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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ASSAY OF LEAVES OF *PERGULARIA DAEMIA* LINN

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ABSRACT
The study reveals the evaluation of Antibacterial activity and phytochemical analysis in different solvents like petroleum Ether, Ethanol, Methanol, Acetone and distilled water of leaves of *pergularia daemia* linn. The antibacterial efficacy of leaves extract of *pergularia daemia* performed by using disc diffusion method against the test micro-organisms like *Escherichia coli, Salmonella typhii, Klebsiella pneumoniae, Pseudomonas aureginosa, Staphylococcus aureus and Shigella flexinari.* The results show the maximum inhibitory activity in petroleum ether extract and ethanol extract was relatively higher than other leaf extracts against *Shigella flexinari* and *Staphylococcus aureus* respectively. The amoxicillin was used as standard. The preliminary phytochemical screening shows the presence of Alkaloids, flavonoids, Saponins, Tannins, Carbohydrates and amino acids.

Keywords: *Antibacterial Assay, Phytochemical Screening, Pergularia Daemia*

INTRODUCTION
Natural products play an important role in prevention and cure of human diseases from many contraries. These natural products are the sources of many useful drugs which are often used in variety of conditions. Most of the medicinal plants used in traditional medicine to cure sickness and diseases. The indigenous system of medicine i.e. Ayurveda, Unani have been in existence in several centuries.

In homeopathy 70% of the medicines are prepared from plants. India is rich in indigenous herbal resources which grow on their varied topography and under changing agro climate conditions with the growth of almost 20000 plants are of medicinal value (Chaudhari, 1980).

Pergularia daemia linn. (Aclepediaceae) is an important foetid smelling, lactiferous twinner with hispid stems found in all parts of India along roadsides. Leaves simple, opposite, cordate, velvety, pubescent with milk latex, margin ciliate, flowers are greenish yellow or dull white, tinged with purple in corymbose clusters flowering may occur many times each year between Aug. and Jan. in central India. Fruits follicles with long beak and soft spines, seeds many, velvety, pubescent on both sides.

Medicinal Uses

Traditionally the plant is useful as anthelmintic, laxative, antipyretic and expectorant and also used in infantile diarrhoea. This drug was also strongly recommended for malarial intermittent fevers (Kirtikar, 1983).

The latex may also be used as a fish poison and gives relief from toothache (Patrik, 1992) (Herbbar, 2004) the Indian Ayurvedic system of medicine reported various uses of its aerial parts like anti-fertility (Golam, 2001), wound healing (Kumar, 2006), anti-diabetic (Wahi, 2002), hepatoprotective (Sureshkumar, 2006), cardiovascular effect (Dhar, 1973) and antibacterial activity (Senthil, 2005). The leaf paste mixed with castor oil is applied to joints in inflammation, liver complaints, spleen enlargement.

The leaf juice is given in asthma and applied to rheumatic swellings in combination with lime or ginger. The leaves are also used in preparation of medicinal oil given in rheumatism, amenorrhea, dysmenorrhea.

The purpose of the present study is to evaluate the preliminary phytochemical screening and antibacterial activity with MIC of various extracts of leaves of *pergularia daemia.*

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves of *Pergularia daemia* were collected from Ahemedpur tahsil of Latur district and Authenticated by Botanical Survey of India, Pune. The collected sample was washed with tap water to remove soil particles and dirt. The plant material was shade dried and powdered by using electronic grinder.

Preparations of Extract

About 30-40 gm of powder of leaves was extracted with different solvents by using Soxhlet apparatus until the extractive was clear. The extracts were concentrated with water both at 50^0 c then the semisolid extracts were stored in refrigerator at 4^0c for further use.

Phytochemical Screening

The extracts were subjected for phytochemical screening by dissolving them in respective solvents (1gm/ml). The extracts show the presence of alkaloids, flavonoids, suponins, tannins, terpeniods, carbohydrates and amino acids (Harbone, 1973) (Trease, 1989).

Test for Alkaloids: To the test solution, add 1% HCL and any of mayor's reagents, Wagner's reagent or dragendroff"s reagent added. A creamish or brownish red or orange precipitate indicates the presence of alkaloids.

Test for flavonoids: To the test solution of each extracts added 5 ml of dilute ammonia solution followed by addition of concentrated $H_2\,so_4$. A yellow color in each extract indicates the presence of flavonoids.

Test for Tannins: Few drops of ferric acid solution were added to the test solution and there is the formation of brownish green or blue black coloration indicates presence of Tannins.

Test for saponins: Extracts were diluted in 5ml distilled water and shakes vigorously and formation of a stable persistent froth which indicate presence of saponins.

Test for Terpenoids: To the test solution 2 ml of chloroform and 3 ml of H_2so_4 was carefully added. Formation of a reddish brown color at the interface indicates the presence of terpenoids.

Test for Carbohydrates: The test solution was boiled on water bath and 1ml of fehlings solution A and B were added respectively. A red precipitate indicates presence of carbohydrates.

