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Phytochemical investigation of Leaves of Pergularia daemia (Forssk.)Chiov

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ABSTRACT
Pergularia daemia (Forssk.) Chiov, a perennial twining herb belonging to the Asclepiadaceae family, is traditionally used for treating respiratory ailments and various other diseases. This study aimed to evaluate the pharmacognostical properties and phytochemical profile of the leaves of Pergularia daemia. Macroscopic examination revealed cordate-shaped leaves with bitter taste, while microscopic analysis showed multicellular covering trichomes and anomocytic stomata. Physicochemical parameters including total ash, acid soluble ash, acid insoluble ash, and extractive values were determined to assess the quality. Preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, carbohydrates, and amino acids. Thin layer chromatographic (TLC) analysis of methanol extract of leaf revealed ten spots under visible light and eleven spots under UV light, indicating diverse chemical constituents. High Performance Thin Layer Chromatography (HPTLC) fingerprinting further confirmed the presence of multiple phytoconstituents, including quercetin. 19.334 µg quercetin was found in 1 gm leaf extract of Pergularia daemia.

KEYWORDS: Flavonoids, phytochemical screening, TLC, HPTLC, Quercetin.

I. INTRODUCTION

The use of plants as therapeutic agents is as old as human civilization. Early humans observed the behavior of animals to identify plants with healing properties, gradually transforming these observations into organized systems of traditional medicine [1, 2]. In India, the Ayurveda classics laid a comprehensive foundation for herbal healing, while Chinese and Japanese Kampo medicine developed in parallel. Dioscorides' De Materia Medica later became a cornerstone of European Pharmacognosy. These traditions illustrate the global and enduring significance of medicinal plants [3]. Herbal remedies remain central to health care for nearly 80% of the world's population, according to the World Health Organization. Even in modern pharmacology, many pivotal drugs, morphine from Papaver somniferum, aspirin from Salix alba, quinine from Cinchona bark, and digitalis from Digitalis purpurea are of plant origin [4]. The growing demand for safer, naturally derived therapeutics continues to drive the search for novel phytochemicals with potent biological activities. Herbal drugs consist of whole plants or specific plant parts such as leaves, roots, or flowers used in crude or processed form. Quality assurance is crucial botanical identification, proper cultivation or wild collection, and controlled drying and storage prevent adulteration and degradation. Analytical methods such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and spectroscopic "fingerprinting" provide chemical profiles that ensure consistency between batches .International guidelines from the WHO and European Medicines Agency outline standards for good agricultural and collection practices, validated manufacturing procedures, and rigorous safety testing[4,5].

Pergularia daemia (Forssk.)is a perennial, twining herb belonging to the family Asclepiadaceae and is commonly known in Tamil as Veliparuthi [6,7]. It grows widely across tropical and subtropical regions of India, Africa, and the Arabian peninsula, often thriving along roadsides

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and hedgerows up to altitudes of about 900–1000 m .The plant exudes a characteristic milky latex, bears cordate (heart-shaped) leaves, and produces small greenish-white or cream flowers with fringed lobes .Traditional medical systems attribute a wide spectrum of therapeutic properties to this species [8-10]. The aerial parts are used as anthelmintic, antiseptic, expectorant, and antivenom agents. Folk remedies employ the plant for ailments such as asthma, bronchitis, rheumatic swelling, delayed childbirth, and postpartum hemorrhage[11-12]. Leaves and roots are prescribed for dysmenorrhea, amenorrhea, infantile diarrhea, and skin disorders [13-15]. The latex is applied externally for boils and sores, while stem and bark preparations are used to reduce fever and treat malarial infections. Such diverse applications underscore the plant's importance in indigenous medicine[16].
The leaves of Pergulariadaemia (Forssk.) Chiov.are shown in Figure 1.

Fig. 1. Leaves of Pergularia daemia (Forssk.) Chiov

Extensive phytochemical investigations reveal that P. daemia contains a variety of secondary metabolites flavonoids (including hyperoside and quercetin derivatives), triterpenes, saponins, cardenolides, alkaloids, tannins, and phenolic acids[17-20]. These compounds are associated with multiple experimentally validated bioactivities such as antioxidant[21-23], anti-inflammatory[24], analgesic, antidiuretic, hepato protective, anticancer, diuretic, anthelmintic, and anti-ulcer activities [25-26].

