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HPTLC profiling of two ethno medicinally important species of *Calatropis*

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ABSTRACT

Calatropis is one of the common and ethnomedicinally most important genus belonging to family Asclepideaceae. Two species of *Calatropis* namely *Calatropis procera* and *Calatropis gigantia* are found in India. In the present investigation root, stem and leaf extracts of both the species were compared using HPTLC. The densitometric analysis showed slight difference in the fingerprints of both the medicinal species. Rf values and peaks of densitogram also showed chemical variation. Therefore HPTLC fingerprint analysis carried out in the absence of any standard was found to be informative enough to identify and to evaluate phytochemical variations present in between these two species.

Key words: *Asclepiadaceae* , *Calatropis*, HPTLC.

INTRODUCTION

Calatropis is small genus of about 6 species of shrubs or small trees distributed tropical and subtropical Africa, Asia and Central America. In India only two species of *Calatropis* namely *Calatropis procera* and *Calatropis gigantia* have been reported so far. Both the species closely resembles each other in structure and find similar uses *(Kirtikar et al., 1994).* The *Calatropis procera* is commonly having purple flower whereas *Calatropis gigantia* has white coloured flowers. The main physical difference in both the species is floral colour, therefore it is hard to recognize the species without flowering, for identification certain chemical parameters needs to be used *(Verma, 2013).*

Medicinal plants are said to be backbone of traditional remedy. The traditional medicines related to treatment of both human and animal diseases with plant derived preparations is providing valuable knowledge for treatment *(Nwosu and Okfar, 1995).* Traditional literature contains large number of medicinal plants including *Calatropis* that can be used against various diseases. *Calatropis gigantia* can be used against diseases like diabetes, atherosclerosis, ischeamic heart diseases, disorders induced by free radicals and other reactive oxygen species *(Chandrabhan et al., 2011).*

C. gigantia having antidibetic properties provide useful source for the development of drugs in the treatment of diabetes from ancient times. 'Swarnabhasma' an ayurvedic preparation containing *Calatropis gigantia* is extensively used by Ayurvedic physicians for treatment of bronchial asthama, rheumatoid arthritis and nervous disorders.

Calotropis gigantea has the following potential pharmacological properties; wound healing antidiarrhoeal, CNS depressant activity, antipyretic and analgesic, anti-inflammatory, analgesic activity. The roots of *Calotropis gigantea* have been used inleprosy, eczema, syphilis, elephantiasis, ulceration, and cough in the Indian system of traditionalmedicine. It contains alkaloids, tannins, phenols, resins, tetra and pentacyclic triterpenoids, cardiac glycosides. Its use in hepatitis has been illustrated in Indian System ofMedicine. The folks and Vaidyas have clinically used it successfully *(Chandrabhan et al., 2011).* Similarly different parts of *Calatropis procera* are traditionally used to cure various types of diseases such as fevers rheumatism, indigestion, cough, cold, eczma, asthma, elephantiasis, nausea, vomiting, leprosy and diarrhoea *(Arjun et al., 2014).* The latex is used for treating ringworms, guinea worm blisters, scorpion stings. Hence both the species of *Calatropis* have wide spectrum of disease curing ability. Non flowering plants of *Calatropis* are morphologically more or less similar and therefore finds difficult to identify correctly. In order to make correct identification of various plant parts and to prepare its phytochemical fingerprint we have made an attempt to compare HPTLC profile of both the species.

METHODOLOGY

Collection of Plant Material

For the present study two species of *Calatropis* i.e. *Calatropis procera* and *Calatropis gigentia* were selected. The plant materials like roots, stem and leaves of both species were collected from the open field separately in separate polythene bags. The materials were brought to laboratory and is dried separately under the shade until dryness. After complete drying the plant material were powdered using mechanical grinder.

Extraction of plant Materials

About 20 gm of grinded materials were extracted separately using 70% ethanol in a Soxhlet Extractor (Borosil) for about six hours. After extraction the extracts were evaporated to dryness. The dried extracts were redissolved in 5 ml methanol and filtered using Whatmann filter. The filtered extracts were later used for HPTLC analysis.

