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PRELIMINARY PHYTOCHEMICAL SCREENING AND HPTLC FINGERPRINT PROFILE OF *TRAGIA PLUKENETII*

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ABSTRACT: Herbal medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history. The validation of these novel products needs powerful analytical devices tailored for the study of herbal extracts in order to assess composition and face their natural complexity as a resource. The last item is important and crucial for the capacity and utility of the analytical results that means that each product should be analyzed with the right approach. Having in mind these arguments, the present study was selected HPTLC as useful tool for the analysis. The present study is mainly focused to establish the fingerprint profile of *Tragia plukenetii* R. Smith using high performance thin layer chromatography (HPTLC) and Preliminary phytochemical screening. Preliminary phytochemical screening of the extract showed the presence of alkaloids, triterpenes, phenolic compounds and steroids. HPTLC finger printing of chloroform extract of leaf revealed 15 peaks with R_f values in the range of 0.11 to 1.0. It can be concluded that HPTLC fingerprint analysis of leaf extract of *Tragia plukenetii* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

INTRODUCTION: Since ancient times, plants have been used as an important source of medicines due to their pharmaceutically significant contents of bioactive components [1]. In order to meet their basic needs, man has always had a close relationship with plants [2]. Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment [3].

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More than 422000 species of flowering plants have been reported from all over the world which 5000 species among them are used for medicinal purposes [4]. Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [5].

There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [6].

Various extraction methods and analytical methods as spectrophotometry, high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC–MS) and Fluorescence Transmission-Infrared Spectroscopy (FT-IR) are developed for the determination of about plant active compounds [7]. High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis.

HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug [8]. HPTLC also facilitates repeated detection of chromatogram with same or different parameters [9, 10].

The present research deals with the phytochemical investigation and development of HPTLC fingerprints of ethanol extract of *Tragia plukenetii* R. Smith belongs to the family *Euphorbiaceae. Tragia plukenetii* R. Smith (Tamil name: Karunkanchori) the root is diaphoretic and alterative and is given for fevers to cause perspiration [11].

MATERIALS AND METHODS: Mature and healthy plants of *Tragia plukenetii* were collected from Southern Western Ghats in the district of Tirunelveli, South India. The specimens were identified, comparing the characteristics of floral and vegetative characters in the 'Flora of the Presidency of Madras [12] and 'Flora of Tamilnadu Carnatic [13]. Voucher specimens are documented in the herbarium of St. Xavier's College (XCH), Palayamkottai, Tamil Nadu, India.

Preparation of ethanolic extract: A weighed quantity of powder was subjected to continuous hot percolation in soxhlet apparatus with ethanol at 65-70^{o}C. The extracts were evaporated under reduced pressure using rota flash evaporator until all the solvent had been removed. The yield of the extract was 10% w/w. when compared to the dried starting material.

Preliminary phytochemical screening: The extracts obtained from the selected plant was subjected to qualitative tests for the identification of various plant constituents by the methods described by [14] **(Table 1).** The preliminary phytochemical screening is a qualitative chemical evaluation which indicates spectrum of chemical constituents in the chosen plant. The chemicals and solvents used throughout the investigation were of analytical grade.

HPTLC studies (High Performance Thin layer Chromatography): HPTLC analysis on ethanolic extract of *Tragia plukenetii* was carried out in (WI/SOP/CSM/C 008, PD Sethi HPTLC 1996) Captain Srinivasa Murti Research Institute of Ayurveda and Siddha Drug Development (Central Council for Research in Ayurveda and Siddha, New Delhi) Ministry of Health & Family Welfare, Govt. of India. Arignar Anna Government Hospital Campus, Arumbakkam, Chennai- 600106.

HPTLC methodology: One gram of extract of each added with 25 ml of ethanol. Boiled for three minutes and made up to 15 ml in graduated tube. The solution was filtered and 10 microlitre of was applied on Merck aluminum plate $60F_{254}$ precoated with silica gel of 0.3 mm. thickness and the plate was developed in Toluene: Ethyl acetate: formic acid (5:1:5:0:5). The plate was air dried and scanned at UV 254 nm using deuterium lamp in Camag HPTLC instrument provided with win cats.