II. MATERIALS AND METHOD

PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of Pergularia daemia (Forssk.) Chiov were collected from Periyanaickenpalayam, Coimbatore (Tamil Nadu), and authenticated by the Botanical Survey of India, Coimbatore, Tamilnadu. The leaves were shade-dried, coarsely powdered, and used for pharmacognostical and phytochemical studies.

PHARMACOGNOSTICAL STUDY

Pharmacognostical analysis included macroscopic evaluation of the sample, wherein colour, odour, taste, shape, and size were observed. The colour was visually examined, and the odour was checked by sensory evaluation**.** Microscopic examination**,** including transverse section and powder microscopy**,** was carried out to study the anatomical characteristics.

MICROSCOPICAL STUDY

T.S of Pergularia daemia (Forssk.) Chiov

Transverse section of leaf of Pergularia daemia (Forssk.) Chiov was studied The leaves were cut into thin transverse section using a sharp blade and the sections were stained with 1% phloroglucinol and conc. hydrochloric acid and the microscopical characters were observed under microscope [27].

POWDER MICROSCOPY

The shade dried, powdered leaves were used for powder microscopic analysis. The organoleptic characters were observed and to identify the different characteristic features, staining reagents were used. Powder was stained with 1% phloroglucinol and conc. hydrochloric acid and observed under microscope. All the lignified cells stained with pink colour.

PHYSICOCHEMICAL ANALYSIS

Physicochemical parameters[28] such as total ash, acid-insoluble ash, water-soluble ash, and extractive values such as ethanol soluble extractive value and water soluble extractive values were determined as per standard WHO guidelines. Ash values are used to determine the quality and purity of a crude drug and to establish the identity of the crude drug. The extractive values are the indicatives of the approximate measures of their

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chemical constituents and the nature of the constituent present in the crude drug. Taking into consideration the diversity in chemical nature and the properties of the content of drugs, solvents like ethanol and water were used for determination of extractive values.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

EXTRACTION

About 5 g of dried coarsely powdered leaves of Pergularia daemia (Forssk.) Chiov was macerated with 50 ml of methanol in round bottom flask. It was kept for 24 h with random shaking. Then it was filtered, the marc was reextracted with 30 ml of methanol and filtered. The filtrates were collected, combined and concentrated by evaporation. The dried extract was subjected for phytochemical screening.

Phytochemical screening was performed using standard chemical tests for alkaloids, flavonoids, tannins, terpenoids, saponins, carbohydrates, proteins, and amino acids[29]. Fluorescence analysis of powdered leaf was carried out. The powder was treated with various reagents and observed visible light, short UV light (254 nm), and long UV light (365 nm) to find out any fluorescence property of the leaf.

THIN LAYER CHROMATOGRAPHIC (TLC) ANALYSIS

TLC was performed on the aluminium backed precoated silica gel 60 F_{254} plates (Merck). 100 mg of methanol extract of Pergularia daemia was dissolved in 10 ml of methanol and the solution was used to perform TLC analysis. Toluene-ethyl acetate-formic Acid (5:4:0.2) was used as mobile phase. The separated compounds were detected in daylight, short UV light and long UV light. The Rf values were calculated for each spot[30,31].

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

High performance thin layer chromatographic (HPTLC) analysis[32] of methanol leaf extract was carried out for quantitative estimation of quercetin. Chromatographic studies were performed using the following conditions:

The stationary phase used was aluminum backed precoated silica gel 60F_{254} HPTLC plates. Toluene-ethyl acetate-formic Acid (5:4:0.2) was used as mobile phase. The volume of mobile phase was kept up to 10 ml; chamber saturation time: 30 min; migration distance: 80 mm; migration time: 30 min; wavelength of detection 254 nm and 366 nm; scanning speed: 20 mm/s; data resolution: 100 mm/step; and band width: 6mm. A Camag video documentation system was used for imaging and archiving the thin layer chromatograms. The object was captured by means of a highly sensitive digital camera. Image acquisition processing and archiving were controlled via Win CATS software.

