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PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF LEAVES EXTRACTS OF PERGULARIA DAEMIA FROM DAVANGERE REGION

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ABSTRACT

Phytochemical analysis of the dried leaves of *Pergularia daemia* (asclepiadaceae) indicates the presence of a steroids, tannins, saponins, alkaloids and reducing sugars. The Phamacological interest of these compounds coupled with the use of this plant is traditional medicine promoted the authors to check for its possible antimicrobial activity. The extracts (pet ether, chloroform and ethanol) were found to possess maximum activity against infectious pathogens. The zone of inhibition was observed with four antimicrobials' with some exceptions. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Keywords: Antimicrobial activity, asclepiadaceae, phytochemical analysis, *Pergularia daemia*

Introduction:

The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. Plants are the main source of most complex organic molecules. These molecules are showing wide structural diversities and are serving as templates for many semi synthetic and synthetic drug molecules. Plant derived drug molecules are frequently showing their role in treating the disease conditions with minimal side effects comparing to the synthetic molecules (Mallikharjuna, P.B. et al., 2007). It is generally considered that compounds produced naturally rather than synthetically will be biodegraded more easily and therefore be more environmentally acceptable. Thus naturally antioxidants, antiviral, fungicidal agents and nutrients have gained popularity in recent years and their use and positive images among consumers are spreading. In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have been developed due to the indiscriminate use of commercially antimicrobial drugs commonly used in the treatment of infectious diseases (Micheal J Pelczer, chan ECS, Noel, RK. 1998) In order to find new therapeutic agents , plants that have antimicrobial activity have attracted attention(Jigna, P, Sumitra, VC.2007).

Pergularia daemia is a perennial twining herb belongs to the family asclepiadaceae. It was mainly found in tropical and sub- tropical areas secreting milky latex. Leaves are thin, broadly ovate and heart-shaped 2-12 cm long, covered with soft hairs. P. daemia is known as "Veliparuthi" in Tamil, "Uttar-avaruni" in Sanskrit, and

"Utranajutuka" in Hindi. Traditionally, The literature review shows that it was reported to have anti-fertility (Golam, S. 2001), hepatoprotective (Sureshkumar, SV. 2007), wound healing (Kumar, B. 2006), anti diabetic(Wahi, AK. 2002) and anti-inflammatory activity (Bhaskar, VH and Balakrishnan, N. 2009). of this plant have been reported in folk and Ayurvedic medicine.

MATERIALS AND METHODS:

Plant material:

The leaves of *Pergularia daemia* were collected in the month of May-June from the fields around the area of Kuraki in Davangere district, Karnataka. The plant was authenticated by Prof. Thippeswamy Department of Botany, Davangere University, Davangere.

Preparation of extracts:

Leaves were shade dried and coarsely powdered. The powdered plant material (30g) was successfully extracted using soxhlet extractor by the solvents viz., pet ether(60-80^{0}C), chloroform and ethanol, according to their increasing polarity respectively. The extract obtained was filtered and evaporated to dryness under reduced pressure in rotary vacuum evaporator(Tanwer, B.S. 2010).

Phytochemical investigation:

A small portion of the extract was used for the phytochemical tests for compounds which include tannins(Archana, P 2012), flavonoids (Sati, S. C. & Kumar, P.2015),, alkaloids(Savithramma, N. 2011and Akinseye, O. R.2017,),, saponins(Tofighi, Z.2016),, and steroids(Panchal Mital, D. & Jha, C. V.2021), in accordance with the methods of with little modifications. Exactly 1.0 g of plant extract was dissolved in10 ml of distilled water and filtered (using Whatman No 1 filter paper) A blue colouration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract. Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. A millilitre of the filtrate was treated with few drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloid. About 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the flavonoids (Yadav, RNS. 2011). The results for preliminary phytochemical evaluation are depicted in table-1.

Table 1 . Phytochemical analysis

S.No	Phytochemicals	Petroleum ether extract	Chloroform extract	Ethanol extract
1.	Tannins	-	+	+++
2.	Saponins	-	-	++
3.	Flavonoids	-	-	++
4.	Quinones	+	-	+++
5.	Betacyanins	-	-	++
6.	Anthocyanins	-	-	-
7.	Steroids	+	-	+++
8.	Alkaloids	-	+	+
9.	Glycosides	-	-	+
10.	Terpenoids	+	-	++
11.	Cardiacglycosides	-	-	-
12.	Phenols	-	+	++
13.	Coumarins	-	-	++

Where as:+++:Strongly present,++:Mildly present ,+ Present and – Absent.

