that was once thought as a forage plant and for agricultural use is now showing tremendous activities upon analysis. From its activity as an antibacterial to anti cancerous and from horse feed to its postmenopausal effects the plant *Trifolium pratense* is rich in its activity and antioxidants. The components like biochanin A and isoflavonoides, genistein, daidzein, irilone etc. have been isolated from the flowering tops roots and leaves. Further studies such as anti-fungal and antiviral studies must be done the plant to further reveal its therapeutical activities.

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