HPTLC Analysis of Extracts

HPTLC fingerprinting of two species were carried out as per the method described by *Anupama et al. (2015).*

Three microliters of the ethanolic extract was applied (band length 8.0 mm) on a precoated TLC aluminum sheets of silica gel G60 F254 of 200 µm thickness plate 10 x10cm (Merck, Mumbai) using Linomat 5 TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100µL syringe. Prior application, the plate was pre-washed with methanol AR and dried at 60°C. TLC plates were developed using the mobile phase Toluene: Ethyl acetate: Formic acid (7.5:2.5:0.5) in a Camag HPTLC twin-trough chamber (10 x10cm). The chamber was saturated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. Plate was developed up to 85.0 mm and dried under stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner 4 in the absorption mode (multi wavelength Scanning) operated by WINCATS software (version 1.4.8). After scanning the spectra and tables thus obtained were analyzed to interpret the results

RESULTS.

Herbals plants are effective source of traditional and modern medicines, useful for primary health care. Plants are richest source of bioactive organic chemicals on earth. The active metabolites like phytochemicals from the medicinal plants were under exploration for the development of novel and biodegradable effective drugs as an alternative to the ineffective contemporary medicine *(Suchita et al., 2014).* HPTLC profiling of *Calatropis* species shows most promising results.

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Calatropis procera* root *showed* four polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.08 to 0.88 in which highest conc. of the phytoconstituents was found to be 56.08% and its corresponding Rf value was found to be 0.08 respectively. This is recorded in Table 1. The corresponding HPTLC chromatogram is presented in Figure 1 which shows four peaks of phytoconstituents.

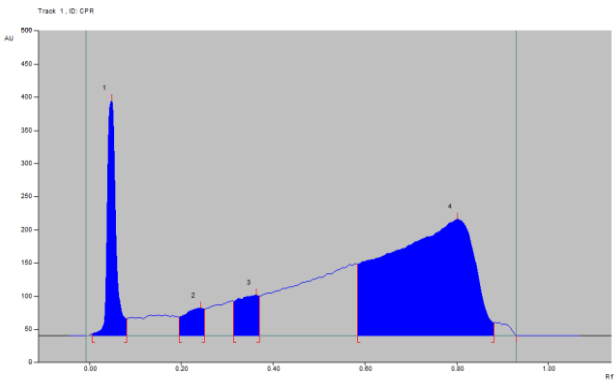

Fig 1: HPTLC profile (Peak Display) of *Calatropis procera* roots

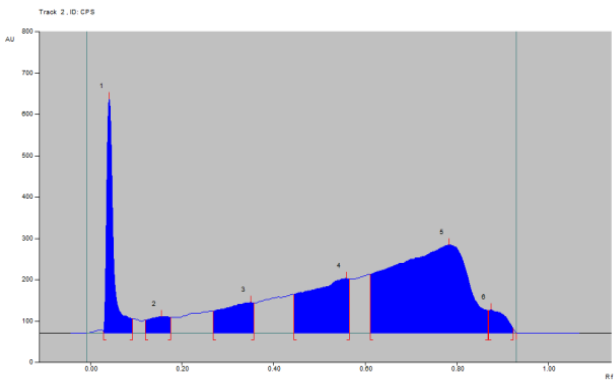

Fig 2: HPTLC profile (Peak Display) of *Calatropis procera* stem.

Table 1: HPTLC profile (Peak Table) of *Calatropis procera* roots

Track 1, ID: CPR

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.9 AU	0.05 Rf	354.8 AU	56.08 %	0.08 Rf	25.3 AU	5592.1 AU	15.14 %
2	0.20 Rf	28.3 AU	0.24 Rf	41.4 AU	6.54 %	0.25 Rf	40.5 AU	1490.4 AU	4.04 %
3	0.32 Rf	52.0 AU	0.37 Rf	60.8 AU	9.61 %	0.37 Rf	59.7 AU	2358.3 AU	6.39 %
4	0.58 Rf	107.7 AU	0.80 Rf	175.7 AU	27.77 %	0.88 Rf	19.5 AU	27489.4 AU	74.44 %