CHROMATOGRAPHIC SEPARATION

A volume of 5 µL extract solution was spotted on the HPTLC silica gel plate, 6 mm band length, using a Camag automatic TLC sampler spotting device. The TLC plate was developed in the ascending mode in a twin-trough chamber presaturated for 30 mins with mobile phase. Linear ascending plate development was performed until a migration of distance 8 cm from the origin was reached. The plate was removed from the chamber, air dried, and scanned in the absorbance/reflectance mode of a Camag TLC scanner. Peak area data were recorded using Camag Win CATS software. Quercetin was used as standard for the quantitative analysis.

CALIBRATION CURVE

A standard solution (Quercetin) of various concentrations were prepared. Volume of 2–10 µL was applied on precoated HPTLC silica gel plates. After development, plates were scanned densitometrically at appropriate wavelengths, and Rf values were recorded. The concentration of the target analysts in the separated bands were determined from the intensity of the reflected light indicated, and the produced peak areas were correlated to the analyst concentrations using six-level linear calibration curves. The employed statistical analysis ensures that the developed method is reproducible and selective[33]. A calibration curve was plotted.

III. RESULTS AND DISCUSSION

PHARMACOGNOSTICAL STUDY

Pharmacognostical characters confirmed diagnostic features of P. daemia leaves including cordate shape of leaf. The leaf is green, bitter taste with aromatic odour. The leaf size was found to be 4 to 4.5 cm long and 3.5 to 4 cm width.

The T.S of Pergularia daemia leaf showed an isobilateral condition with the following important characters.

Upper epidermis is single layered with polygonal cells covered on the outer side by a thick

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cuticle. Only covering trichomes emerge from the epidermis layer. Trichomes are non- glandular, long, multicellular and non-lignified (Figure 2).Lower epidermis shows polygonal paranchymatous cells. Many covering trichomes are present in the lower epidermis. Mesophyll is differentiated into palisade and spongy parenchyma, Palisade is further differentiated into upper palisade and lower palisade. Upper palisade is single layered and elongated columnar cells and present only in the laminar region. Lower palisade cells are smaller than the upper palisade and present only in the laminar region. Spongy parenchyma is thin narrow loosely arranged between the upper and lower palisade. Vascular strands are seen. Vascular bundle is prominent occupying the central portion of the midrib, Xylem as usual is towards the ventral surface and phloem towards the dorsal surface. Below the upper epidermis a strip of collenchyma is seen. Similarly, above the lower epidermis, a strip of collenchyma is seen in the mid region. The surface preparation showed the anomocytic stomata (Figure 3). Powder microscopy revealed multicellular covering trichomes, mesophyll tissue, anomocytic stomata and vascular strands. These findings may be considered as standard for leaf of Pergularia daemia.

Fig. 2. Multicellular covering Trichome

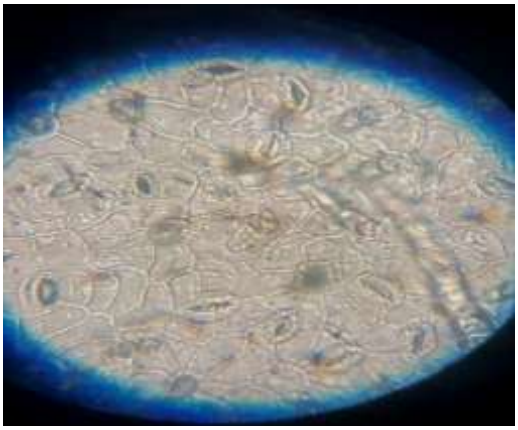

Fig. 3. Anomocytic stomata

PHYSICOCHEMICAL ANALYSIS

The physiochemical parameters such as for total ash, acid insoluble ash and water-soluble ash were found to be 8.66 % w/w, 4.66 % w/w, and 2.33 % w/w respectively. This indicated the acceptable purity. The extractive value using different solvent such as water and ethanol are 8.2 % w/w and 4.8 % w/w respectively Extractive values were higher in water (8.2%) compared to ethanol (4.8%), suggesting predominance of polar constituents.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical screening of Pergularia daemia showed the presence of alkaloids, phenolic compounds, tannins, carbohydrates, proteins, amino acids, flavonoids, saponins, steroids and triterpenoids (Table 1). These classes of compounds are associated with antioxidant, anti-inflammatory, and antimicrobial properties, aligning with traditional uses.

