PRELIMINARY PHYTOCHEMICAL SCREENING AND HPTLC ANALYSIS OF LEAF EXTRACT OF CROTALARIA JUNCEA FROM VIDARBHA REGION, MS, INDIA.

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ABSTRACT
Native shrub Crotalaria juncea from Vidarbha region of central India is explored as it has a part of ethanomedicinal system of tribal community. Present study is aiming to identification and quantification of active constituents in C. juncea. Preliminary phytochemical screening of all three types of aqueous (AqE), methanol (ME) and petroleum ether (PEE) extracts showed the presence of alkaloids, terpenes, tannins, saponnins, glycosides, phenolic compounds and flavonoids. The HPTLC analysis, chromatograph of methanolic leaf extract of Crotalaria junceaat 366 nm showed total 10 peaks while at 540 nm revealed 06 peaks with the Rf values of the peaks ranging from 0.01 to 0.94 resp. It has concluded that in all three types of extracts contain not a single compound but a mixture of compounds and so it is proven thatthe pharmacological activity shown by them are due to the collective effect of all the compounds in combined.
KEYWORDS: phytochemical; chromatograph; ethanomedicinal.

INTRODUCTION
Natural herbal products are playing important roles in treating and preventing human diseases as well as providing nutrition (Rautet al., 2009). In India, several medical systems have evolved and prominent among these systems are Ayurveda, Siddha and the Unani Systems of Medicine. In different civilizations the contribution of floral biodiversity to health care has been well documented (Posey, 1999). Because of the accelerated local, national and international interest in recent years the demand for medicinal and aromatic plants has increased manifolds and pharmaceutical industry views plant wealth as a source of income. Due to easy availability, no side-effects, and sometimes only source of health care, the demand for medicinal plants is increasing in both developing and developed countries. In recent days, plant based nutritional products are also making boom. Crotalaria is one of most important genus of Fabaceae, prominently herbaceous in nature. Crotalaria is the largest legume genus in India, having 93 species. Most species of Crotalaria are being used as wild medicinal plants (Vijaykumar et al., 2003). It was also reported to have curative properties (Kirtikar and Basu, 1935 and Chopra et al., 1956). Crotalaria juncea L., a native of India, is a fast growing annual crop. It is an important source of natural fibre. Traditionally its fiber is used in preparation of ropes, twines, fishing nets, tat-patties, handmade paper etc. (Tripathi et al., 2012). It has been
identified as the most promising indigenous raw material for manufacturing of high quality tissue paper, cigarette paper and paper for currency. It is one of the most outstanding green manure crops suited to almost all parts of the India (Ram and Singh, 2011). In Hawaii, sun hemp (Tropic Sun) has

added 150 to 165 kg of nitrogen per hectare to the soil when grown for 60 days and then incorporated in test plots (Rotar and Joy, 1983). Species from this genus possesses medicinal properties and is also used as forage to a limited extent. The present study was done with contemporary methods describing the identification and quantification of active constituents in the Crotalaria juncea L., Which is expedient for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques applying appropriate standards. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time. Crotalaria juncea L. (Fabaceae) is an enormous shrub, which holds a vital place in local tribal medicine. It is broadly used by native ayurvedic medical practitioners and local tribal population of Vidarbha region for treating jaundice, paralysis and intestinal worm. In this present study the Preliminary phytochemical screening of Crotalaria juncea L .has been done with leaf extraction and identify the chemical constituents HPTLC fingerprinting technique is used. Leaf extracts had been performed which may be used as markers for quality evaluation and standardization of the medicine.

MATERIALS AND METHODS

The present study is focused on the pharmacognostic, phytochemical and HPTLC fingerprinting analysis of some Crotalaria juncea found in Vidarbha region of Maharashtra State (India).

Study Area: Vidarbha region is the eastern part of Maharashtra state, situated in the central position of India. It comprises two major divisions (Nagpur and Amravati) out of total six divisions of Maharashtra. Amravati divisions former name was Berar region (in local- Varhad). Vidarbha comprises total 11 districts (Map1). This region holds 31.6% of total area and 21.3% of population of Maharashtra.