Table 2: HPTLC profile (Peak Table) of *Calatropis procera* stem

Track 2, ID: CPS

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	3.3 AU	0.04 Rf	566.2 AU	52.40 %	0.09 Rf	34.7 AU	7212.8 AU	13.25 %
2	0.12 Rf	30.9 AU	0.16 Rf	40.0 AU	3.71 %	0.18 Rf	37.5 AU	1514.0 AU	2.78 %
3	0.27 Rf	53.2 AU	0.35 Rf	73.8 AU	6.83 %	0.36 Rf	72.6 AU	4205.4 AU	7.73 %
4	0.45 Rf	93.4 AU	0.56 Rf	131.7 AU	12.18 %	0.57 Rf	30.5 AU	9845.6 AU	18.09 %
5	0.61 Rf	142.4 AU	0.78 Rf	213.0 AU	19.72 %	0.87 Rf	54.2 AU	29965.1 AU	55.05 %
6	0.87 Rf	54.4 AU	0.88 Rf	55.8 AU	5.17 %	0.93 Rf	11.2 AU	1688.8 AU	3.10 %

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Calatropis procera* stem showed six polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.09 to 0.93 in which highest conc. of the phytoconstituents was found to be 52.04% and its corresponding Rf value was found to be 0.09 respectively. This is recorded in Table 2. The corresponding HPTLC chromatogram ispresented in Figure 2 which shows six peaks of phytoconstituents.

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Calatropis procera* leaf showed seven polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.11 to 0.86 in which highest conc. of the phytoconstituents was found to be 40.52% and its corresponding Rf value was found to be 0.11 respectively. This is recorded in Table 3. The corresponding HPTLC chromatogram is presented in Figure 3 which shows seven peaks of phytoconstituents

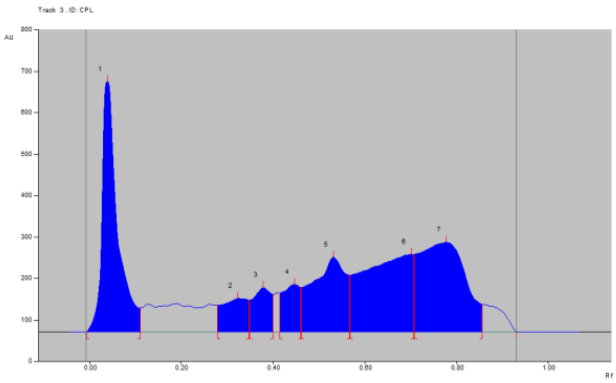

Fig 3: HPTLC profile (Peak Display) of *Calatropis procera* leaf.

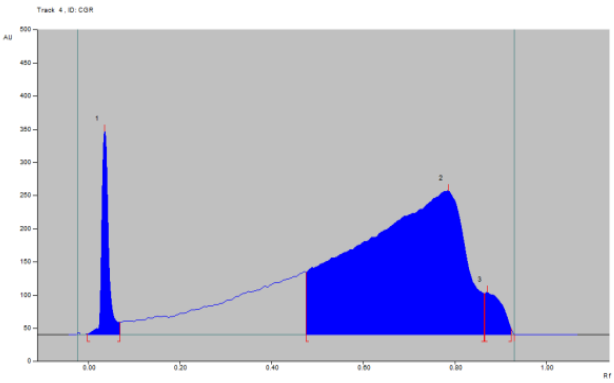

Fig 4: HPTLC profile (Peak Display) of *Calatropis gigantia* root.