Test for Proteins: To the extracts few drops of concentrated nitric acid added. Formation of yellow colour shows the presence of proteins.

Test Micro-organisms

The microbial strains *E. coli* MTCC (443), *Salmonella typhii* MTCC (734), *Klebsiella pneumonae* MTCC (2653), *Pseudomonas aureginosa* MTCC (424), *Staphylococcus aureus* MTCC (96), *Shigella flexinari* MTCC (1457), were obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of Discs

10mm discs were prepared and sterilized in autoclave. These discs were soaked in different concentrations of extracts like Petroleum ether, Ethanol, Methanol, Acetone and Distilled water. The standard drug Amoxicillin was used as control.

Determination of Antibacterial Activity

Antibacterial activities of the *Pergularia daemia* leaves extract were determined by agar disc diffusion method (Kirby, 1996). Nutrient agar was prepared for the study. Each plate of Nutrient agar was swabbed with each bacterial strain by using sterile glass spreader. The soaked dried discs were placed on the surface of each inoculated plate. The plates were allowed for diffusion for half an hour and then transferred to incubator at 37^0c for 24 hours. Standard disc of Amoxicillin were also placed as positive control and acetone was used as negative control. The antibacterial activity of leaves extracts was determined by measuring the diameter of zone of inhibition in mm.

Minimum Inhibitory Concentration

For determination of MIC, different concentrations of plant extracts prepared ranges from 2mg/disc to 10 mg/disc. Each concentration of plant extracts were tested against the different bacterial pathogens and the inoculated plates were incubated for 24 hours. Antibacterial activity was assigned by measuring the zone of inhibition around the disc. Amoxicillin was used as standard.

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RESULTS AND DISCUSSION

The preliminary phytochemical screening of *pergularia daemia* leaves extracts shows the presence of phytochemical compounds like Alkaloids, flavonoids, Saponins, Tannins, Terpenoids, Carbohydrates, Amino acids in all the extracts. The Alkaloids were not found in the leaves extract of ethanol. The saponins were also not detected in methanol and acetone leaves extract (Table -1).

Table – 2 shows the antibacterial activity of all the extracts with different concentrations against all the bacterial strains. The Petroleum ether extract shows significant result against all bacterial strains that it shows maximum zone of inhibition (36mm) against *shigella flexinari* at its higher concentration (10mg/disc), (35mm) against *pseudomonas aureginosa*, (33mm) against *klebsiella pneumonae*, (31mm) against *salmonella typhii*, (29mm) against *staphylococcus aureus* and (26mm) against *E.coli*.

As the concentration of extracts increases the zone of inhibition also increases. The Ethanol extract also shows marked inhibition at its higher concentration (10mg/disc) it shows effective zone of inhibition (31mm) against *salmonella typhii* and *staphyiococcus aureus* respectively. The distilled water extract also shows marked zone of inhibition at (10mg/disc) (28mm) against *staphylococcus aureus*. The Methanol and Acetone extract of *Pergularia daemia* leaves shows moderate activity against all the bacterial strains (Table -2).

Discussion

The results of present study shows that the Petroleum ether and Ethanol leaves extract of *Pergularia daemia* shows much more antibacterial effect against all the bacterial strains.

Table 1: Antibacterial activity of *Pergularia daemia* leaves

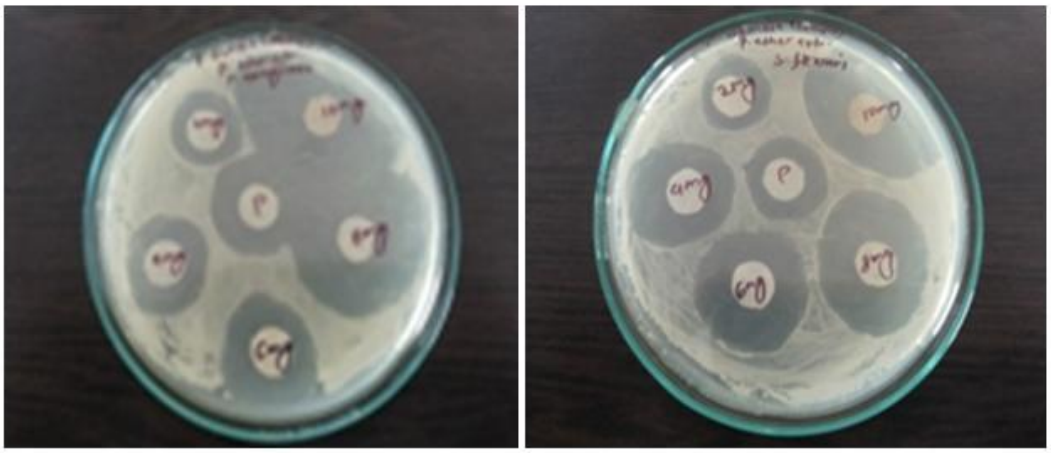
Plants	Solvents	Conc.	Pathogens (zone of inhibition in mm)					
		Mg/disc	*E.coli*	*S.typhi*	*K.pneumoniae*	*P.aeruginosa*	*S.aureus*	*Sh.flexinary*
***Pergularia daemia* (leaves)**	**Petroleum Ether**	2	14	_	15	19	_	19
		4	19	16	19	21	15	23
		6	23	21	26	24	21	27
		8	24	22	28	32	23	32
		10	26	31	33	35	29	36
	Ethanol	2	_	18	_	_	18	_
		4	_	20	16	_	22	_
		6	_	22	22	_	24	17
		8	17	28	24	16	26	20
		10	19	31	26	19	31	24
	Methanol	2	_	_	_	_	_	13
		4	13	_	_	_	_	14
		6	17	11	14	15	14	18
		8	19	13	16	17	19	20
		10	23	17	19	23	22	24
	Acetone	2	13	_	_	11	15	13
		4	14	14	14	12	17	15
		6	15	16	17	14	18	17
		8	18	18	18	16	19	18
		10	20	21	21	19	21	19
	Aqueous	2	_	_	_	_	_	_
		4	_	_	_	_	16	_
		6	_	16	_	_	18	_
		8	15	19	19	14	26	17
		10	20	23	26	16	28	20
	Standards		28	30	30	24	26	24

