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RESEARCH ARTICLE

PHYTOCHEMICAL AND ANTIMICROBIAL INVESTIGATIONS ON THE AERIAL PLANT PARTS OF *PERGULARIA DAEMIA* FORSK. (ASCLEPIADACEAE): A PERENNIAL TWINNING HERB

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ABSTRACT

Scientific investigations of medicinal plants have been initiated in many parts of our country because of their contribution to health care. The medicinal properties of plants are due to the presence of phytochemicals. The phytochemicals have anti-oxidant and anti-microbial properties. The resistance to antibiotics by bacteria is increasingly becoming a concern to public health. Currently used antibiotic agents fail to bring an end to many bacterial infections due to super-resistant strains. Medicinal plants represent a rich source of antimicrobial agents from which new drugs can be obtained. The phytochemical constituents of plants are desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources. The present study has been carried out on the phytochemical, anti- oxidant and anti-bacterial character of aerial plant parts of *Pergularia daemia*. The phytochemical result shows that the flavonoids, phenols and tannins were present abundantly in the Ethanolic extracts of aerial plant parts. Both total phenol and tannins are high in ethanolic extract. The antioxidant activity of the plant was done by using DPPH radical scavenging assay. The antimicrobial activity of ethanolic extract of *Pergularia daemia* aerial plant parts was tested against *Klebsiella pneumoniae* and *Citrobacter freundii.*

Keywords: *Pergularia daemia*, aerial plant parts, flavonoids, phenols,tannins, antioxidant, antimicrobial

1. INTRODUCTION

India is represented by its rich culture, traditions and natural biodiversity. It offers a unique opportunity for drug discovery researchers. Throughout the world there are of about 20,000 medicinal plants; out of that India harbors of about 15 percent (3,000-3,500) of medicinal plants. About 90 percent of these are found growing wild in different climatic regions of the country. Recently herbal medicines are getting more important for the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycemic agents (1,2). According to World Health Organization, medicinal plants are the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants.

Pergularia is a genus of the botanical family Apocynaceae. *Pergualria daemia* is a perennial twinning herb that grows along the roadsides. *P. daemia* has been traditionally used as an anthelmintic, laxative, antipyretic expectorant and also used to treat infantile diarrhea and malarial intermittent fevers. It is widely distributed in the tropical and subtropical regions of the world. Various phytochemicals including terpenoids, flavonoids, steroids and alkaloids have been isolated and identified from the various parts of the plant (leaves, stems, shoots, roots, seeds, and fruits). *P. daemia* is widely used by various tribal communities in Western Ghats of India for the treatment of a variety of ailments, while predominantly the roots of the plant have been used to treat liver disease and jaundice.

2. PLANT DESCRIPTION

2.1. Systematic Position

Class : Magnolidopsida
Order : Gentiales
Family : Asclepidaceae
Genus : *Pergularia*
Species : *daemia*

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Fig. 1. Leaves of *Pergularia daemia*

Fig. 2. Fruits of *Pergularia daemia*

Habit : Perennial herb
Stem : Herbaceous, climbers, and presence of milky latex.
Leaves : Simple, opposite, membranous, 3-9 cm long, broadly ovate, orbicular or deeply cordate, acute or short-acuminate at apex, pubescent beneath, petioles 2-9 cm long.
Inflorescence : Axillary drooping umbellate cyme.
Flowers : Pedicellate, greenish-yellow or dull-white, hispid, borne on long peduncles as drooping clusters.
Fruits : A pair of follicles, base inflated, covered with soft spines, and seeds are covered by velvety hairs.

2.2. Ethnomedical Information

P. daemia is used as a pungent, cooling, anthelmintic, laxative, and antipyretic agent. It cures asthma and ulcers. It is useful in eye complaints, urinary discharges, leucoderma, uterine complaints, and inflammation and facilitates parturition. It has anti-venom, anti-malarial, anti-inflammatory, anti-oxidant, anti-rheumatic, anti-diabetic, and anti-microbial properties.

