



Qualitative and Quantitative Preliminary Phytochemical Study of *Trifolium tomentosum* L. - a medicinally promising herb

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Abstract:-

The present study is about the Preliminary phytochemical screening of the crude extract of *Trifolium tomentosum* L. plant specimen belongs to the Fabaceae (Leguminaceae) family exposed to find out the occurrence of various bioactive components. The specimen shows the occurrence of Phenol, alkaloids, Flavonoids, Proteins Carbohydrate, Glycosides, and Saponins mostly whereas the tannin and terpenoids are not reported. While the Carotenoids and Steroids found in trace amount.

Keywords: - *Trifolium tomentosum* L., Phytochemical, Alkaloid, Flavonoids, Saponins, Tannin.

Introduction:-

Trifolium L., the clover genus, is one of the largest and important genera in Fabaceae (Leguminaceae) family with about 250-300 species having wide distribution in temperate and subtropical regions of both hemispheres (Bisby *et al.*, 1994.). The Mediterranean region is very rich in *Trifolium* species (Zohary & Heler, 1984) especially in Turkey where it is widely spread and represented by 103 species (Zohary, 1970). It shows adaption to different agro-ecological regions (Gillet *et al.*, 2001; Ellison *et al.*, 2006).

As per the available information about the traditional medicinal uses of *Trifolium* species in all over the world, it is clear that this plant species have extremely great potential to treat the various ailments as in Turkish folk medicine, some species used as expectorants, analgesics, antiseptics and against rheumatism aches (Baytop, 1984), few species 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). The Oriental and the European cultures as well as the Americans used these herbs for the treatment of eczema and psoriasis (Klejdus *et al.*, 2001, Figueiredo *et al.*, 2007).

The estrogenic properties of some species of this genus 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), aerial part of *T. alexandrinum* L. is used as a cattle feed and the seeds are used as an antidiabetic treatment (Khaled *et al.*, 2000).

The recent findings on chemical screening concern with 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, Burda & Oleszek, 2001,).

Trifolium species as *T. burchellianum*, *T. fragiferum*, *T. hybridum*, *T. minus*, *T. philistaeum* var. *filifolium* and *T. purpureum* *T. alexandrinum*, *T. echinatum*, *T. incarnatum*, *T. repens* and *T. scabrum*. *T. pratense* exhibited biologically activities including anti-inflammatory 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.*, 2006, 2008a 2008b; Wang *et al.*, 2007; Tangpu *et al.*, 2005; Polkowski *et al.*, 2004; Atkinson *et al.*, 2004; Clifton-Bligh *et al.*, 2001; Hidalgo *et al.*, 2005; Fugh- Berman and Kronenberg, 2001; Beck *et al.*, 2005, Joanna Kolodziejczyk-Czepas, 2016 and Aldo Tava *et al.* 2019). Still to this date there is no any relevant information about the phytochemical analysis of *Trifolium tomentosum* L species, hence this plant specimen is taken for the preliminary phytochemical investigation.

Material and Methods:

Plant material

The aerial parts (Leaf, Steam and Flower) of *Trifolium tomentosum* L. were collected in required quantity from the forests of Bhandara district, (latitudes 20⁰39' and 21⁰38' North and longitudes 79⁰27' and 80⁰42' East, Specimen collected by J. V. Gadpayale- JVGBH-0104 (Herbarium voucher specimen number). The plant specimen is clearly identified with the relevant available literature of Flora of Maharashtra State: Dicotyledones, Vol. II: (Combretaceae to Ceratophyllaceae), Botanical Survey of India (Singh *et al.*, 2001) & Zohary & Heler (1984).