Table 3: HPTLC profile (Peak Table) of *Calatropis procera* leaf

Track 3, ID: CPL

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	1.0 AU	0.04 Rf	603.2 AU	40.52 %	0.11 Rf	57.4 AU	17186.1 AU	23.38 %
2	0.28 Rf	63.7 AU	0.32 Rf	80.8 AU	5.42 %	0.35 Rf	76.7 AU	3690.0 AU	5.02 %
3	0.35 Rf	76.8 AU	0.38 Rf	106.9 AU	7.18 %	0.40 Rf	90.8 AU	3555.0 AU	4.84 %
4	0.42 Rf	93.2 AU	0.45 Rf	114.3 AU	7.68 %	0.46 Rf	07.2 AU	3585.5 AU	4.88 %
5	0.46 Rf	107.2 AU	0.53 Rf	180.1 AU	12.09 %	0.57 Rf	36.6 AU	10755.8 AU	14.63 %
6	0.57 Rf	136.8 AU	0.70 Rf	187.5 AU	12.59 %	0.71 Rf	86.8 AU	16492.8 AU	22.44 %
7	0.71 Rf	186.8 AU	0.78 Rf	216.1 AU	14.52 %	0.86 Rf	66.9 AU	18240.4 AU	24.81 %

Table 4: HPTLC profile (Peak Table) of *Calatropis gigantia* root.

Track 4, ID: CGR

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	1.1 AU	0.04 Rf	306.7 AU	52.24 %	0.07 Rf	18.1 AU	3883.0 AU	8.17 %
2	0.48 Rf	94.2 AU	0.79 Rf	216.6 AU	36.90 %	0.87 Rf	61.5 AU	41616.3 AU	87.59 %
3	0.87 Rf	61.7 AU	0.87 Rf	63.7 AU	10.86 %	0.93 Rf	9.8 AU	2013.4 AU	4.24 %

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Calatropis gigantia* root showed three polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.07 to 0.93 in which highest conc. of the phytoconstituents was found to be 52.54% and its corresponding Rf value was found to be 0.07 respectively. This is recorded in Table 4. The corresponding HPTLC chromatogram is presented in Figure 4 which shows three peaks of phytoconstituents.

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Calatropis gigantia* stem showed five polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.11 to 0.92 in which highest conc. of the phytoconstituents was found to be 48.34% and its corresponding Rf value was found to be 0.11 respectively. This is recorded in Table 5.

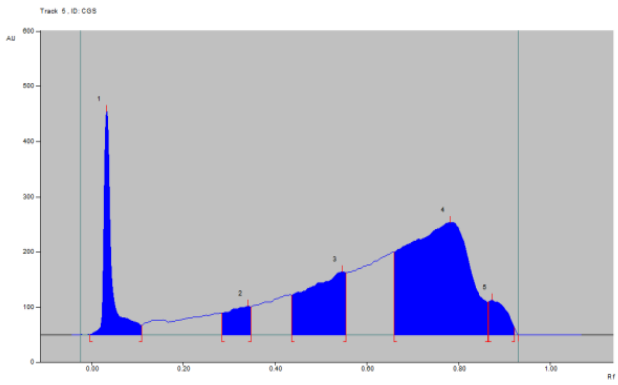

Fig 5: HPTLC profile (Peak Display) of *Calatropis gigantia* stem

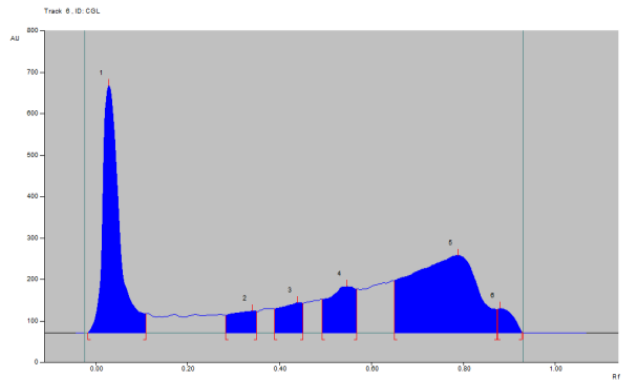

Fig 6: HPTLC profile (Peak Display) of *Calatropis gigantia* leaf.

