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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMICROBIAL STUDY OF PERGULARIA DAEMIA

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Abstract

Traditional plant-based medicines play a crucial role in global healthcare, especially in resource-constrained regions where pharmaceutical options are limited. Pergularia daemia, commonly known as Trellis-vine, is one such medicinal plant with a rich history of ethno-medicinal use. In this study, we explore the pharmacological potential of P. daemia, focusing on its diverse properties, including antifungal, antioxidant, antimicrobial, anti-inflammatory, analgesic, antipyretic, fertility-enhancing, hepatoprotective, and anticancer effects. Additionally, we investigate its traditional applications in treating gastric ulcers, menstrual disorders, anemia, and respiratory ailments. Despite its widespread use, scientific documentation remains scarce. Therefore, our research aims to qualitatively and quantitatively assess the phytochemical composition, antioxidant activity, and antimicrobial properties of different P. daemia extracts.

Introduction:

According to the World Health Organization (WHO), more than 80% of the global population in poor and underdeveloped countries rely primarily on traditional plant-based medicines for their primary health care needs because pharmaceuticals are not available or are relatively expensive[1].

Herbal medicine is a part of alternative treatment method and it includes the use of different medicinal plants and their extracts. This type of treatment is the most effective and safe mode for treating the patients. Herbal medicines are directly synthesized from various parts of plants such as leaf, stem, roots, barks, flowers, seeds, etc. In Ayurvedic system, herbal compositions are mostly prepared through polyherbal formulations. Also, they proved a significant effect against various chronic ailments. The herbal preparations are named as

powders (Churna), decoction (Kwatha), resins (Guggul), oil (Taila) and infusion (Phanta) etc.[2]In this study, the medicinal plant of interest is Pergularia daemia, which belongs to the Asteraceae family. It is a slender, hispid, fetid smelling perennial climber with small, white flowers and is commonly found in central India and Africa. It is used in ethno-medicine and has been proved to be pharmacologically active with potential medicinal significance as an antifungal, antioxidant, antimicrobial, anti-inflammatory, analgesic, antipyretic, fertility, hepatoprotective and anticancer agent. It is also used in treating gastric ulcers, uterine and menstrual complaints. On the other hand, the leaves of this plant are also used as an effective medicine for anemia, leprosy, arthritis, hemorrhoids, amenorrhea, dysmenorrheal, infantile diarrhea, body pain, asthma, bronchitis and whooping cough[3,4]. Latex of this plant is used to treat boils and sores. While, the stem is used to treat cold, malarial fever and pyretic[5]. From the literature review it is cleared that there is a dearth of scientifically documented literature on the medicinal benefits of this abundant plant; hence, this study was undertaken to estimate the phytochemicals qualitatively and quantitatively, antioxidant investigation and antimicrobial activities of different extracts of Pergularia daemia.

Materials and methods:

Plant collection:

The fresh plants of P. daemia were collected in the month of April 2012 from the village Soyata district Washim, Maharashtra, India.

VERNACULAR NAMES:

Table 1. Vernacular names of the plant Pergularia daemia[3]

Language	Name
Marathi	Utarn
Hindi	Utaran, Sagovam, Aakasan
Sanskrit	Kurutakah, visamika, kakajangha
Gujarathi	Chamardudhi
English	Hariknot plant
Tamil	Uttamani, Seendhal kodi, Veliparuthi
Telugu	Dustapuchettu, Jittupaku, Gurtichettu
Kannada	Halokoratige, Juttuve, Talavaranaballi

Preparation of the extract:

The leaves and stem of Pergularia daemia were washed with water to remove dust and soil particles and dried under shade and fine powder was prepared by using mechanical grinder. Dried powdered sample was then extracted in Soxhlet extractor using methanol, ethanol, chloroform and petroleum ether. Water extract was prepared separately. Extracts were filtered using Whatman Filter paper No.1 and stored in air tight container for further analysis.

Qualitative Phytochemical Screening:

Preliminary phytochemical testing was carried out by using following tests [6, 7].

Test for reducing sugars (Fehling's test)

1ml of extract, equal quantities of Fehling solution A and B were added and heated. Formation of brick red precipitate indicates the presence of reducing sugars.

Test for cardiac glycosides (Keller killiani test):

1ml of extrct, 5ml of distilled water was added and evaporated to dryness. Then to the Sample 2ml of glacial acetic acid containing trace amount of ferric chloride solution was added. Then 1ml of concentrated sulphuric acid was added along the sides of the tube. Formation of brown ring underlayed with blue colour indicates presence of cardiac glycosides

Test for tannins (Ferric chloride test):

1ml of extract, 1ml of ferric chloride solution was added. Formation of blue, black or brownish green colour indicates the presence of tannins.