Preparation of the plant extracts

Freshly collected plant materials were washed with distilled water and chopped into small pieces then shade dried so as to bring down the initial large moisture content to enable its prolonged storage life. After drying, they were grinded to powder, which was later used for the preparation of solvents extracts like petroleum ether, chloroform, ethanol, methanol, hot water. Phytochemical tests were carried out on the extract of the powdered specimens using standard procedures (Harbone, 1998; Sadashivam, *et al.*, 2015)

Qualitative Analysis

The crude extract of whole aerial parts of the plant were qualitatively screened for the occurrence of various secondary metabolites such as alkaloids, phenol, flavonoids, tannins, terpenoids, steroids, carotenoids, saponins and glycosides in addition with Carbohydrate and proteins.

Quantitative phytochemical analysis

Quantitative phytochemical analysis was done for the detection of total carbohydrate by Anthrone method, total protein by Bradford method (Sadasivam & Manickam 2015), Tannin, Phenol, Alkaloid (Fazel *et al.*, 2008) and Flavonoids (Boham & Kocipai-Abyazan 1974).

Results and observations:-

A) Morphology of *Trifolium tomentosum* L.

Trifolium tomentosum Linn. Sp. pl. 771. 1753; Hossain in Notes Roy., Bot. Gard. Edinb. 23: 453. 1961; Bhellum & Magotra, A cata. Fl. Pl. Doda, Kishtwar and Ramban Districts, Kash. Himal. 69. 2012. Babu, Ind. For. 95(2):102.1969. Raizada, Suppl. Fl. Upper Gang. Plain 60. 1976. Sharma, Geobios new reports 5:53. 1986; Punj. plants 31,1990.7. *T. fragiferum* sensu Sharma & Kachroo, Fl. Jammu I: 135. 1981 non. Linn.

A diffuse, glabrous, annual herb; stem subterete, decumbent below, ascending upwards; lower leaves long petioled, leaflets digitately 3-foliolate, 1-2 x 1-1.5cm, obovate, rounded-truncate-emarginate, acutely denticulate-dentate; stipule 12-15mm long, with scarious lower halves and adnate to the petiole, ovate-lanceolate; heads axillary, pedunculate, globose, 5-8mm across; peduncles 1-2 cm long, shorter than their subtending leaves, recurved in fruiting; flowers subsessile; calyx 2-3mm long, densely hairy at the back, accrescent and globose in fruit, with 11 mm long inwardly bent and hidden upper calyx teeth; corolla purplish 4- 5mm long, macroscent, the standard obovate notched; style glabrous; pod ovoid-ellipsoid, 2-seeded, shorter than the inflated fruiting calyx.

Flowering and Fruiting: January to April.

