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Phytochemical Evaluation and HPTLC Fingerprint Profile of *Peltophorum Pterocarpum* (L.) Merr. Flower Extract

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ABSTRACT

Peltophorum pterocarpum traditionally been used as astringent, anti-inflammatory, carminative purgative, demulcent and anthelmintic. Phytochemical analysis of the plant showed the presence of alkaloids, carbohydrates, proteins, phytosterol, phenol, flavonoids etc. In the present study, an attempt was made to quantify the flavonoid quercetin in the flower extract. TLC was done to confirm the presence of quercetin and HPTLC method has been developed for quantification of quercetin in the ethanol flower extract. TLC silica gel 60 F 254 plate was used as stationary phase and the solvent system toluene: chloroform: ethanol (5.5:3.5:1) as the mobile phase. Quantitative analysis was carried out in the absorbance at 200,254,366 nm. A good linear relationship 0.99926 was obtained between the concentration ranges of 100-600 ng.

Keywords: Peltophorum pterocarpum, anti-inflammatory, quercetin, TLC, HPTLC.

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The substances having medical value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Products of primary metabolism such as aminoacids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, and terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance [1]. Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some need to be explored [2]. The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bio-activities [3]. Identification and quality evaluation of crude herbal extracts is a fundamental requirement. It is an accepted fact that the qualitative analysis of crude herbal extracts constitutes an important and reliable part of quality control protocol as any change in the quality of extract directly affects the constituents. Standardization and quality control of herbal drugs is very complicated because herbal products contain a group of phytoconstituents and are very capable of variation. There is the

variability within the same plant material or between the different parts of the same plant. The variability may be from grower to grower, crop to crop and also depends on the harvest and post harvest handling. On the other hand herbal drugs have multiple phytoconstituents including active, inactive, unknown which are dietary rather than therapeutic [4]. Hence, methodologies that can generate a fingerprint of each extract in large collections would be useful to detect stability of the same extract over time. Preferably, the method should be based on electronic storage, retrieval and analysis of the data [5]. 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 study about plant active compounds [6]. 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. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters [7, 8, 9]. *Peltophorum pterocarpum* (Yellow flame tree) belongs to family Leguminosae. It is a semi-wild Indian copper pod. It is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections and it is widely used in traditional medicinal system of India. It has been reported to possess hepatoprotective, anti-inflammatory, antifungal and antibacterial properties [10]. The present research deals with the phytochemical investigation and development of HPTLC fingerprints of ethanol extracts of *Peltophorum pterocarpum* flowers which can be used for identification, authentication and characterization.

MATERIALS AND METHODS

Collection and Identification of Plant Material: Peltophorum pterocarpum belonging to family Leguminosae was selected on the basis of traditional applications and pharmacological reports. The flowers of *Peltophorum pterocarpum* were collected locally from different areas of Kalyan (Maharashtra), India in the month of October and November 2013. The flowers was dried in shade at room temperature and mechanically crushed.

Reagent and Chemicals: All organic solvents petroleum ether, chloroform, ethyl acetate, ethanol, toluene, n-hexane and inorganic reagents $FeCl_3$, CH_3COOH, NaOH, $NaHCO_3$ used were of Qualigens analytical grade. Column chromatography was carried out using Qualigens Silica gel (60-120 mesh). Thin layer chromatography was done using TLC plate (7.5" x 2").

Preparation of Plant Extract: Dried and crushed flowrs of *Peltophorum pterocarpum* (1 Kg) was extracted with ethanol for 24 h and with waters for 16 h. This water extract is transferred into a separatory funnel and treated with chloroform then organic phase is removed using rotary evaporator. Some amount of water extract refrigerated as such. After evaporation of solvent in vacuum, dark yellow coloured gummy mass (60 g) was obtained. The solubility of residue was checked in different solvent. The residue was analysed with TLC using different solvent system to get an idea about the number of components present. The solvent system used for this purpose are petroleum ether – diethyl ether (5:5 v/v), di-ethyl ether-chloroform (5:5 v/v), diethyl ether – carbon tetra chloride (5:5 v/v), petroleum ether – ethyl acetate (7:3 v/v) and different composition of petroleum ether – chloroform ranging from (8:2 to 2:8 v/v) but the most suitable solvent system was found to be petroleum ether – ethyl acetate (9.9:0.1 v/v).