The preliminary phytochemical analysis of different solvents extracts of *Pergularia daemia* revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, Quinones, steroids, terpenoids and saponins as illustrated in table-1.

Antimicrobial activity:

The cup-plate method was used for evaluating antimicrobial activity of the crude extracts

Micro organisms used:

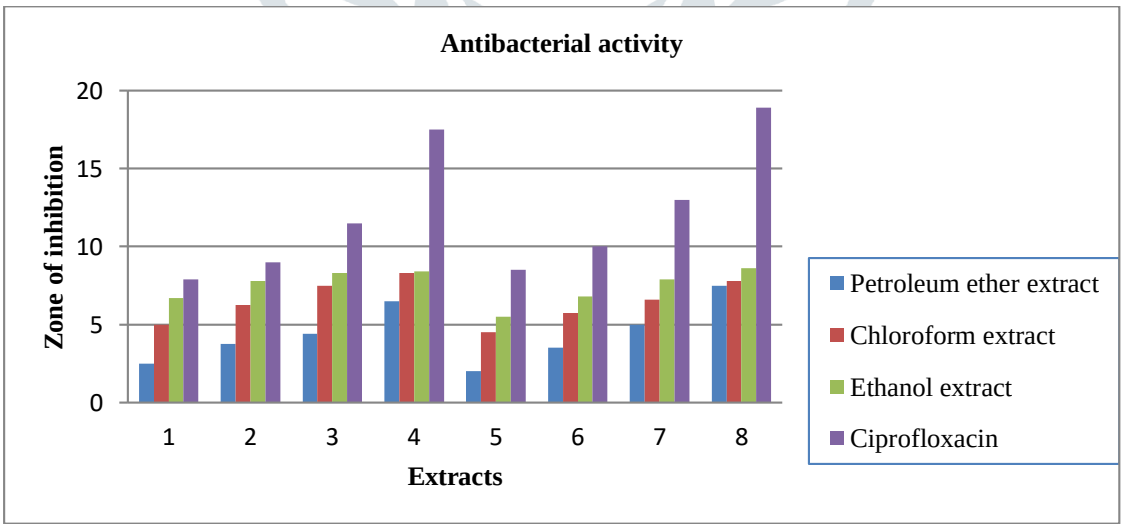
The bacterial cultures viz., *E.coli* (NCIM 2945) *S.aureus* (NCIM2127) and the fungal cultures viz., *A.niger* (NCIM798) and *C.albicans* (NCIM 3102). These cultures were procured from National Collection of Industrial Microorganism (NCIM), Pune, India.

Antibacterial activity:

The pet ether, chloroform & ethanolic extracts of *Pergularia daemia* were tested by the cup-plate method (Bukar, AM, 2015.) Different concentrations of the extracts (5%, 10%, 15%, 20%) was prepared. The petri plates were poured with Nutrient agar medium and allowed for solidification. The test microorganisms (*E. coli* & S. *aureus*) were swabbed on the petriplate containing media. The four wells were prepared using cork borer. Different concentrations were filled in these wells. Then the plates were incubated at 37^0C for 24h along with the standard being Ciprofloxacin andcontrol DMSO respectively. The diameter of the inhibition zones were measured in mm (Chanda, SY.2010). The results were tabulated in table-2

Table- 2. Antibacterial activity of extracts of *Pergularia daemia*

Extract	Zone of inhibition (in mm)							
	E. coli				*S. aureus*			
Concentration	5%	10%	15%	20%	5%	10%	15%	20%
Petroleum ether extract	2.5	3.75	4.40	6.5	2.0	3.5	5.0	7.5
Chloroform extract	5.0	6.25	7.5	8.3	4.5	5.75	6.6	7.8
Ethanol extract	6.7	7.8	8.3	8.4	5.5	6.8	7.9	8.6
Ciprofloxacin	7.9	9.0	11.5	17.5	8.5	10.0	13.0	18.9
Control(DMSO)	……..				……..			