Test for tannins (Lead acetate test):

1ml of extract, 1ml of lead acetate was added. A formation of white precipitate indicates the presence of tannins.

Test for saponins (Froth test):

1 ml of extract, 5 ml of distilled water was added and shaked vigorously. Formation of froth indicates the presence of saponins.

Test for phenols (Lead Acetate test):

1ml of extract, 1 ml of lead acetate solution was added. Formation of precipitate indicates the presence of phenols.

Test for alkaloids (Mayer's test):

1ml of extract, 1 ml of Mayer's reagent (Potassium iodide solution) was added. Formation of whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Test for flavanoids (Alkaline reagent test):

1 ml of extract, few drops of dilute ammonium solution and few drops of concentrated hydrochloric acid were added. A yellow colouration indicates the presence of flavanoids.

Test for terpenoids (Salkowski test):

1 ml of extract, 2ml of chloroform and few drops of sulphuric acid were added. Formation of reddish brown ring indicates the presence of terpenoids.

Test for steroids (Libermann Burchard test):

1ml of extract, 2ml of acetic anhydride and 2ml of concentrated sulphuric acid were added. Formation of violet to blue or green colour indicates the presence of steroids.

Quantitative Analysis of Phytochemicals

Estimation of Alkaloids:

5 gm of sample was added to 200 ml of 10% acetic acid in ethanol in a beaker. The beaker was tightly covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quater of the original volume. The entire solution was precipitated by the drop wise addition of concentrated ammonium hydroxide solution. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is alkaloid, which was dried and weighed[7].

Estimation of Flavanoid:

10 gm of sample was added to 100 ml of 80% aqueous methanol in a beaker. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was then evaporated to dryness and weighed[7].

Estimation of total Phenol:

Few grams of sample were boiled with 50 ml of ether for 15 minutes for the extraction of phenols. To the 5ml of extract, 10 ml of distilled water, 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The samples were left for 30 minutes. This was measured at 505 nm[7].

Antioxidant activity

Scavenging of DPPH ((2,2-diphenyl-1-picrylhydrazyl) free radical by the plant extract was examined by known method [8,9].Stck solution 1mg/ml concentration of extract was prepared in methanol. Dilutions were made to obtain concentrations of 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.62 µg/mL, 7.81 µg/mL, 3.90 µg/mL, 1.99 µg/mL and 0.97 µg/mL. 1 ml diluted solution of each concentration was then mixed with 1mL

methanolic solution of DPPH of concentration 1mg/mL .After 30 min of incubation in the dark at room temperature, the absorbance was recorded at 517 nm with a spectrophotometer (UV 1800) A control was a mixture of 1 mL methanol and 1mL methanolic DPPH . Percentage inhibition was calculated using the equation below.

% inhibition = (A of control - A of sample/ A of control) × 100

Antibacterial Activity:

Culture of three human pathogenic bacteria made up of two gram negative and one gram positive bacteria were used for antibacterial assay. Salmonella typhi, Escherichia coli were the gram negative species and Staphylococcus aureus was the gram-positive species. Antibacterial activity of the plant extract was determined using a Kirby- Bauer disk diffusion method.100 µl of the test bacteria were grown in 10ml of fresh media until they reached a count of approximately 10^8 cells of bacteria. Then 100µl of microbial suspension was spread on to the Agar Plates and sterile wells were made with the help of sterile cork borer. The extract of Pergularia daemia was added to the wells in aseptic conditions. The above Plates were incubated at 37^0C for 24 hours and the diameters of the inhibition zones were measured. Each antibacterial assay was performed in triplicate & Mean Values were reported. Standard antibiotic Amoxicillin served as positive controls for antimicrobial activity[10].

RESULTS & DISCUSSION:

Phytochemical screening of the crude extract:

The phytochemical screening of different solvent extracts of Pergularia daemia results (Table 1) showed the presence of alkaloids, flavonoids, tannins, phenolic compounds, steroids, terpenoids and saponins.Polar solvents showed high concentrations of phytochemicals for both qualitative and quantitative analysis.

Table:2 Phytochemical screening of Pergularia daemia (Arial Part)

Phytochemical	water	Ethanol	Petrolium Ether	Ethyl Acetate
Alkaloids	+	+	-	+
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Terpenoid	+	+	+	+
Saponin	+	+	-	-
Phenols	+	+	-	+
Tannin	+	+	-	-
Cardiac Glycoside	+	+	+	+
Reducing Sugar	+	+	+	+

+ present & -absent

Table:3 Quantitative phytochemical analysis of the leaves of Pergularia daemia

Phytochemical	Methanol	Ethanol	Petroleum Ether	Chloroform	Water
Alkaloids	6.78	7.18	1.90	2.04	8.54
Flavonoids	2.09	2.34	2.12	0.12	4.09
Phenols	14.56	13.44	4.22	9.12	7.12