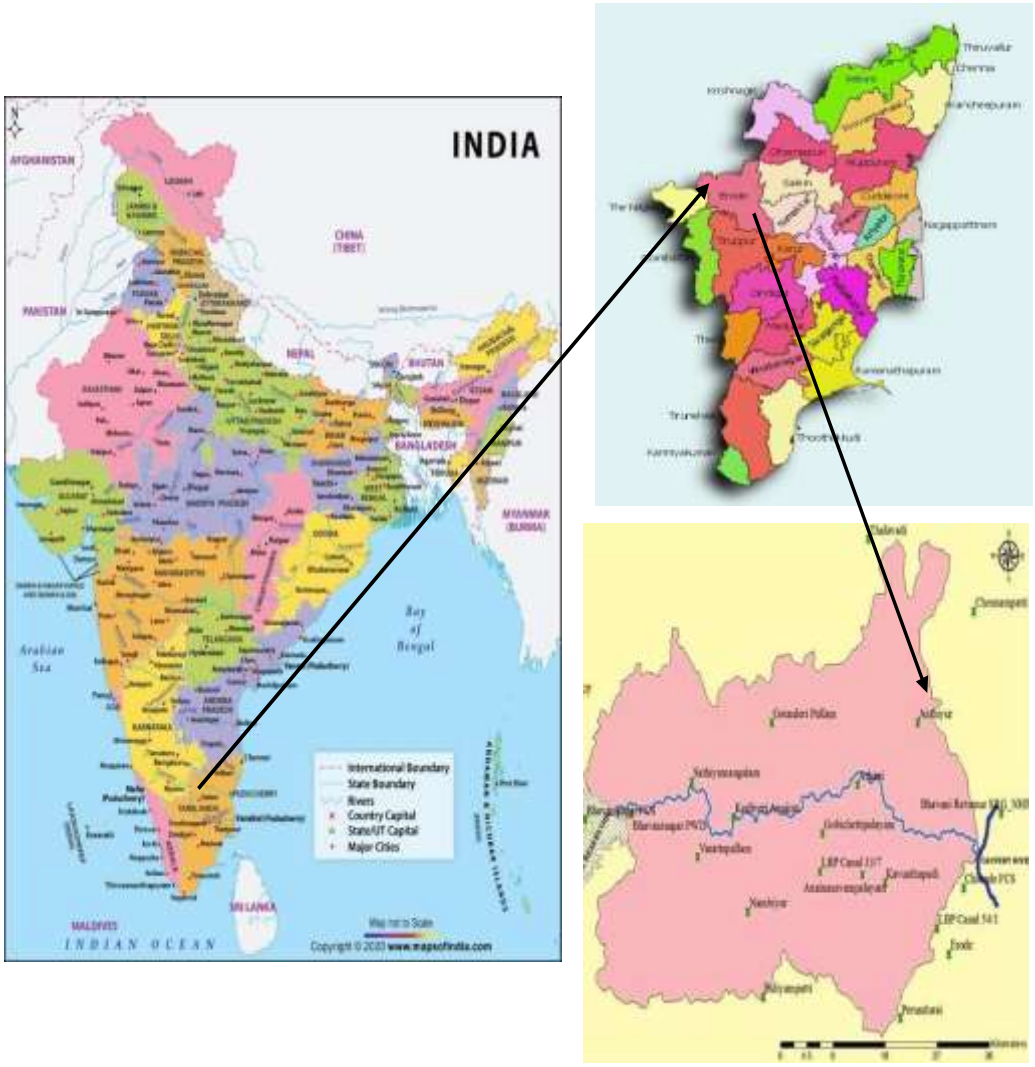
3. MATERIALS AND METHODS

The plant materials were collected during the month of December 2021 from Anthiyur, Erode District. Anthiyur is located at 11.58°N 77.60°E. It has an average elevation of 251 m (823 ft).

Freshly collected aerial plant parts (leaves and stems) of *P*. daemia was collected, cleaned, shade dried and powdered mechanically and stored in an air-tight container for further studies.10 gram of the powdered sample was kept steeped for 72 hours in the solvents like petroleum ether, chloroform and ethanol and filtered through Whatmann No. 1 filter paper. The filtrate was then collected and concentrated by evaporation and used for further qualitative and quantitative phytochemical studies.

3.1. Preliminary qualitative phytochemical analysis

Qualitative phytochemical analysis was done by using the procedure of (3). The presence of alkaloids, flavonoids, glycosides, tannins, phenols, fixed oils, fats, and saponins was analyzed qualitatively.



3.2. Quantitative phytochemical analysis

3.2.1. Determination of Total Flavonoid Content (TFC):

The total flavonoid content was determined spectrophotometrically using the method adopted by Zhishen *et. al*. (4). 0.5 mL of appropriately diluted extract solution was mixed with 2.0 ml. of distilled water and subsequently with 0.15 ml of 5% sodium nitrite solution and maintained for 6 min. Then, 0.15 ml. of 10% Aluminium chloride solution was added and allowed to stand for 6 min, and finally, 2.0 mL of 4% sodium hydroxide solution was added. Final volumes of the contents were made up to 5.0 ml. with distilled water and were mixed thoroughly. After 15 min of incubation at laboratory temperature, the absorbance was determined against blank at 510 nm. The total flavonoid content was determined by using a standard curve with Rutin. The mean of the three values was expressed as milligrams of Rutin equivalents (mg RE)/ g extract on a dry weight basis.

