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Finger printing analysis of the flavonoid from leaves *Pergularia Daemia* Forsk using HPTLC analysis.

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Abstract
Objectives The present study was carried out to identify the flavonoid from petroleum ether and methanol extract of leaves of *Pergularia daemia* Forsk using HPTLC technique
Method- Preliminary phytochemical testing was done and HPTLC study was carried out by the CAMAG HTLC scanner equipped with Linomat sample applicator [Switzerland]. Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software [1.4.6 camag] with the help of tungsten lamp.
Results- Preliminary Phytochemical testing petroleum ether of leaves of Pergularia daemia showed the presence of steroids, terpenoids, triterpenoids and methanol extract showed alkaloids, glycosides, tannins, flavonoids and carbohydrates. HPTLC fingerprinting of flavonoids of petroleum ether extract reveled five poluvent phytoconstituent [5 peaks] and corresponding ascending order of R_f values in the range of 0.09 to 0.84 While methanol extract of leaf showed polyvent phytoconstituent [10 peaks] and corresponding ascending order of R_f value in the range of 0.07 to 0.84 .
Conclusion- With the above testing and HPTLC analysis, we concluded that petroleum ether and methanol extract of leaf of *Pergularia daemia* Forsk shows the presence of flavonoids.

Keywords: leaf extract of *Pergularia daemia* [Forsk], Phytochemical screening, flavonoids, HPTLC fingerprinting.

1. Introduction
Natural product has always remained a profile source for the discovery of new drug and used since the Vedic period [1]. Ayurveda has been a vibrant system of health care in India and has been practicized since 6000 years back, but growth as an industry has commenced only a few years back. India share a global export of medicine is at around 10% only which is low. Therefore, there is a need to transform Ayurveda into a dynamic, scientifically validated and proof based industry which take its roots from the rich knowledge base of old tradition [2-4].
It is necessary to develop a method for rapid, precise and accurate identification and estimation of active constituents or a marker compound as the qualitative and quantitative target to assess authentic and inherent quality [5, 6]. Through various analytical techniques like TLC, HPLC and HPTLC we the presence of these compounds in plants and also quantify the. HPTLC offers many advantages over other chromatographic techniques such as unsuppressed flexibility, choice of detection, user friendly, rapid and cost effective [7]. This HPTLC is widely used at industrial level for routine analysis of herbal medicines.
Pergularia daemia (Forsk.) Chiov (Asclepiadaceae) is a foetid smelling laticiferous twiner found in the plains throughout the hot parts of India, at an altitude of 1000 m in the Himalayas [1]. This plant is known as "Veliparuthi" in Tamil," Uttaravaruni" in Sanskrit and "Utranjutuka" in Hindi "Uttarni" in Marathi.. Pergularia species are widely distributed in the old world tropics and subtropical region from southern and tropical Africa and Asia, have multiple applications in different folk medicine, including the Indian Ayurvedic system [2]. Traditionally the plant Pergularia daemia is used as anti-helmintic, laxative, antipyretic and expectorant, also used to treat infertile diarrhea and malarial intermittent fevers Latex of this plant used for toothache [4-6]. Stem bark remedy for cold [8] and fever. [9] Aerial parts of this plant the various pharmacological activities like hepatoprotective [10] analgesic, anti-pyretic and anti- inflammatory. Phytochemically the plant has been investigated for cardenoloids, alkaloids, and saponins [11]. The plant was found to contain various triterpenes and steroidal compounds, [12] and have been documented for antifertility, [2] wound healing, [3] antidiabetic, [4] hepatoprotective, [5] cardiovascular effect, [6] antibacterial activity [7]. In this present study the preliminary phytochemical screening of leaf extract of Pergularia daemia Forsk has been done to identify the chemical constituent and HPTLC fingerprinting of Pergularia daemia

Forsk extract has been performed which may be used as marker for quality evaluation and standardization of drug. Qualitative and quantitative standardization of flavonoid was performed using HPTLC.

2. Material and Method-

2.1 Plant material

The leaves of *Pergularia daemia* Forsk were collected from the area of railway station at Yeola, Dist Nashik in the month of September and were authenticated from Prof. Sandenshive H.O.D. Dept. of Botany SSGM Arts, Commerce and Science College, Kopargaon. The plant material was dried and powdered to a coarse particle size to mesh 40

2.2 Extraction of Plant material

The powdered leaves were charge into thimble of soxhlet apparatus and extracted using petroleum ether [40-60 ^{0}C] as a solvent and extraction was continued till a solvent in siphon tube becomes colourless. The extract was separated and the marc was dried and further extracted with methanol [60-80 ^{0}C] as a solvent extraction continued till a solvent appears to be colourless in siphon tube. Both extracts were evaporated till get a thick, pasty mass. The extracts were finally air dry and percentage yield were calculated. And both extracts were subjected to following investigation