Documentation: Quantitative results were obtained by adding together the areas of every peak in the chromatogram, and expressing each as a percentage of the total. The results were always given as a percentage. All work performed was documented in a project worksheet. The output was recorded as a series of peaks - each one representing a compound in the mixture passing through the detector and absorbing UV light.

RESULTS AND DISCUSSION:

Preliminary phytochemical Screening: The aerial part powders with various extracts were subjected to preliminary phytochemical screening and presented in **Table 1.** The aerial part powder with various extracts showed positives for alkaloids, phenol, triterpenoids and steroids. Terpenoids therapeutically exhibits activities like analgesic, carminatives, anthelmintics, antiseptic, counter irritant [15].

Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers. Tannins can cause regression of tumors that are already present in tissue [16]. Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities [17].

Polyphenols (phenolic acid, stilbenes, tannins, isoflavones green tea catechins) have been reported to inhibit the reproduction and growth of many fungi, yeasts, viruses and bacteria [18]. Phenolics acid possesses diverse biological activities, for instance, antiulcer, anti- inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic, and antidepressant activities [19].

TABLE: 1. PHYTOCHEMICAL SCREENING OF *TRAGIA PLUKENETII*

Extracts	Steroids	Triterpenoids	Reducing Sugars	Alkaloids	Phenols	Flavonoids	Catachins	Saponins	Tannins	Anthroquinones	Amino acids
Petroleum ether	+	+	–	–	+	–	–	+	–	–	–
Chloroform	+	+	-	+	–	–	–	–	–	–	–
Benzene	+	+	–	–	+	–	–	–	–	–	+
Ethanol	+	+	–	+	+	+	–	+	+	–	–

HPTLC Studies: Among different chemical solvents, ethanol extracts have shown more positive results. The HPTLC finger print scanned at wavelength 400 nm for ethanol extract of *Tragia plukenetii* was shown 15 peaks **(Table 2)** (**Plate 1**) with maximum R_f (0.11, 0.22, 0.26, 0.31, 0.40, 0.44, 0.51, 0.56, 0.59, 0.67, 0.74, 0.78, 0.85, 0.93, 1.0 with a percentage area of 0.60, 4.30, 4.24, 2.11, 23.07, 4.04, 8.41, 4.59, 1.75, 7.86, 6.76, 8.66, 10.50, 3.08, 10.02) in the mobile phase tolune : ethyl acetate : formic acid (5:1.5:0.5). Two major peaks were revealed at R_f 0.40 and 0.85 with the area percentage of 23.07 and 10.50 **(Table 2).** HPTLC fingerprint studies confirmed the results of phytochemical screening by the presence of various coloured bands at different wavelengths with specific solvent systems, symbolizing the presence of particular phytocompounds.

TABLE 2: HPTLC DATA - *TRAGIA PLUKENETI*

Peak	Start position Rf	Start Height	Max Position	Max Height	Max%	End Position	End Height	Area Au	Area%
1	0.07	0.2	0.09	10.0	0.93	0.11	0.2	166.2	0.60
2	0.18	1.6	0.20	63.6	5.93	0.22	27.4	1182.0	4.30
3	0.22	27.9	0.24	57.3	5.33	0.26	11.2	1164.0	4.24
4	0.26	11.2	0.28	31.8	2.97	0.31	3.8	580.0	2.11
5	0.31	3.8	0.36	239.4	22.3	0.40	37.5	6340.3	23.07
6	0.40	37.5	0.41	44.6	4.15	0.44	0.4	1109.70	4.04
7	0.45	0.5	0.48	86.8	8.09	0.51	14.2	2312.10	8.41
8	0.51	14.4	0.53	47.5	4.42	0.56	18.3	1262.0	4.59
9	0.56	18.6	0.58	28.6	2.67	0.59	26.6	481.9	1.75
10	0.59	26.8	0.62	60.3	5.61	0.67	0.4	2159.4	7.86
11	0.68	0.2	0.72	80.2	7.47	0.74	40.0	1858.6	6.76
12	0.74	41.1	0.76	106.5	9.92	0.78	34.7	2379.2	8.66
13	0.78	34.9	0.81	96.3	8.97	0.85	5.5	2886.5	10.50
14	0.88	0.1	0.91	36.1	3.36	0.93	31.9	846.8	3.08
15	0.93	31.9	0.97	84.7	7.89	1.00	4.1	2754.1	10.02