Collection of Plant Material and processing: Plant material of Crotalaria juncea L. required for the study was collected randomly from various location of Akola, Amravati and Buldhana Districts by doing several seasonal survey, during 2015-2017. The plant is photographed, identified and then collected for the experimental purpose. Collected plant identification had been done with the help of floras (Naik, 1998 and Singh and Karthikeyan, 2001).

Preliminary Phytochemistry- Preliminary phytochemical study of each powdered sample was done using the established methods of Harborne (1973), Krishanaiah et al. (2009) and Koche et al., (2010). For the phytochemical analysis, all chemicals used were of analytical grade (AR grade) and manufactured by SD fine, India. For phytochemical analysis, the extracts were prepared by taking 2gms of each dried powder into separate 100 ml conical flask and 50ml of each solvent (Aqueous, Methanol, Petroleum ether) was added. The conical flasks were plugged with cotton plugs, labeled and allowed to stand for about 2 hrs and then filtered using Whatman No.1 filter paper. Thus, the filtrates obtained were used as test solutions. After the addition of specific reagents to the tests of alkaloids, phenolic, flavonoids, terpenoids, tannins, glycosides, steroids, proteins were detected by visual observation of color change or by precipitate formation.

HPTLC analysis:

High performance thin layer chromatographic (HPTLC) analysis of all samples was done. The powdered samples were sent to Mr. Biologist Analyticals, Pune for analysis. The samples were

extracted in methanol by Soxhlet extraction method and then concentrated. The final concentration of the extract was 01 mg /ml. The analysis was done using the HPTLC system of Shimadzu coupled with CAMAG derivatizer and WINCAT software. The mobile phase used for the study was Toluene: Ethyl acetate: Glacial acetic acid (05: 04: 0.8) (All chemicals were procured from Rankem India., AR grade). The wavelengths used for the study were 366nm and 540nm.The HPTLC operations were done at 28oC ± 2oC. For comparison, Gallic acid (Sigma make) was used as standard. Further, unidentified peaks of the chromatographs were correlated with the RF values of other standard phenolic compounds RF values reported in standard journals (Hingse et al., 2014 and Dinakaran et al., 2018).

RESULT AND DISCUSSION

The preliminary phytochemical analysis of powdered material of Crotalaria juncea is presented in table 1. The observations showed that the species selected for study are rich in phytochemical composition. In all three types of extracts aqueous (AqE), methanol (ME) and petroleum ether (PEE) etc. Most of the phytochemicals were observed positive in Methanol extract are alkaloids, phenolics, flavonoids, tannins, glycosides, steroids and proteins. While in Aqueous extract it was positive for flavonoids, glycosides, saponnis and in petroleum ether extract only two phytochemicals terpenoids and tannins were observed positive.

HPTLC analysis of methanol leaf extract of Crotalaria juncea at 366 nm: The HPTLC chromatograph of methanolic leaf extract of Crotalaria juncea at 366 nm showed total 10 peaks with the Rf values ranging from 0.01 to 0.94 (Fig.2.1 and table 2). Out of these 10 peaks, two peaks (peak no. 4 and 5) with Rf values 0.67 and 0.69 respectively are correlated with Rf values of standard Caffeic acid and Coumaric acid respectively. The peak area of these two peaks was 12329.2 and 7239.3. However, remaining eight peaks remain unidentified. HPTLC analysis of methanol leaf extract of Crotalaria junceaat 540 nm: At 540 nm, the HPTLC chromatogram of C. juncea showed 06 peaks. The Rf values of the peaks ranging from 0.01 to 0.94 (Fig. 4.27 and table 4.4a). The peak values of all six peaks in the chromatogram were 0.01, 0.24, 0.69, 0.79, 0.88 and 0.94. Of these, only one peak (peak number 3) matched with the standard gallic acid (Rf Value 0.69). It has the peak area of 2423.9. Remaining peaks were unidentified (Fig. 3.1 and table 3)

CONCLUSION

Currently, the interest in study of herbal Medicines is growing rapidly, especially as a part of drug discovery programs. We have shown interest to isolate the pure constituents responsible for the above mentioned pharmacological action. While performing HPTLC, the result showed that there are many compounds in Crotalaria juncea. Which revealed that in all three types of extracts aqueous (AqE), methanol (ME) and petroleum ether (PEE) extracts contain not a single compound but a mixture of compounds and so it is proven that the pharmacological activity shown by them are due to the collective effect of all the compounds in combined.

