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Research Article

Preliminary Phytochemical Investigations of Three Species of Traditional Medicinal Plants of Tribal Regions of Maharashtra (India)

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ABSTRACT

Since ancient period, plant based traditional systems of medicines such as Ayurveda, Siddha, Unani, Homeopathy have greatly contributed towards primary health care of human beings. At present also many herbal drugs are being used not only in various formulations of traditional systems but also in modern medicines. The present need of the hour is to authenticate, standardize the drug and scientifically prove its efficacy. Researches are now on to study scientifically active ingredients of traditional medicines. However, lacunas have been remained in many plants, in their scientific evaluation of active ingredients. In view of this, present study of proximate preliminary qualitative phytochemical analysis of *Euphorbia ligularia*, *Tectaria coadunata* and *Clematis hedysarifolia* was carried out using standard conventional methods. For this study methanolic, aqueous and petroleum ether extracts were used. Preliminary phytochemical screening of all the three species indicated the presence of carbohydrates, tannins, anthraquinone and coumarin glycosides, steroids and flavonoids. Results of TLC and HPTLC studies of *Clematis hedysarifolia* revealed that extract contains wide range of active ingredients. The present findings are very encouraging and indicate that these plants need further extensive study for its therapeutic effect.

Keywords: Active phytochemicals, Qualitative analysis, Traditional medicines, Tribal region, Maharashtra.

INTRODUCTION

With the advent of human civilization on the earth, plant based traditional systems of medicines such as Ayurveda, Homoeopathy, Unani etc. developed and greatly contributed towards primary healthcare of mankind. The use of medicinal plants as source of medicine has been documented right from the Vedic period. *Rigveda* (500 BC), *Yajurveda* and *Atharvaveda* (4500BC-2500BC) have recorded medicinal uses of 67, 81 and 290 species respectively. Furthermore, *Charak Samhita* (700 BC) and *Sushrut Samhita* (200 BC) have described properties and usage of 1100 and 1230 species of traditional medicines. Plants have become important source of traditional medicine because plants contain numerous biologically active ingredients, that produce definite physiological action on human body and many of these have been shown to exhibit anti-inflammatory, anti-allergic, antioxidant etc. properties and therefore they have been and are being used to treat chronic and other infectious diseases. The traditional medicines continued to be used not only for primary healthcare of poor and middle-income group people in underdeveloped and developing countries but also in well developed countries where modern medicines are predominantly used in the national healthcare system. World Health Organisation (WHO) report says that more than three quarter of the world's population depend on traditional medicine for their primary healthcare. India being very vast country, having many geographical and climatic regions, has very rich phytodiversity. Flora of India has ca 17000 species of higher plants. Out of which more than 7000 species have been used by traditional healers for the treatment of human as well as animal diseases. At present in India, many herbal drugs in crude form are being used not only in various formulations of traditional systems but also in modern (allopathic) medicines. The study of phytochemicals is necessary for the discovery of potential new compounds and also in finding out new sources of ingredients (Fransworth,1966)[1]. Even though these traditional medicines are widely used, they have received very little attention in the modern research and development and efforts to upgrade the traditional health practices are very meagre. The present need of the hour is to authenticate the plant materials i.e. correct botanical identification, standardize the drug and scientifically prove its efficiency. Review of previous literature shows work on analysis of phytochemical constituents of *Clematis gouriana* (Arul H.S.J, 2014)[2] and *in vitro* antimicrobial activity and phytochemical screening of *Clematis* species of Ethiopia (Hawaze S. *et al*, 2012)[3]. Phytochemical and microbial studies of leaf extract of *Euphorbia nerifolia* showed presence of flavonoid,

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Table 1: Phytochemical analysis of methanolic, petroleum ether and aqueous extracts of the plant species studied.

Extract	Chemical groups	Reagent / Tests	Observation Colour if Positive	Name of the Plant Species		
				Clematis hedysarifolia	*Euphorbia ligularia*	*Tectaria coadunata*
Methanol	1. Carbohydrate	Benedicts	Red ppt	P	P	P
	2. Tannins and Phenolic compounds	5% FeCl3	Dark Brown colour	P	A	A
	3. Antraquinone	Borntranger	Pink Colour	A	P	P
	4. Coumarin glycosides	2N NaOH	Dark Yellow Colour	A	P	P
	5. Flavonoids	Shinoda	Red-Pink Colour	P	P	P
	6. Steroids	Libermans	Bluish Green	A	P	P
Pet –Ether	1. Carbohydrate	Benedicts	Red ppt	P	P	P
	2. Tannins and Phenolic compounds	5% FeCl3	Dark Brown colour	A	P	P
	3. Antraquinone	Borntranger	Pink Colour	A	P	P
	4. Coumarin glycosides	2N NaOH	Dark Yellow Colour	A	A	A
	5. Flavonoids	Shinoda	Red-Pink Colour	A	A	P
	6. Steroids	Libermans	Bluish Green	P	A	A
Aqueous	1. Carbohydrate	Benedicts	Red ppt	P	P	P
	2. Tannins and Phenolic compounds	5% FeCl3	Dark Brown colour	P	A	A
	3. Antraquinone	Borntranger	Pink Colour	A	A	A
	4. Coumarin glycosides	2N NaOH	Dark Yellow Colour	P	A	A
	5. Flavonoids	Shinoda	Red-Pink Colour	P	A	A
	6. Steroids	Libermans	Bluish Green	A	A	A

Table 2: TLC and HPTLC of chemical constituents and probable activity of *Clematis hedysarifolia*.