Table 5: HPTLC profile (Peak Table) of *Calatropis gigantia* stem

Track 5, ID: CGS

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	0.6 AU	0.03 Rf	404.6 AU	48.34 %	0.11 Rf	17.5 AU	5781.1 AU	13.99 %
2	0.28 Rf	39.6 AU	0.34 Rf	51.8 AU	6.19 %	0.35 Rf	50.4 AU	2179.8 AU	5.27 %
3	0.44 Rf	72.1 AU	0.55 Rf	113.9 AU	13.61 %	0.56 Rf	12.6 AU	7967.4 AU	19.28 %
4	0.66 Rf	149.9 AU	0.78 Rf	203.6 AU	24.33 %	0.87 Rf	59.2 AU	23391.1 AU	56.59 %
5	0.87 Rf	59.6 AU	0.88 Rf	63.0 AU	7.52 %	0.92 Rf	13.9 AU	2012.4 AU	4.87 %

Table 6: HPTLC profile (Peak Table) of *Calatropis gigantia* leaf.

Track 6, ID: CGL

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.5 AU	0.03 Rf	595.9 AU	55.24 %	0.11 Rf	46.4 AU	17655.6 AU	33.27 %
2	0.28 Rf	42.1 AU	0.34 Rf	52.7 AU	4.88 %	0.35 Rf	51.8 AU	2435.7 AU	4.59 %
3	0.39 Rf	58.1 AU	0.44 Rf	72.8 AU	6.75 %	0.45 Rf	71.6 AU	3045.1 AU	5.74 %
4	0.49 Rf	80.9 AU	0.55 Rf	111.8 AU	10.37 %	0.57 Rf	05.4 AU	5444.8 AU	10.26 %
5	0.65 Rf	126.2 AU	0.79 Rf	186.6 AU	17.30 %	0.88 Rf	56.6 AU	22863.6 AU	43.09 %
6	0.88 Rf	56.9 AU	0.88 Rf	58.9 AU	5.46 %	0.93 Rf	2.5 AU	1618.5 AU	3.05 %

The corresponding HPTLC chromatogram is presented in Figure 5 which shows five peaks of phytoconstituents. The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Calatropis gigantia* leaf showed six polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.11 to 0.93 in which highest conc. of the phytoconstituents was found to be 55.24% and its corresponding Rf value was found to be 0.11 respectively. This is recorded in Table 6. The corresponding HPTLC chromatogram is presented in Figure 6 which shows six peaks of phytoconstituents.

DISCUSSION

The medicinal plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Different parts of the plant have immense

potential to cure various diseases and disorders . It is used in various polyherbal preparations. *Calotropis* is used alone and sometimes with other plants to cure variety of human and animals ailments *(Kumar et al., 2013).*

Some plants are of a great importance due to their special constituents. These constituents have chemical substances that produce definite physiological action in human body when consumed in some fixed amount. Generally in plants these bioactive constituents are alkoids, tannins, flavonoids, and phenolic compounds. HPTLC finger printing is a valuable quality assessment tool for the evaluation of botanical materials, it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods as the spots are well resolved. The HPTLC method is simple, rapid, accurate, reproducible, selective and economic, can be used for quality control analysis and for quantitative determination of the plant material *(Palani and Natesan, 2011).*

In current investigation we compared HPTLC fingerprint of root, stem and leaves of both species. Roots of *Calatropis procera* and *C. gigantia* showed presence of five and three polyvalent compounds with Rf values ranging from 0.01 to 0.88 and 0.07 to 0.93 respectively. Similarly stem of *Calatropis procera* and *C. gigantia* showed presence of six and five polyvalent phytoconstituents with Rf values ranging from 0.09 to 0.93 and 0.11 to 0.92 respectively. The phytochemical comparison of leaf extracts showed presence of seven and six polyvalant compounds with Rf value ranging from 0.11 to 0.86 and 0.11 to 0.93 respectively. The results reveal that the extract of *Calotropis* have a number of chemical constituents, which may responsible for many therapeutical activities. Findings of present investigation clearly showed that the two species of *Calatropis* shows different chemical composition. Therefore the fingerprint developed during our study can be used for the correct identification of different plant parts of *Calatropis* before using for medicinal purpose.

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