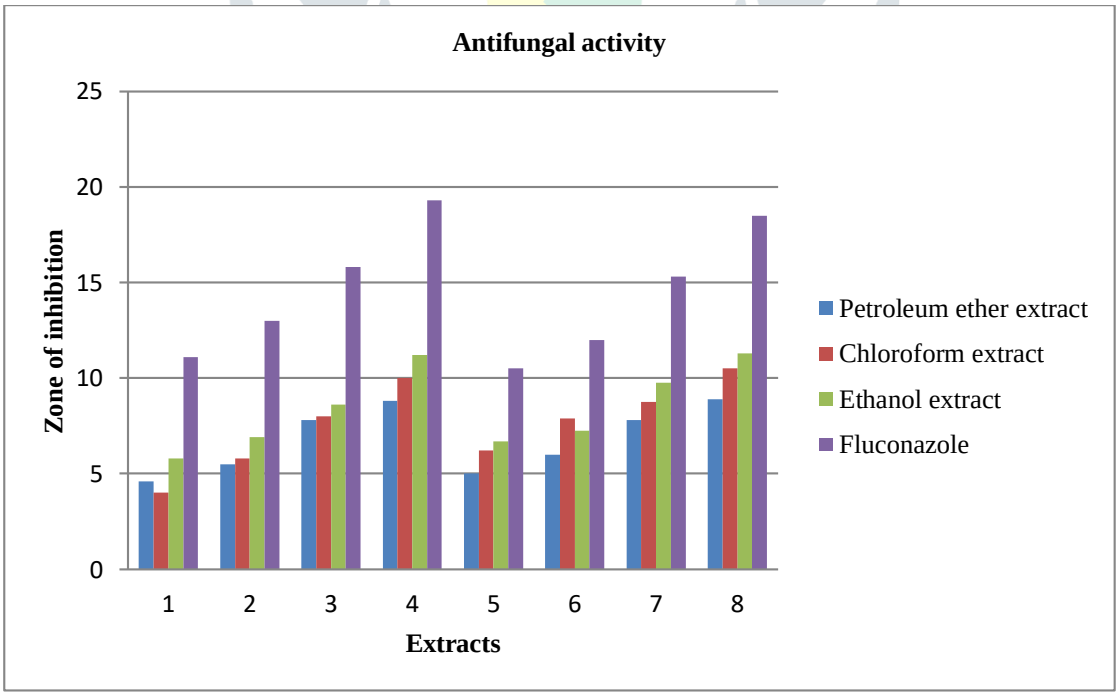


Antifungal activity:

The pet ether, chloroform & ethanolic extracts of *Pergularia daemia* were tested by the cup-plate method (Chopra, I .2000 and CLSI. 2015). Different concentrations of the extracts (5%, 10%, 15%, 20%) was prepared. The petriplates were poured with Nutrient agar medium and allowed for solidification. The test microorganisms (*A. niger* & *C. albicans*)were swabbed on the petriplate containing media. The four wells were prepared using cork borer. Different concentrations were filled in these wells. Then the plates were incubated at 28^0C for 48h along with the standard and control Flucanazole and DMF . The diameter of the inhibition zones were measured in mm. Results were tabulated in table -3

Table- 4. Antifungal activity of extracts of *Pergularia daemia*

Extract	**Zone of inhibition (in mm)**							
	A. niger				***C. albicans***			
Concentration	5%	10%	15%	20%	5%	10%	15%	20%
Petroleum ether extract	4.6	5.5	7.8	8.8	5.0	6.0	7.8	8.9
Chloroform extract	4.0	5.8	8.0	10.0	6.2	7.9	8.75	10.5
Ethanol extract	5.8	6.9	8.6	11.2	6.7	7.25	9.75	11.3
Fluconazole	11.1	13.0	15.8	19.3	10.5	12.0	15.3	18.5
Control (DMF)	----				----			



RESULTS AND DISCUSSION:

The present Phytochemical screening revealed exist several classes of secondary metabolites such as alkaloids, polyphenols, flavonoids, coumarins, saponins, tannins, triterpenes and steroids. Several molecules are active on pathogenic microorganisms. Exist such metabolites in the tested plant extracts can give a preliminary explanation on their antimicrobial activities. All the extracts have showed antibacterial and antifungal activity against the organisms. The results revealed that chloform and ethanolic extracts exhibited considerable zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*. The ethanolic extract has showed moderate activity against both the organisms at 20% concentration. All the extract were compared with the standard drug Ciprofloxin. The different concentration of all the extracts also exhibited potent antibacterial activity. Antifungal activity was carried out against *Aspergillus niger* and *Candida albicans*. The ethanol extract showed maximum zone of inhibition at all the concentrations. The pet ether and chloroform extract showed potent antifungal activity. All the extracts were showed comparable activity with the standared drug Flucanazole. This study has shown the scientific basis for the therapeutic uses of traditional plants. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

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