Name of species and plant parts used	Ethnic / Medicinal usage by tribals	Chemical compound present and their R*f* value and area %	Probable activity of chemical compound
Clematis hedysarifolia (Leaves, stem and branches)	. Fits / epilepsy 2. Throat problem (Throat ache)	. Phenolic acid- Rf: 0.503 (area53.55%) 2. Chlorgenic acid- Rf: 0.65 (area17.59%) 3. Antraquinone – Rf: 0.90 (area 17.02%) 4. Steroids: Rf: 0.82 (area30.36%)	Anti-inflammatory and anti-allergic activity due to higher amount of phenolic acids

tannins, saponin, terpenoid etc. (Kumarswamy M. *et al*, 2011)[4]. Sharma and Pracheta (2013)[5] worked on preliminary phytochemical evaluation of leaves of *E. nerifolia*. Mori H.*et al* (2013)[6]have studied pharmacognostical evaluation of rhizome of *Tectaria coadunata*. In the present study, therefore, we have worked on preliminary phytochemical analysis of i) Stem extract of *Euphorbia ligularia*, ii) Fronds of *Tectaria caudunata*and, iii) Stem, branch and leaf extract of *Clematis hedysarifolia*.

MATERIALS AND METHODS

Collection of plant material and authentication

In the present study plant material of following three species has been collected. *i) Euphorbia ligularia* Roxb. Family – Euphorbiaceae. Its local/vernacular name is *Saber*. Tribals use stem and branches in arthritis. Stem is vertically cut into two halves and one part is tied/applied on the aching joints. The plant material was collected from Vanganpada, Nandurbar Dist (Plate 1). *ii)Tectaria coadunata* (Wall. ex Hook. & Grev.) C. Chr. Family-Tectariaceae. Its local name is *fern*. Tribals use fronds for cough and general weakness. One teaspoonful powder of fronds is taken with a glass of water once a day for 5-6 days. The plant material was collected from Lonavala-Khandala, Pune Dist (Plate 1). *iii)Clematis hedysarifolia* DC., Family-Ranunculaceae. Its local name is *Morvel, Bendricha vel*. Tribals use its leaves in the treatment of fits and epilepsy. The leaves of *Morvel* and *Medvan* (*Dioscorea*) crushed together and its 2-3 drops are put in the nose. After sneezing affected person gets relief (Plate 1). Authentication (botanical identification) of plant

Clematis hedysarifolia

Euphorbia ligularia

Tectaria coadunata

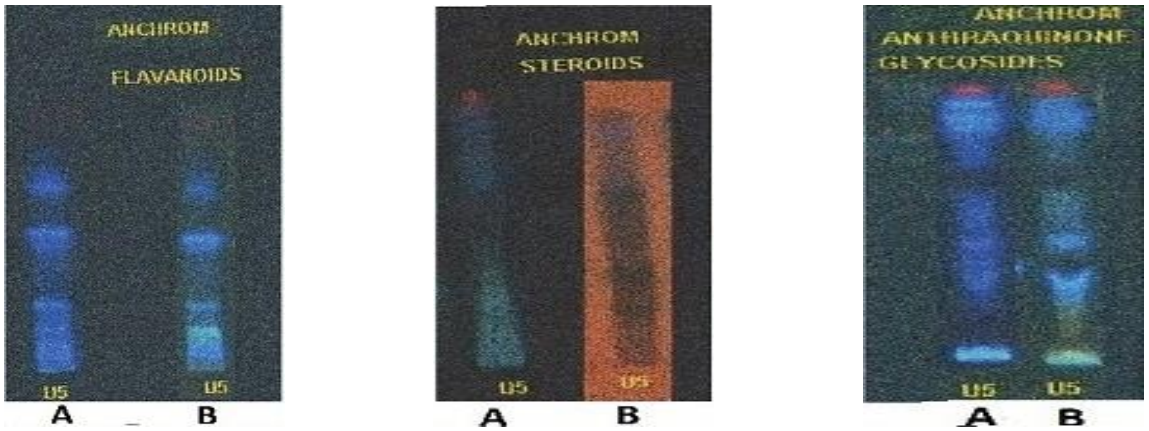

Plate 1: Chromatogram of *Clematis hedysarifolia* observed under HPTLC at 366 nm A. Before derivatisation B. After derivatisation

material of all the three species has been confirmed by Prof. S.Y. Kamble, Senior author and former Dy. Director Botanical Survey of India, Pune. The herbarium of voucher plant specimens have been prepared and deposited in the herbarium of Botany Department, Yashwantrao Mohite College, Erandwane, Pune.