1. Preliminary phytochemical screening.
2. HPTLC fingerprinting of flavonoids.

2.3 Preliminary phytochemical screening

The preliminary phytochemical screening of leaf extracts of *Pergularia daemia* Forsk were carried out with standard protocol [13] the result were presented in Table No. 1

2.4 HPTLC studies were carried out by following the method described in Harbone [14] and Wagner [15] *et al.*

2.4.1 Sample preparation

Petroleum ether and methanol extracts obtained were evaporated under reduce pressure using rota-evaporator. Each extract residue was re-dissolved in 5 ml of chromatographic grade petroleum ether and methanol, which was used for sample application on pre-coated silica gel 60F_{254} aluminum sheets.

2.5 Developing solvent system

A number of solvent systems were tested for extract. But the satisfactory resolution was obtained in the solvent ethyl acetate: formic acid: glacial acetic acid: water [10:0.5:0.5:1.3]

2.6 HPTLC chromatographic condition, sample application, development of chromatogram and detection of spot.

The sample solution was spotted in the forms of bands of width 8.00 mm with a Camag microlitre syringe on pre-coated silica gel aluminum pate 60F_{254} [20 cm X10 cmX25µm thickness; E Merck, Darmstad, Germany, supplied by Anchrome Technologist, Mumbai] using a Camag Linomat V [Switzerland] . The plates were activated at 120 ^{0}C for 20 min. prior to chromatography. A constant application rate of 1.0 µl/s was employed and space between two bands was 5 mm. the slit dimension was kept at 6.0 mm X 0.45 mm and 10 mm/s scanning speed was employed. The slit band width was set at 20 nm, each track was scanned thrice and base line was corrected. The mobile phase for fingerprinting of flavonoids consist of ethyl acetate: formic acid: glacial acetic acid: water in the volume ratio of [10:0.5:0.5:1.3 V/V] and anisaldehyde sulphuric acid was used for derivatization of flavonoids. 20 ml of the mobile phase was used per chromatography. Linear ascending development was carried out in 20cm X10 cm through glass chamber [Camag, Muttenz. Switzerland] saturated with filter paper whatman no.:1 in the middle phase. The optimized chamber saturation time for mobile phase was 20 min. at room temperature [25 ^{0}C±2] at a relative humidity of 60% ±5 the length of chromatogram rum was 8.0cm. subsequent to the scanning, TLC plate was dried in a current of air with the help of air dryer. Densitometry scanning as performed with Camag TLC saner IV in the reflectance absorbance mode at 540 nm and operated by win CATS software [1.4.6 Camag] with the help of tungsten lamp. Subsequent to the development; TLC plate was dipped in anisaldehyde sulphuric acid reagent followed by drying in oven at 110 ^{0}C. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas with linear regression [16-25].