Table 1. Preliminary phytochemical screening of Pergularia daemia

Sl.No.	Name of test	Aqueous extract	Ethanol extract
1.	**Test for Alkoloids**		
	a) Dragendroff's test	-	+
	b) Wagner test	-	+
	c) Mayer test	-	+
	d) Hager test	-	+
2.	**Test for phenolic compounds and tannins**		
	a) Ferric chloride test	+	+
	b) Lead acetate test	+	+

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	c) Test for Phenolic flavonoids	+	+
3.	**Test for carbohydrates**		
	a) Molish test	+	+
	b) Fehling's test	+	+
4.	**Test for amino acids and proteins**		
	a) Millon's test	+	-
	b) Ninhydrin test	+	-
	c) Biuret test	+	-
	d) Xanthoproteic test	+	-
5.	**Test for steroids and terpenoids**		
	a) Salkowski test	-	+
	b) Liebermann-Burchard test	-	+
6.	**Test for flavonoids**		
	a)Alkaline reagent test	+	+
	b)Shinoda test	+	+
	c)Test for phenolic flavonoid	+	+
7.	**Test for saponins** a)Foam test	+	-

Fluorescence analysis showed characteristic colour changes (green to fluorescent green, brown, and black) under different reagents and UV conditions, which can serve as a simple and reproducible identification tool.

THIN LAYER CHROMATOGRAPHIC (TLC) ANALYSIS

TLC profiling revealed 10 distinct spots under visible light (Rf 0.08–0.98), 11 spots under short UV (Rf 0.15–0.98), and 9 spots under long UV (Rf 0.04–0.95), confirming chemical diversity. HPTLC analysis provided better resolution and quantification of phenolic and flavonoid constituents. Multiple peaks with distinct Rf values were observed, validating the presence of bioactive compounds in methanolic extract. The reproducibility of chromatographic fingerprints supports its use for quality control and standardization. The TLC representation of Pergularia daemia is shown in figure 4.

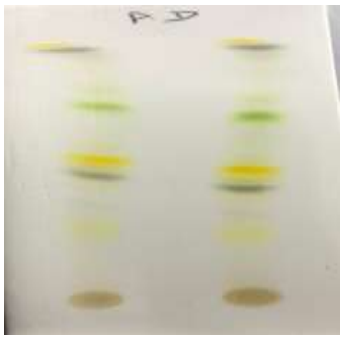

Fig. 4. TLC representation of Pergularia daemia

HPTLC analysis

QUANTITATIONOF QUERCETIN

The presence of quercetin was detected and quantified by HPTLC. The limit of detection (LOD) was found to be 20 µg and limit of quantitation (LOQ) of quercetin 50-250 µg (Table 2).Quantity of quercetin present in Pergularia daemia extract was found to be 19.334 (µg/gm). Chromatogram of quercetin and the Pergularia daemia extract were given in figure 5 and 6 respectively. Earlier, quercetin was also identified in the leaf of Nerium oleander by HPTLC method[34].

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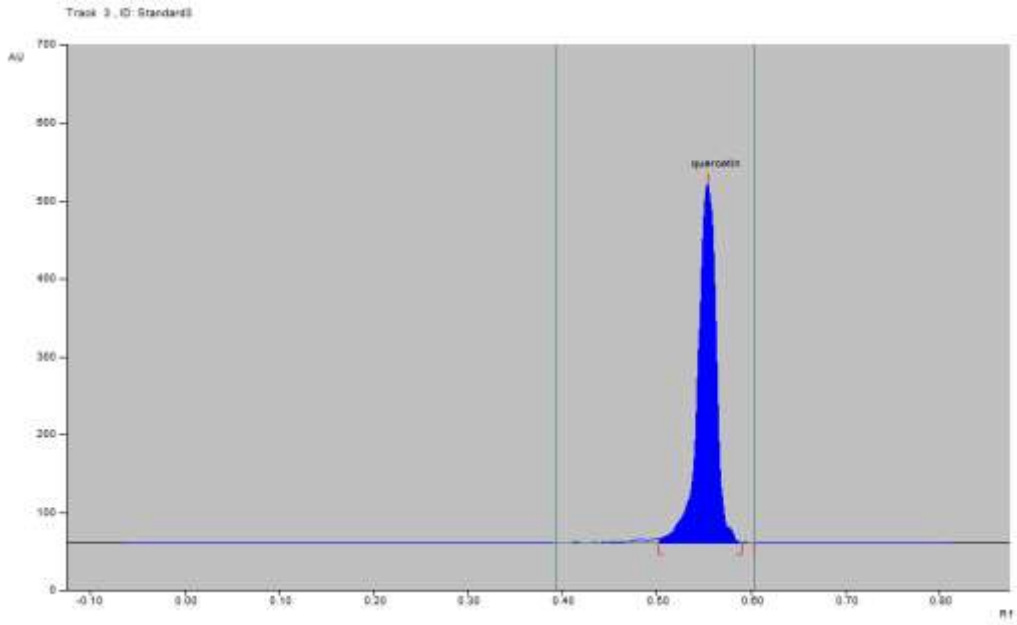