3.2.2. Determination of Total Phenolic Content (TPC):

The amount of Total Phenolic content in extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g Gallic acid equivalents (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of Gallic acid were prepared in methanol. 0.5ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10-fold diluted Folin- Ciocalteu reagent and 2ml

of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was reading 760 nm spectrophotometrically. All determination was performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. This blue color is measured spectrophotometrically. Thus, the total phenolic content can be determined (5).

3.2.3. Determination of total Tannins Content (TTC):

The Total Tannins Content was determined by Folin and Ciocalteu method. 0.1 ml of the sample extract was added with 7.5 ml of distilled water and adds 0.5 ml of Folin Phenol reagent; 1 ml of 35% sodium carbonate solution was added and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. The Blank was prepared with water instead of the sample. A set of standard solutions of Gallic acid is treated in the same manner as described earlier and read against a blank. The results of tannins are expressed in terms of Gallic acid mg/g of extract (6).

3.3. Anti-oxidant activity

3.3.1. In- vitro Antioxidant (DPPH) radical scavenging activity of ethanolic leaf extract of P. daemia

The hydrogen donating capacity was assessed by using the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) method (7). Briefly, a solution of 0.1mM DPPH was prepared using methanol. The aliquots of samples were mixed with 5.0 ml DPPH solution. The reaction mixture was shaken and incubated at 270ºC for 20 minutes at dark, and absorbance was measured at 517nm. Percent DPPH discoloration (% inhibition) of the sample was calculated using the equation:

[(Abs control – Abs sample) / Abs of control] x 100.

3.4. Antibacterial activity

The ethanolic extracts of the aerial parts of *Pergularia daemia* were tested for their antibacterial properties.

3.4.1 Bacteria used: Citrobacter freundii and *Klebsiella pneumoniae*.

3.4.2. Sterilization

All glasswares were well sterilized in the pressure cooker for 1 hour. Instruments like forceps and inoculation needles were sterilized over flame after dipping in alcohol. The media was sterilized in the pressure cooker for 20 minutes.

3.4.3. Method of inoculation

Sterilized Petri plates (4 inches) containing sterile medium were well swabbed with the selected microorganisms in such a way so as to get thorough coverage of uniform thick lawn of growth of the inoculum.

3.4.4. Incubation

The cultures were maintained at room temperature in the laboratory for a period of 24 hours for bacteria.

3.4.5. Nutrient Agar medium (NA):

S. No.	COMPOSITION	QUANTITY (gm)
1.	Sodium chloride	3.0
2.	Peptone	5.0
3.	Beef extract	3.0
4.	Agar	15.0
5.	Distilled water	1000 ml
6.	pH	6.8 – 7.2

3.4.6 Procedure

The antibacterial activity of the ethanol extracts of aerial plant parts of *Pergularia daemia* was determined with the Agar Well Diffusion Method. Wells were bored in to the agar using a sterile cork borer (6mm diameter). Different concentration of extracts (25 µl, 50 µl and 100 µl) were poured into the wells and incubated at 37ºc for 24 hours. Controls were maintained. After the incubation period, the zone of inhibition was measured.

4. RESULTS AND DISCUSSION

The qualitative phytochemical analysis of the aerial plant parts of *Pergularia daemia* is summarized in Table 1. The aerial plant part extracts of *Pergularia daemia* shows the presence of alkaloids, flavonoids, lignin, phenol, steroids, tannins, and terpenoids. Alkaloids, steroids, phenols and tannins were found in all the three solvent systems. Flavonoids were present only in chloroform and ethanolic extract. Lignins were

present only in petroleum ether extract. Terpenoids were present in both petroleum ether and ethanolic extracts whereas amino acids, proteins, and saponins were found to be absent in all the three solvent systems.

Table 1. Qualitative phytochemical analysis of aerial part extracts of *P. daemia*.

S. No	PHYTOCHEMICAL COMPOUND	PETROLEUM ETHER	CHLOROFORM	ETHANOL
1.	Alkaloids	**+**	**++**	**++**
2.	Amino acids	**-**	**-**	**-**
3.	Flavonoids	**-**	**+**	**+++**
4.	Lignin's	**+**	**-**	**-**
5.	Phenols	**+**	**+**	**++**
6.	Proteins	**-**	**-**	**-**
7.	Saponins	**-**	**-**	**-**
8.	Steroids	**+**	**+**	**+**
9.	Tannins	**+**	**+**	**+**
10.	Terpenoids	**+**	**-**	**++**

+++ Abundant, ++ Moderate, + poor, - absent.