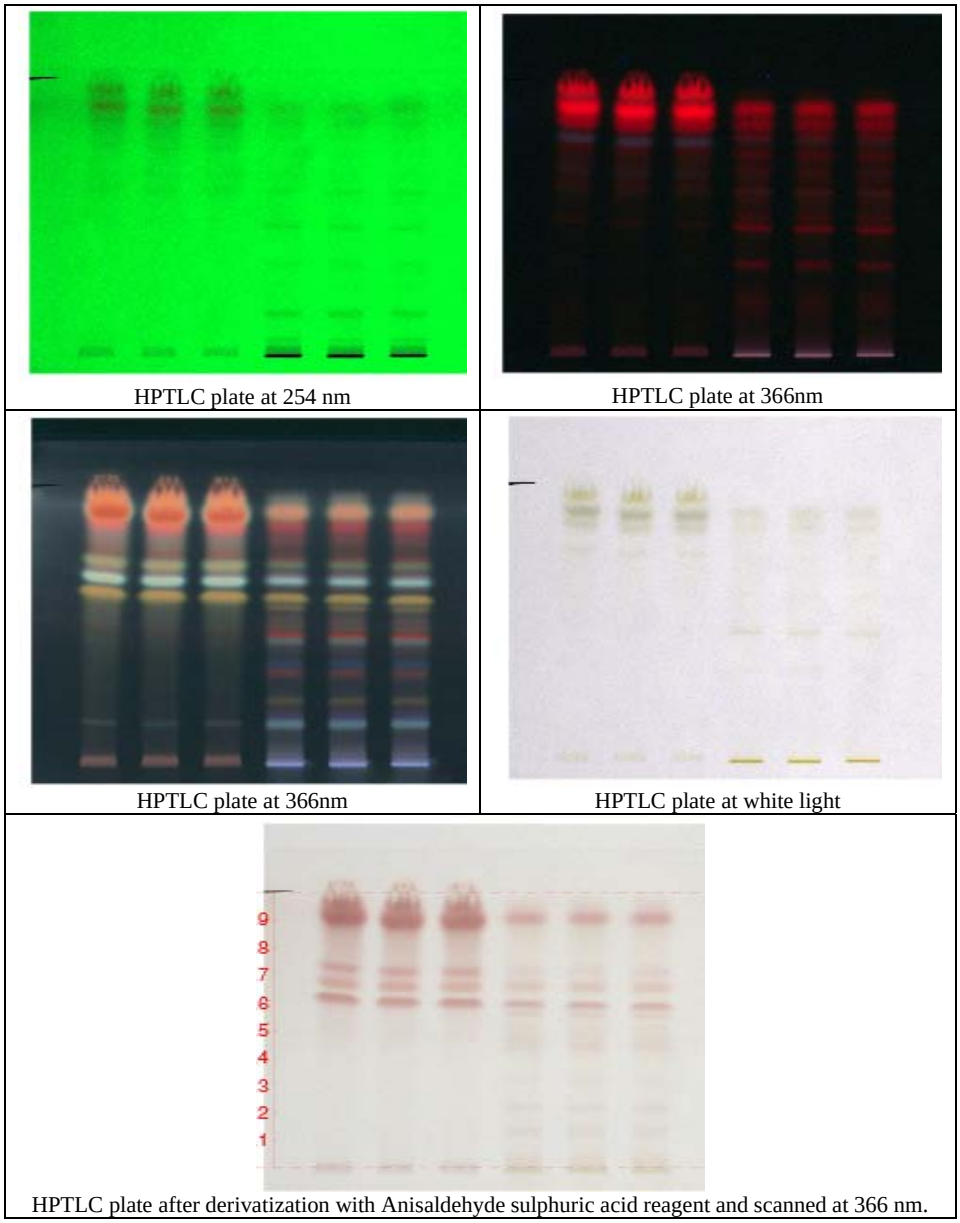
3. Result and discussion

Preliminary phytochemical analysis of petroleum ether extract of *Pergularia daemia* Forsk showed the presence of steroids, terpenoids, triterpenoids and glycosides while methanolic extract of *Pergularia daemia* Forsk Table No.1. the chromatogrammed shown in figure no.1 indicate that all sample constituents were clearly separated without any tailing and diffuseness.

Table 1: Preliminary phytochemical screening of petroleum ether and methanol extract of leaf of *Pergularia damia* Forsk

Phytoconstituents	Test performed	Pergularia daemia Forsk	
		Petroleum ether extract	Methanol extract
Steroids	Salkowski reaction Liberman Burchard reaction	++ ++	+ +
Triterpenoids		++	--
Glycosides	Balgets test Killer –killani test Legal test Brontager test	+ + +	++ ++ ++ ++
Saponins	Foam test	-	-
Carbohydrates	Molish test Barfoeds test Fehlings test Benedicts test	++ ++ ++ ++	++ ++ ++ ++
Alkaloids	Mayer's Haggeors test's Dragendroff's	+ + +	++ ++ ++
Flavonoids	Ferric chloride Shinoda test	++ ++	+ +
Tannins	Ferric chloride 5% Gelatin test	+ +	++ ++
Proteins	Million's test Xanthoprotic Biuret test Ninhydrin test	+ + + +	+ + + +

++ Higher concentration, + present, - absent.



Track 1-3: Petroleum ether extract of leaf, **Track 4-6:** methanol extract of leaf

Fig 1: HPTLC fingerprinting profile of leaf of *Pergularia daemia* Forsk

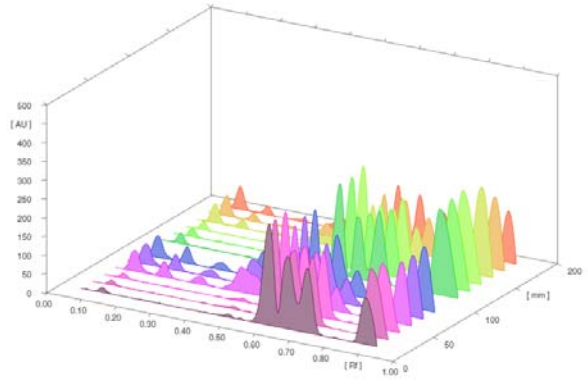


Fig 2: Plot of fingerprint of flavonoids of leaf of *Pergularia daemia* Forsk

The result from HPTLC fingerprint of flavonoids scanned at wavelength 540 nm for petroleum ether extract of leaf of *Pergularia daemia* Forsk showed that there are five polyvent phytoconstituent and corresponding ascending order of rf values from 0.09 to 0.84 in which highest concentration of phytoconstituent was found to be 34% and its corresponding Rf value was found to be 0.60 respectively and was recorded in Table no. 2. The corresponding HPTLC chromatogram was present in Fig No.3