HPTLC fingerprint analysis not only gives the idea for the authentication of the plant extracts and its constituents but also provides the parameters for quality of herbal formulations. HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant.

The chromatographic fingerprint, therefore is suitable for monitoring the identity and purity profile of a plant extract. In addition to qualitative detection, HPTLC technique also provides semi quantitative information about the major active phytoconstituents present in a plant extract, thus enabling an assessment of plant extract quality.

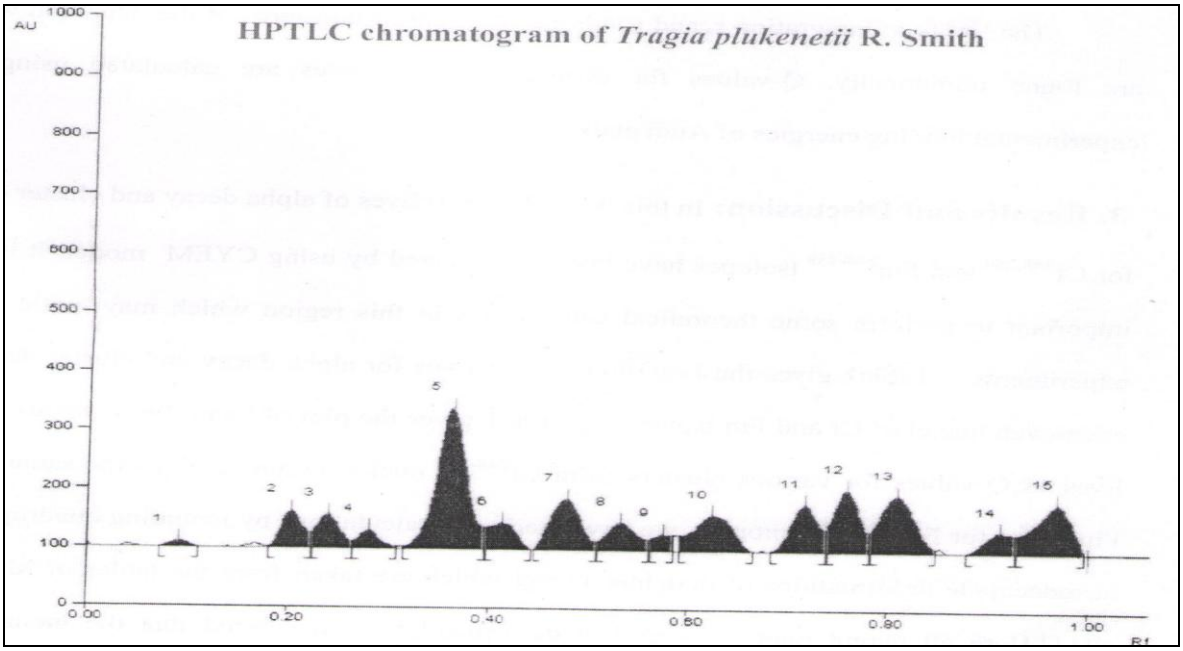

PLATE 1: HPTLC CHROMATOGRAM OF *TRAGIA PLUKENETII* R. SMITH

CONCLUSION: Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixing standards to this plant. In conclusion, the results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. It can be concluded that HPTLC fingerprint analysis of leaf extract of *Tragia plukenetii* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant population.

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