4.1. Quantitative determination of total flavonoids, tannins and phenols of P. daemia aerial part extracts.

The total phenol content of *P. daemia* aerial part extracts ranged from 54.8 ± 0.04, 43.5 ± 0.03, and 60.4 ± 0.05 mg/g of Gallic acid. The Gallic acid was used to draw a standard calibration curve and the result was expressed as Gallic acid equivalents which showed an abundant amount of phenolic content. The total flavonoid content of *P. daemia* aerial part extracts ranged from 31.1 ± 0.04, 27.4 ± 0.02, and 49.5 ± 0.01 mg/g Rutin. The total tannin content of *P. daemia* aerial part extracts ranges from 39.3 ± 0.02, 52.7 ± 0.04, and 59.8 ± 0.03. Both total phenol and tannins are high in Ethanolic extract (Table 2).

Table 2. Quantitative analysis of aerial plant part extracts of *Pergularia daemia.*

Solvents	Total phenols mg GAE /g	Total flavonoids mg RE /g	Total tannins Mg GAE /g
Petroleum ether	54.8 ± 0.04	31.1 ± 0.04	39.3 ± 0.02
Chloroform	43.5 ± 0.03	27.4 ± 0.02	52.7 ± 0.04
Ethanol	60.4 ± 0.05	49.5 ± 0.01	59.8 ± 0.03

4.2. Antioxidant activity

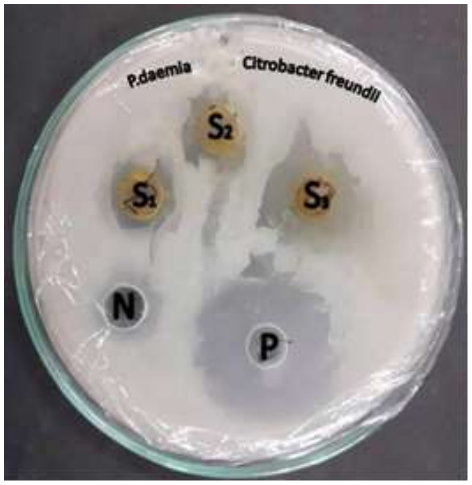
The antioxidant activity of Petroleum ether, Chloroform, and Ethanolic aerial plant part extract was done by using DPPH radical scavenging assay. The antioxidant activity of the plant is compared with standard Rutin.

Table 3. Antioxidant activity

Solvents	Concentration	% Inhibition	IC_{50}
Petroleum ether	100	27.37	519.63
	500	48.11	
	1000	87.53	
Chloroform	100	35.40	611.46
	500	40.88	
	1000	85.14	
Ethanol	100	35.93	550.46
	500	45.41	
	1000	84.93	

4.3. Anti-bacterial Activity of ethanolic extract of aerial plant parts of P. daemia:

The ethanolic extract of *Pergularia daemia* aerial plant parts was tested for antibacterial activity against *Citrobacter freundii* and *Klebsiella pneumoniae*. The Minimum Inhibitory Concentration (MIC) of the ethanol extracts were 25 µl, 50 µl, and 100 µl. The highest zone of inhibition was produced by ethanolic extract of leaves of *Pergularia daemia* against *Klebsiella pneumonia* (MIC like 25, 50, and 100 µl) than *Citrobacter freundii* for the same. The zone of inhibition for *Citrobacter freundii* was measured as 7mm, 9mm, and 13mm for 25, 50, and 100 µl of MIC respectively. The zone of inhibition for *Klebsiella pneumoniae* was measured as 9mm, 11mm, and 13mm for 25, 50, and 100 µl of MIC.



PC, Positive control; NC, Negative control; S1, 25 µl of extract; S2, 50µl of extract; S3, 100µl of extract.

5. CONCLUSION

Plants especially medicinally important plants are useful to mankind for curing various ailments. The plant *Pergularia dameia* that is taken under study is a medicinally viable plant that contains substantial amount of secondary metabolities like phenols, flavonoids and tannins; which is needed to cure major diseases prevailing in the world. It is also confirmed that the plant has a high standard of antioxidant capacity as well as antimicrobial property. So this plant *Pergularia dameia* can be considered and utilized widely in the field of medicine for the preparation of novel drugs. In various fields such as pharmaceutical, nutraceutical and drug designing industries this plant serves as a potent resource. Further investigations on this plant can be done elaborately to find out the major curative properties.

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