Fig. 5. HPTLC chromatogram of quercetin

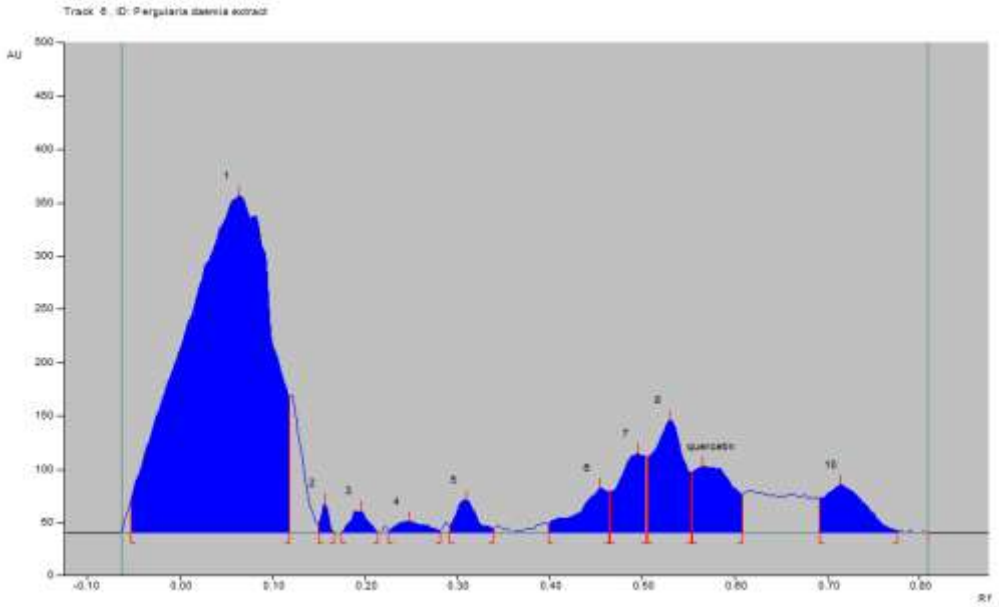

Fig. 6. HPTLC chromatogram of Pergularia daemia

Table 2. HPTLC of Quercetin in Pergularia daemia

S.no	Experimentals	Value
1.	Rf value of quercetin	0.54
2.	Limit of detection	20 µg
3.	Limit of quantificaton	50-250 µg
4.	Quantity of quercetin present	19.33 µg/gm

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IV. CONCLUSION

The present study establishes the pharmacognostical, physicochemical, phytochemical, and chromatographic characteristics of Pergularia daemia (Forssk.) Chiov leaves. Diagnostic features such as cordate shape, multicellular trichomes, and isobilateral lamina were confirmed through macroscopic and microscopic studies. Physicochemical constants provide baseline parameters for quality control, while phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and carbohydrates, supporting its traditional medicinal uses. Fluorescence analysis, TLC, and HPTLC profiling further validated the chemical diversity and demonstrated reproducible fingerprint patterns useful for authentication and standardization. Quercetin, one of the leading flavonoid was identified by HPTLC method. The quantity of quercetin in Pergularia daemia leaf extract was found to be 19.334 µg/gm. Overall, the findings highlight P. daemia as a potential source of bioactive compounds with therapeutic relevance, and they provide a scientific foundation for future pharmacological and clinical investigations.

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