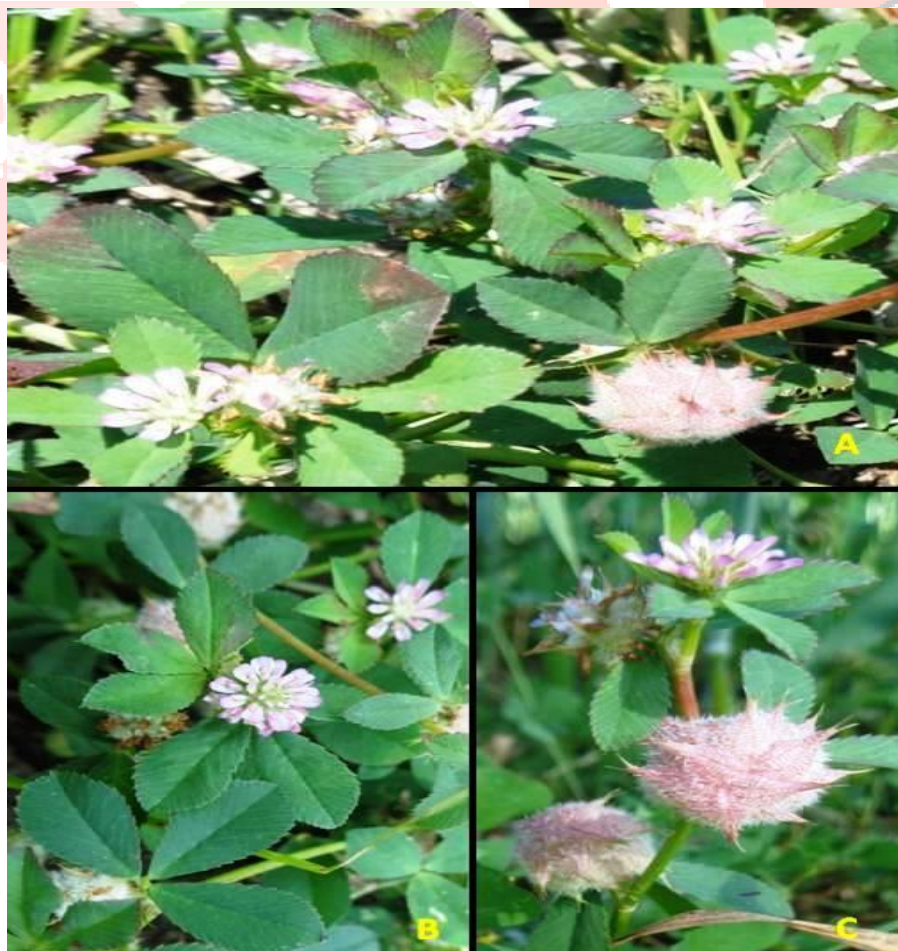


Photo plate of *Trifolium tomentosum* L: - A- Habit of plant, B- Flowering twig, C- Infructescence

B) Preliminary phytochemical analysis of aerial parts of *Trifolium tomentosum* L.:-

The extract obtained after extraction of plant material were carried out for phytochemical screening which revealed the present of various active phytoconstituents. The results are enumerated in Table No. 1. and Table No. 2.

Table 1: Preliminary phytochemical analysis of *Trifolium tomentosum* L.:-

Sr. no.	Test	Aerial plant material of <i>Trifolium tomentosum</i> L. for Preliminary phytochemical analysis in following extracts				
		PE	CL	HW	E	M
1	Phenol	++	++	+++	++	++
2	Alkaloid	++	+	++	++	++
3	Tannin	---	---	---	---	---
4	Flavonoids	++	++	+++	++	++
5	Carotenoids	+	+	+	+	+
6	Protein	+	++	++	++	++
7	Carbohydrate	++	++	++	++	++
8	Terpenoids	---	---	---	---	---
9	Glycosides	+	++	++	+	+
10	Saponins	+	+	++	+	+
11	Steroids	+	---	+	---	---

PE: Petroleum Ether, CL: Chloroform, HW: Hot Water, E: Ethanol, M: Methanol.

Table 2:- phytochemical estimation of *Trifolium tomentosum* L. (mg/g):-

Plant material	Phenol	Alkaloid	Flavonoids	Protein	Carbohydrate	Glycosides	Saponins
Aerial plant material	43.68	0.1526	15.70	0.1526	0.6543	0.5080	0.0033

The Preliminary phytochemical analysis of Aerial parts of *Trifolium tomentosum* L. for in said extracts showed the presence of Phenol, alkaloids, Flavonoids, Proteins Carbohydrate, Glycosides, and Saponins mostly whereas the tannin and terpenoids are not reported. While the Carotenoids and Steroids found in trace amount.

Conclusions

This work find out the phytochemical composition of different plants parts (Aerial parts) of the *Trifolium tomentosum* L. by using different solvents extracts. It was observed that all factors affected the chemical composition of the extracts, both quantitatively and qualitatively, concerning their total phytochemical contents. The results obtained for the aerial plant parts, it was clear that the plant is rich in Phenol, alkaloids, Flavonoids, Proteins Carbohydrate, Glycosides, and Saponins validating the use of plants for pharmaceutical/ medical purposes. This study may useful for the further pharmaceutical and R& D industry.

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