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Tables and Figures:

Phytochemical	*Crotalaria juncea*		
	Aqueous extract	Methanol extract	Petrol um ether extract
Alkoloids	-	+	-
Phenolics	-	+	-
Flavonoids	+	+	-
Terpenoids	-	-	+
Tannins	-	+	+
Glycosides	+	+	-
Steroids	-	+	-
Saponnins	+	-	-
Proteins	-	+	-

Table 1: Preliminary Phytochemistry of leaf extract of Crotolariajuncea collected from study area.

Map 1. – Vidarbha Region

Peak no.	Conc.	Wave length	Rf value	Area	Comp. identified as
1	10µl	366	0.01	9731.7	Unidentified
2	10µl	366	0.24	1387.4	Unidentified
3	10µl	366	0.43	2204.6	Unidentified
4	10µl	366	0.67	12329.2	**Caffeic acid**
5	10µl	366	0.69	7239.3	**Coumaric acid**
6	10µl	366	0.79	4292.2	Unidentified
7	10µl	366	0.83	1652.3	Unidentified
8	10µl	366	0.88	1350.0	Unidentified
9	10µl	366	0.91	990.6	Unidentified
10	10µl	366	0.94	652.5	Unidentified

Table-2: Different parameters of HPTLC Chromatograph of methanol leaf extract at 366nm.

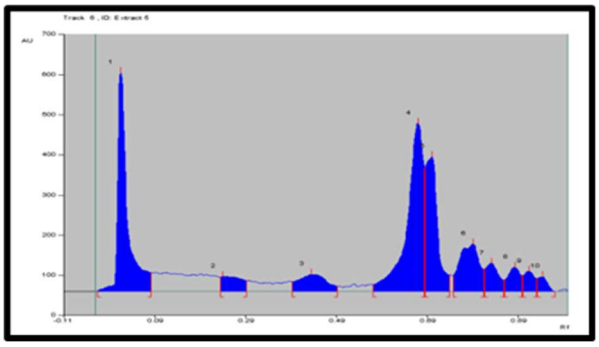

Fig. 2.1: HPTLC chromatograph of methanol leaf extract of Crotalaria junceaat 366 nm.

Peak no.	Conc.	Wavelength	Rf value	Area	Comp. identified as
1	10µl	540	0.01	2984.0	Unidentified
2	10µl	540	0.24	1292.1	Unidentified
3	10µl	540	0.69	2423.9	**Gallic acid**
4	10µl	540	0.79	1686.0	Unidentified
5	10µl	540	0.88	518.4	Unidentified
6	10µl	540	0.94	229.3	Unidentified

Table-3: Different parameters of HPTLC Chromatograph of methanol leaf extract at 540 nm.

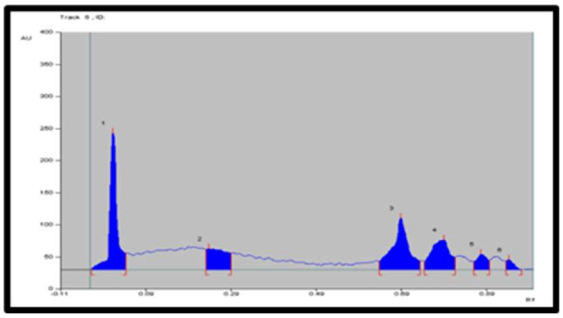

Fig. 3.1: HPTLC chromatograph of methanol leaf extract of Crotalaria junceaat 540 nm.

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