Antioxidant activity:

2,2-diphenyl-1-picrylhydrazyl (DPPPH)radical scavenging activity:

The percentage inhibition of P. daemia over DPPH free radicals varied from 32.33% to 87.03%. Polar solvent extract showed better scavenging activity than the extract in non-polar solvent. The IC_{50} (µg/mL) is the minimum concentration of the extract required to scavenge 50% of the DPPH radicals. The IC_{50} value of vitamin C with the minimum concentration is just around 7.8 µg/mL, which is considered to be chemotherapeutically significant (IC50 < 10 µg/mL).The parallel examination of vitamin C as a reference with antioxidant activity of plant extracts is given in Figure1 below. Antioxidant activity of an ethanolic extract of P. daemia is at around 9µg/mL and 15µg/mL for petroleum ether extract. This study reveals that the free radicals that might be present in humans can be eliminated by P. daemia to get relief from oxidative stress and degenerative illness. Ethanolic extract from P. daemia has capacity for scavenging DPPH radicals and was even effective at low concentration.

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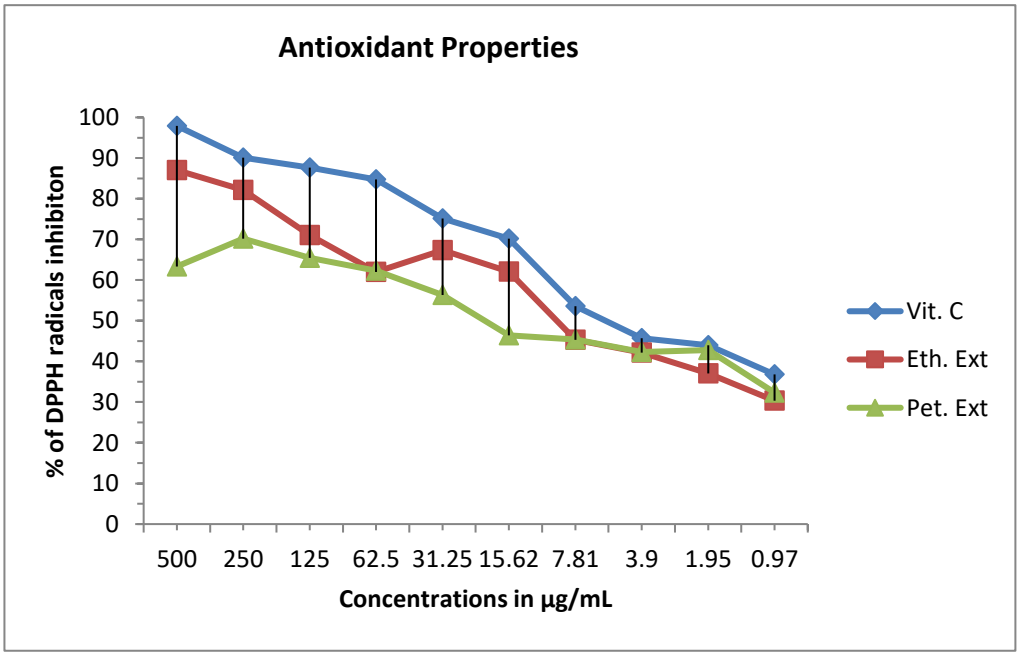

Antimicrobial Activity:

The results from this investigation reveal that the P. daemia water extra offers significant potential against microorganisms.

Table 4: Antibacterial activities of extracts of Pergularia daemia

Extract	Diameter of zone of inhibition of bacteria (mm)		
	E. coli	**S. aureus**	**S. typhi**
Methanol	NA	13	NA
Ethanol	NA	NA	12
Petroleum Ether	11	10	11
Chloroform	NA	NA	21
Water	18	22	29
Amoxicillin	25	NA	32

Conclusion:

In this study, we investigated the phytochemical composition, antioxidant activity, and antimicrobial potential of *Pergularia daemia*. Our findings reveal that this plant possesses a high content of flavonoids and other phenolic compounds. Notably, efficient extraction was achieved using polar solvents. The abundant phenols and flavonoids contributed to the strong antioxidant activity observed in the plant extracts. Furthermore, the polar solvent extracts demonstrated heightened sensitivity against human pathogens compared to less polar extracts. These results align with traditional folk medicine practices, where extraction with water or liquor (both polar solvents) efficiently captures the active ingredients from *P. daemia*. Given its promising properties, *P. daemia* emerges as a potential candidate for drug discovery. We recommend further phytochemical analysis to isolate and identify the specific active compounds responsible for the observed biological activities. By unlocking the secrets within this plant, we may uncover novel therapeutic agents with significant health benefits.

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