Qualitative Phytochemical Analysis The extracts were tested for the presence of bioactive compounds by using standard methods [11, 12].

Flavonoids: Extract mixed with few fragments of magnesium turnings. Concentrated HCl was added drop wise. Appearance of pink scarlet colour after few minutes indicates the presence of flavonoids.

Phenols and Tannins: The sample mixed with 2mL of 2% solution of $FeCl_3$. A blue-green or black coloration indicates the presence of phenols and tannins.

Saponins: 5ml of distilled water mixed with extract in a test tube shaken vigorously. The formation of stable foam is taken as an indication for the presence of saponins.

Alkaloids: 2mL of 1% HCl mixed with crude extract and heated gently. Mayer's and Wagner's reagent was added to the mixture. Turbidity of the resulting precipitate is taken as evidence for the presence of alkaloids.

High Pressure Thin Layer Chromatography (HPTLC) Profile: Chromatographic fingerprint profile of ethanol extracts of Peltophorum pterocarpum were studied by HPTLC.

Sample Preparation and Application: 5 mg mL^{-1} concentration of extracts were prepared in respective solvents of chromatographic grade and then filtered by Whatman filter paper No. 1. Prepared samples of different extracts were applied on TLC aluminium sheets silica gel 60 F 254 (Merck) 07 µL each with band length of 6 mm using Linomat 5 sample applicator set at a speed of 150 µL sec^{-1}.

Developing Solvent System: A number of solvent systems were tried, for each extract for better resolution and maximum number of spots, but the satisfactory resolution was obtained in the solvent Toluene: chloroform: ethanol:: 5.5:3.5:1.

Development of Chromatogram: The automatic and reproducibly developing chamber ADCZ, saturated with Toluene: Chloroform: ethanol (5.5:3.5:1) for 20 min at room temperature upto the distance of 90 mm from the point of application.

Scanning and Detection of spots: The air dried plates were viewed in ultraviolet radiation to mid-day light (Figure 1). Spots were visible without derivatization at 254 and 366 nm wavelengths but best results were shown when TLC plates were sprayed with detection reagent (Anisaldehyde sulfuric acid reagent and plate was heated at 120°C for 5 minutes) and then visualized in UV- visible light range 200-700nm. Scanning was performed by CAMAG HPTLC Densitometer (Scanner 3) in absorbance mode at both 254 and 337 nm, The source of radiation were deuterium and tungsten lamp with slit dimension 26.0 X 0.30 mm. A CAMAG Digistore 2 digital system with win CATS software 1.4.3 was used for the documentation of derivatised plates.

RESULTS AND DISCUSSION

Phytochemical Screening: The Phytochemical tests on methanol and ethyl acetate extracts of P.pterocarpum flowers showed the presence of various phytoconstituents like alkaloids, saponins, flavonoids, phenols and tannins.

HPTLC Profile: The study revealed that P. pterocarpum showed best results in Toluene: Chloroform: ethanol: 5.5:3.5:1 solvent system for the extract. After scanning and visualizing the plates in absorbance mode at 200 nm, 254 nm, 366 nm and multiwavelength scanning 200-700nm after spraying with anisaldehyde sulphuric acid reagent) best results were shown at 366nm. The HPTLC images shown in figure 1 indicate that all sample constituents were clearly separated without any tailing and diffuseness. The results from HPTLC finger print scanned at wavelength 200, 254,366 nm for ethanol extract of P pterocarpum flowers revealed the presence of sixteen to twenty polyvalent phytoconstituents (Table 2).

The Rf values ranged from 0.06 to 0.84. It is also clear from table 2 and the chromatogram as shown in figure 2 and 3 that out of 16 components, the component with Rf values 0.34, 0.38 and 0.84 were found to be more predominant as the percentage area is more with 14.02%, 17.28 and 12.5% respectively. TLC plate showed different colour phytoconstituents of P.pterocarpum ethanol extract. The bands revealed presence of one greenish, three purple, one pink and two light yellowish orange bands showing the presence of steroids, terpenoids and saponins after spraying with anisaldehyde sulphuric acid reagent.