The results from HPTLC fingerprint of flavonoid scanned at wavelength 540 nm for methanol extract of *Pergularia daemia* Forsk showed that there were ten polyvalent phytoconstituent and corresponding ascending order of Rf value starts from 0.07 to 0.84 in which highest concentration was found to be 23.19 and its corresponding Rf value found to be 0.57 respectively and recorded in table no. 4 The corresponding HPTLC chromatogram was present in Fig No.4

Table 2: Rf value for flavonoid of Petroleum ether extract of Pergularia daemia Forsk leaf

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.1	0.12	13.8	1.79	0.15	0.2	236.0	1.14
2	0.54	0.8	0.60	269.6	34.92	0.63	38.6	7037.3	33.88
3	0.63	39.4	0.66	192.9	25.00	0.68	71.3	5508.8	26.52
4	0.68	71.6	0.71	172.1	22.30	0.76	0.0	4548.6	21.90
5	0.84	0.2	0.88	123.5	15.99	0.91	33.1	3441.0	16.57

Table 3: Rf value for flavonoid of methanol extract of Pergularia daemia Forsk leaf

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	0.0	0.11	55.4	6.24	0.17	1.7	1436.3	6.21
2	0.17	1.7	0.20	41.8	4.71	0.24	0.0	842.8	3.65
3	0.25	0.1	0.30	20.9	2.35	0.35	0.0	558.5	2.42
4	0.35	0.1	0.42	66.9	7.54	0.48	35.3	3554.9	15.38
5	0.48	35.5	0.49	39.3	4.42	0.52	21.6	777.1	3.36
6	0.52	21.8	0.54	80.3	9.04	0.55	61.4	1321.4	5.72
7	0.55	61.6	0.57	205.9	23.19	0.60	12.5	4434.1	19.18
8	0.60	12.6	0.64	147.7	16.64	0.67	31.0	3981.1	17.22
9	0.67	31.0	0.69	63.9	7.19	0.75	0.0	1654.1	7.16
10	0.84	0.1	0.88	165.8	18.67	0.91	9.6	4552.8	19.70

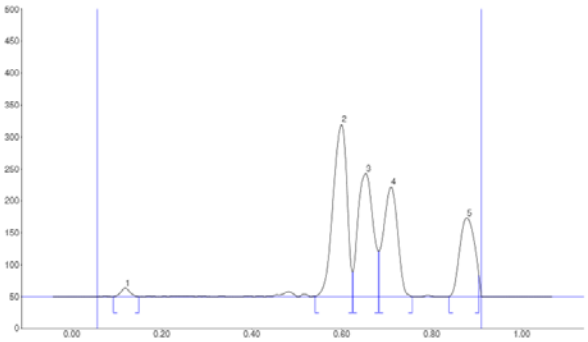


Fig 3: Chromatogrammed of flavonoid in petroleum ether extract of *Pergularia daemia* Forsk.

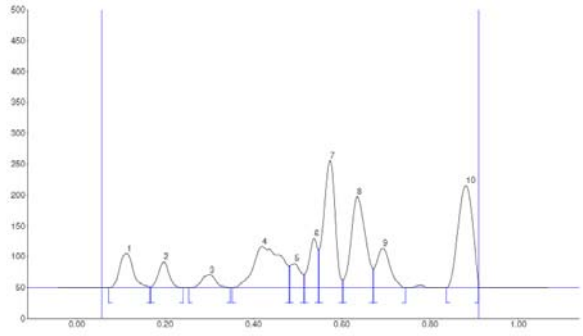


Fig 4: Chromatogrammed of flavonoid in methanol extract of *Pergularia daemia* Forsk.

4. Conclusion

As various adverse effects of synthetic drugs people are demanding natural drugs for safety in recent years there for scientist are search in for alternative medicine to synthetic drugs. Some chronic diseases require long term therapy in that case synthetic drugs may produce side effects. Through various literature surveys we found that flavonoids are having curative property, hence flavonoids have been implemented in various pharmacological actions essential to develop a fingerprint profile for flavonoids of this plant. The developed fingerprint analysis of leaf extract of *Pergularia daemia* Forsk will help to isolate, identify new flavonoid which will offer a possible to discover lead molecule for development of drug.

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