Preparation of Extract

The plant material required for phytochemical analysis i.e stem, fronds, leaves and branches etc. were cut into small pieces, washed in running tap water, shade dried andground into powder by using electronic blender and coarse powder was passed through sieve no. 12 and used for extraction. This powder was soxhlet extracted with petroleum ether and methanol (quligen XLR grade). Solvent was removed by distillation method, extractive value was calculated and extract was used for further study. Methanolic extract was concentrated and dried in vaccum evaporator, extractive value was calculated and then extract was subjected to further studies. For the aqueous extract 25 gm of powder mixed in 350 ml water, stirred continuously for 6 hrs, filtered and used for further studies.

Qualitative Phytochemical Analysis

Preliminary phytochemical tests are qualitative. The methods described by Harborne (1998)[7] were used to test the presence of active ingredients. Preliminary phytochemical investigations were done in methanol, petroleum ether and aqueous extract, to detect the presence of following phytochemical constituents, using reagent/ test, mentioned in parenthesis 1) Carbohydrate (Benedicts), 2) Steroids (Libermans & Salkowskis), 3) Coumarine glycosides (2N NaOH), 4) Anthraquine glycosides (Borntrangers) 5) Tannisand phenols (5% FeCl3), 6) Flavenoids (Shinoda) and 7) Alcoloids (Mayers test). Observations and result sare shown in Table 1.

Thin Layer Chromatographic (TLC) Studies

TLC studies have been done only of*Clematis hedysarifolia*. TLC technique gives an idea about the phytoconstituents in a given extract. It is also a pilot technique for column chromatography. In TLC stationary phase is silica gel and mobile phase is solvent system. The slurry of qualigen silica gel, prepared in water, was evenly spread on TLC glass plates. The plates dried in the air, activated at 105^{o}C for 30 minute in hot air oven, cooled and used for loading the samples. For ascending TLC studies, chromatographic chambers were filled with solvent to a depth of 0.5 cm. The TLC plates were developed allowing solvent system to ascend for 10 cm. The plates removed, dried, observed under UV light and fluorescence colour was noted. The solvent system for 1) Petroliumether extract – Benzene: Acetone (97:3), 2) for methanol extract – Ethyl Acetate: GAA: Formic Acid: Water (100:11:11:26), 3) For aqueous extract – Chloroform: MeOH: Formic acid: Water (12:2:0.4:0.1) were used. In the second step for visualising the spots, plates were kept in iodine chamber. The Rf value was

noted. The plates, after removing iodine, were then sprayed with visualizing agent i.e 1% vaniline sulfuric acid. The plates were heated at 120º – 110º, till colour development. The TLC plates were scanned using scanner 3 at 366 nm and result of size, shape, Rf value andcolour intensity of spot were noted (Table 2, Plate 1).

RESULTS AND DISCUSSION

The preliminary phytochemical screening of three species under study has revealed the presence of secondary metabolites of therapeutically importance. The major phytochemicals detected were carbohydrate, tannins, phenols, anthraquinone glycosides, coumarin glycosides, flavonoids and steroids (Table 1).

- Carbohydrates are present in all extracts of all the three species.
- Tannins are present in the methanolic extract of all species. In petroleum ether extract they are present in *Euphorbia ligularia* and *Tectariacoadunata*. In aqueous extract tannins are present in *Clematis hedysarifolia*.
- In petroleum ether and aqueous extract Anthroquinones are present in *Euphoria* and *Tectaria*species.Inmethanolic extract they are absent in C*lematis hedysarifolia*.
- Coumarins in methanolic extract are present in *Euphorbia* and *Tectaria*speciesand absent in C*lamatis* species. In petroleum ether extract,coumarins are absent in all the species. While in aqueous extract they are present in *Clematis*species.
- Flavonoids inmethanolic extract are absent in C*lematis*species and present in *Euphorbia* and *Tectaria*species. In Petroleum ether extract they are present in *Tectaria*while in aqueous extract they are present in *Clematis*species.
- Steroids in methanolic and petrolium ether extract are absent in *Tectaria* and *Euphorbia* and they are present in *Clematis hedysarifolia*.Sharma and Pracheta (2013)[5] recorded the presence of glycosides, flavonoids, phenolics, alcoloids, terpenoids etc.in the leaf extract of *Euphorbia nerifolia*. Our present report, confirms the presence of carbohydrate,tannins and phenols, glycosides, flavonoids and steroids in the stem extract of *Euphorbia ligularia.*

TLC and HPTLC studies of *Clematis hedysarifolia* revealed presence of following phytoconstituents- phenolic acid (Rf-0.50 and area% 53.55), chlorogenic acid (Rf-0.65, area% 17.59), Anthraquinone glycosides (Rf-0.90, area%17.02) and Steroids (RF-0.82, area%30.36) (Table 2). HPTLC densitometric scan of plates, after derivatisation, showed many bands under UV 366 nm (Table 2. Plate 1). The result of present investigations of the phytochemicals and their probable biological activity has provided scientific support for some of its traditional uses.

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