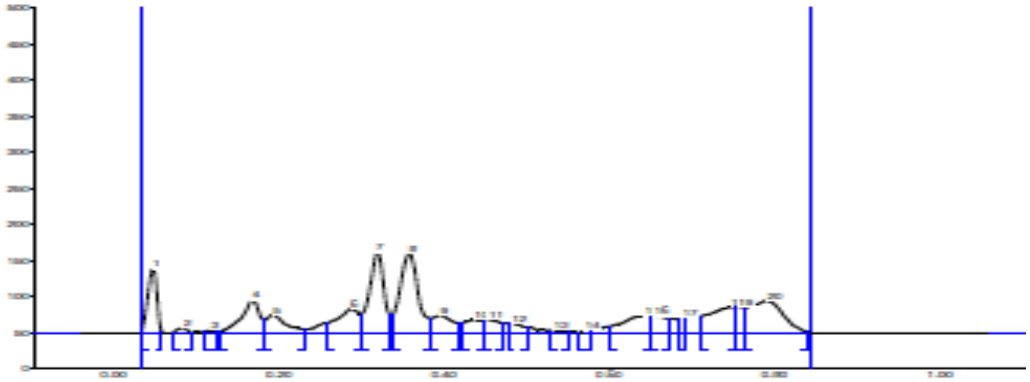

Fig 1. Chromatogram of *Peltophorum pterocarpum* at 254nm

Table 1: Showing peaks at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	6.9	0.05	88.3	12.97	0.06	0.8	926.9	6.97
2	0.07	0.1	0.09	6.9	1.02	0.09	0.2	66.2	0.50
3	0.11	1.4	0.12	3.2	0.47	0.13	2.1	31.6	0.24
4	0.13	2.7	0.17	45.3	6.66	0.18	17.1	932.3	7.01
5	0.18	17.4	0.19	24.6	3.62	0.23	4.5	541.2	4.07
6	0.26	12.5	0.29	32.8	4.81	0.30	25.3	834.6	6.28
7	0.30	25.9	0.32	110.8	16.27	0.34	23.2	1863.3	14.02
8	0.34	23.3	0.36	110.3	16.19	0.38	19.9	2297.2	17.28
9	0.39	19.9	0.40	23.4	3.43	0.42	13.5	523.8	3.94
10	0.42	12.6	0.44	17.9	2.63	0.45	16.7	352.7	2.65
11	0.45	16.8	0.45	18.4	2.70	0.47	13.4	307.4	2.31
12	0.48	12.2	0.48	13.2	1.94	0.50	7.1	210.4	1.58
13	0.53	2.7	0.53	4.0	0.59	0.55	0.6	51.1	0.38
14	0.56	0.0	0.57	2.3	0.33	0.58	1.7	16.8	0.13
15	0.60	8.3	0.64	22.3	3.28	0.65	21.7	688.7	5.18
16	0.65	21.6	0.66	22.6	3.31	0.67	18.9	421.5	3.17
17	0.68	18.0	0.69	19.1	2.80	0.69	18.4	149.4	1.12
18	0.71	22.0	0.75	35.7	5.24	0.75	35.1	1090.7	8.21
19	0.76	35.4	0.76	35.6	5.22	0.77	33.6	314.7	2.37
20	0.77	33.6	0.79	44.4	6.53	0.84	0.9	1670.9	12.57