Table 2: Phytochemical tests of *Pergularia daemia* leaves

Plant Name	Extracts	Alkaloids	Flavonoids	Saponins	Tanins	Carbohydrates	Terpenoids	Amino acids
Pergularia daemia **leaves**	**Petroleum Ether**	+	+	+	-	+	+	+
	Ethanol	-	+	+	+	+	+	+
	Methanol	+	+	-	+	+	+	+
	Acetone	+	+	-	+	+	+	+
	Aqueous	+	+	+	+	+	+	+

+: Present, - : Absent

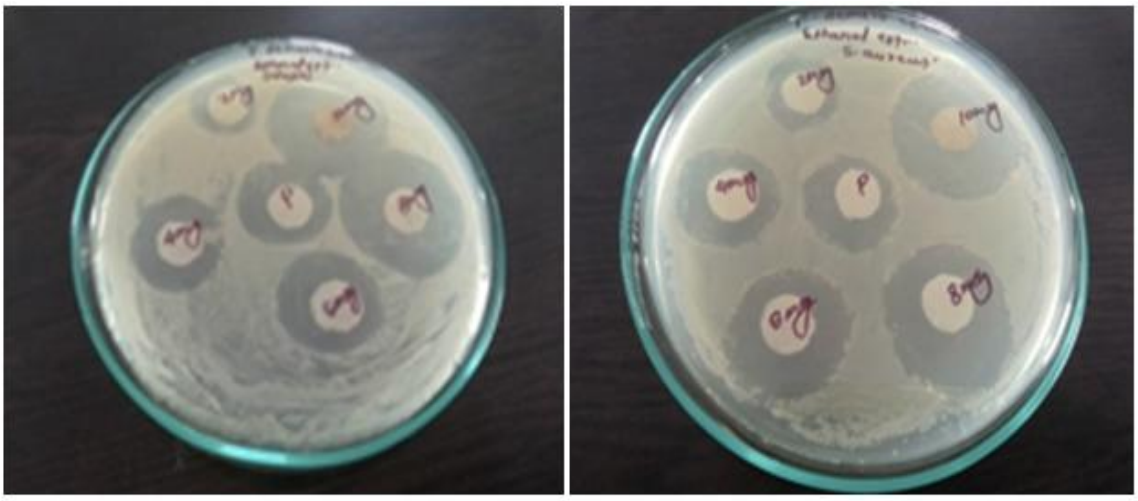
Pergularia daemia leaves extract (petroleum Ether)



Pseudomonas aureginosa *shigella flexinari*

Pergularia daemia leaves extract (Ethanol)

0



S. typhi *S. aureus*

Both the extracts shows maximum zone of inhibition than that of the standard drug Amoxicillin. The Petroleum ether extract was found to exhibit greater antibacterial activity than the Ethanol extract. The antimicrobial activity is probably due to the membrane disruption by terpenes and their complex with extracellular soluble proteins, bacterial cell walls and disrupts microbial membranes (Urzua, 1998).

Earlier studies of *C. Deepika et al* shows the similar observations from the whole plant extract of *Pergularia daemia* (Tenmonzi, 2011). Several reports have shown the antibacterial properties of plant extracts of *Pergularia daemia* (Jogi, 2012), (Chandak, 2010), (Karthiswaran, 2010) *K.karthishwaran and et al*. Plant based antimicrobials have much therputic potential as they serve the purpose with lesser side effects which are associated with synthetic antimicrobials (Iwu, 1999).

Conclusion

The Petroleum ether extract of *Pergularia daemia* shows maximum inhibitory activity against all test organisms as compared to standard drug Amoxicillin. Therefore this study provides evidences for the antibacterial activity of *Pergularia daemia* leaves which could partly contribute to its medicinal use. Further pharmacological and phytochemical investigation may useful for development of new drug in future.

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