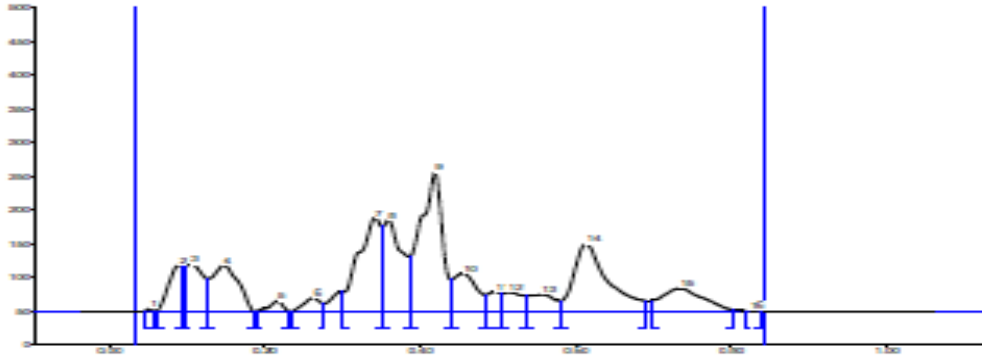

Fig 2. Chromatogram of *Peltophorum pterocarpum* at 366nm

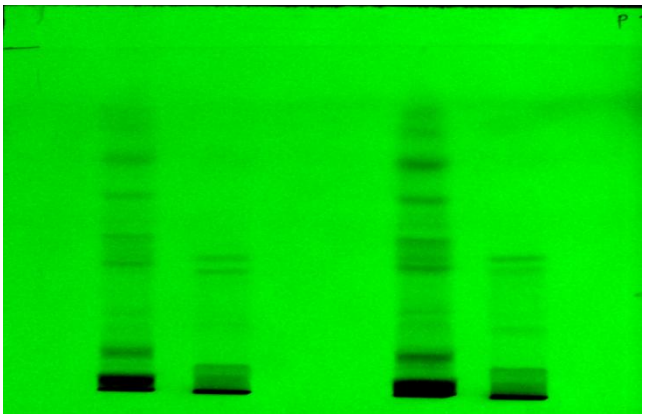

Fig 3. HPTLC finger print of *Peltophorum pterocarpum*

Table 2: Showing peaks at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	0.0	0.05	4.2	0.42	0.06	0.0	25.2	0.09
2	0.06	0.3	0.09	68.2	6.80	0.10	67.4	1172.8	4.22
3	0.10	67.4	0.11	69.9	6.98	0.13	48.9	1621.4	5.84
4	0.13	49.0	0.15	68.5	6.84	0.19	0.0	2180.9	7.85
5	0.19	0.1	0.22	15.7	1.57	0.23	0.2	244.6	0.88
6	0.23	0.0	0.26	19.9	1.98	0.28	11.4	416.6	1.50
7	0.30	28.5	0.34	139.0	13.86	0.35	125.3	3952.3	14.23
8	0.35	126.4	0.36	136.0	13.56	0.39	81.3	2974.8	10.71
9	0.39	81.8	0.42	205.7	20.52	0.44	46.7	5634.7	20.29
10	0.44	46.9	0.46	56.3	5.62	0.48	24.6	1594.5	5.74
11	0.49	24.8	0.50	29.0	2.89	0.51	26.1	492.8	1.77
12	0.51	26.2	0.51	28.6	2.85	0.54	23.2	650.9	2.34
13	0.54	23.2	0.56	25.4	2.53	0.58	15.7	807.3	2.91
14	0.58	15.9	0.61	100.3	10.00	0.69	16.1	4198.0	15.12
15	0.70	16.3	0.74	34.4	3.43	0.80	1.5	1782.2	6.42
16	0.82	0.7	0.83	1.4	0.14	0.84	0.1	18.3	0.07

Phytochemicals are chemical compounds synthesized during the various metabolic processes. Various phytochemicals possess a variety of pharmacological activities. These chemicals are often called secondary metabolites. Some of these are found to have antimicrobial activity and serve as plant defense mechanisms against pathogenic organisms. These compounds are classified as phenols, quinines, flavonoids, tannins, alkaloids, glycosides and polysaccharides. Phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, phenols and tannins in P.pterocarpum ethanolic flowers extract. 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. Our results are in accordance by showing the presence of alkaloids, flavonoids and saponins in the ethanolic extract of flowers of P.pterocarpum. Jyothi et al reported quercetin in Cassia auriculata L. using HPTLC fingerprint profile [13]. The present study is first to report the HPTLC fingerprint of ethanol extracts of P.pterocarpum flowers showing maximum number of components 16 and 20 respectively at 254 and 366nm with solvent system Toluene: Chloroform: ethanol:: 5.5:3.5:1. From the HPTLC studies, it has been found that ethanol extract contain a mixture of compounds. This densitometric HPTLC fingerprint profile may be used as marker for quality evaluation and standardization of the drug. Thus, HPTLC fingerprint profile along with their Rf values were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant.

APPLICATIONS

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 P. pterocarpum extract of flowers 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.

CONCLUSIONS

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. In HPTLC technique, as the sample is applied as a rectangular band it provides more resolution and better separation of spots as compared to the TLC technique because of the shape of the area in which the compounds are present on the plate. 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 semiquantitative information about the major active phytoconstituents present in a plant extract, thus enabling an assessment of plant extract quality. HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. 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 P. pterocarpum extract of flowers 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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