



**EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF VARIOUS
SOLVENT EXTRACTS OF *PERGULARIA DAEMIA* (FORSK.) CHIOV. LEAVES**

G. Savitha, S. Selvamani and S. Balamurugan*

Department of Botany (DDE), Annamalai University, Annamalainagar – 608002, Tamilnadu, India.

ABSTRACT

The main objective of the study was to investigate the phytochemical compounds and antimicrobial activity of different extracts of *Pergularia daemia* (Forsk.) Chiov. Alkaloids, terpenoids, tannins, saponins, flavonoids, glycosides were present and anthroquinones were not present. Different extracts of leaves of *Pergularia daemia* were screened for antimicrobial activity against selected pathogens by agar diffusion method. In future this plant can be subjected to the isolation of major constituent's antimicrobials and to further pharmacological evaluation.

Keywords: Phytochemical, antimicrobial activity, *Pergularia daemia*

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include leaf, stem, flower, fruit and root exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [2]. Although hundreds of plant species have been tested for antimicrobial properties, the majority of them have not been adequately evaluated [3]. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antimicrobial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activities. The *Pergularia daemia* (Forsk.) Chiov. belongs to the family *Asclepiadaceae*. Traditionally, the plant *Pergularia daemia* is used as anthelmintic, laxative, antipyretic and expectorant, and is also used to treat infantile diarrhoea and malarial intermittent fever, latex of this plant is for toothache [4]. Stem bark of this plant is remedy for cold [5] and fever [6]. Aerial parts of this plant are reported to have various pharmacological activities like hepatoprotective [7], antifertility [8], anti-diabetic [9] analgesic and anti-inflammatory [10]. Phytochemically the plant has been investigated for cardenolides, alkaloid and saponins and it has been found that contains triterpenes and steroidal compounds [11]. The present study was undertaken to determine the folklore uses of *Pergularia daemia* (Forsk.) Chiov.

MATERIALS AND METHODS

The present work was carried out at Department of Botany, Annamalai University, Annamalai nagar. The leaves of *Pergularia daemia* (Forsk.) Chiov. were collected from in and around Annamalai University campus. The plant was taxonomically identified and authenticated by Dr.V.Venkatesalu, Head of the Department of Botany (DDE), Annamalai University. The leaves of *Pergularia daemia* (Forsk.) Chiov. was thoroughly washed with water and dried under shade for about ten days the dried plant sample was ground well into a fine powder in a mixture grinder. The powder was stored in sealed polyethylene bags at room temperature before extraction.

Microorganisms used:

The microorganisms used in the study: Gram-negative *Pseudomonas fluorescense*, Gram-positive *Bacillus subtilis* and fungus *Candida albicans* were obtained from stock culture in the Department of Microbiology, Raja Muthiah Medical College & Hospital, and Annamalai University.

Antimicrobial screening of extracts:

Susceptibility tests were carried out. The modified agar well diffusion method [12, 13] was followed to test the antimicrobial activity of the extracts. The medium employed was diagnostic sensitivity agar. The culture were prepared in triplicate and incubated at 37°C for 24 to 72h. 0.2 ml of broth culture of the organism was put in a sterile Petri-dish and 18 ml of sterile molten diagnostic sensitivity agar, was added. Well were bored into the medium using 0.1 ml of the extracts. Streptomycin and Chloramphenicol & Fluconazole were used as the standard antimicrobial agents at a concentration of 10mcg/disk, 30mcg/disc respectively. The plates were kept in sterilized inoculation chamber for 2 h to facilitate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37°C for 24 h and the diameter of zone of inhibition of microbial growth was measured in the plates in millimeters.

Preliminary phytochemical analysis:

The extracts were analyzed for the presence of Alkaloids, Terpenoids, Tannins, Saponins, Flavonoids, Anthraquinones, and Glycosides [14].

Alkaloids: About 0.2 g of the extracts was warmed with 2% H₂SO₄ for two minutes. It was filtered and few drop of Dragencloffs reagent were added. Orange red precipitated indicates the Presence of alkaloids.

Tannins: Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride added to the filtrate. Dark green solutions indicate the presence of tannins.

Anthraquinones: About 0.5 g of the extracts was boiled with 10% HCL few minutes in a water bath. It was filtered and allow to cool. Equal volume of CHCl₃ was added to the filtered. Few drops of 10% NH₃ were added to the mixture and heat. Formation of rose-pink color indicates the presence of anthraquinones.

Glycosides: The extracts was hydrolyzed with HCL solution and neutralized with NaOH solution. A few drop of few Fehling's solution A and were added. Red predicate indicates the presence of glycoside.

Saponins: About 0.2 g of the extracts was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy of small bubbles) shows the presence of saponins.

Flavonoids: Extracts of about 0.2 g was dissolved in diluted NaOH and HCL was added A yellow solution that turns colorless, indicates the presence of flavonoids.

Terpenoids: (Salkowski test): 0.2 g of extracts was mixed with 2 ml chloroform (CHCl₃) and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to

indicate positive results for the presence of terpenoids.

RESULTS AND DISCUSSION

Phytochemical screening of hexane, chloroform, ethyl acetate, methanol, and water extract of *Pergularia daemia* (Forsk.) Chiov. is shown in table 1. The susceptibility of test microorganism to the crude extracts of *Pergularia daemia* is shown in table 2. The qualitative analysis of the leaf extracts of *Pergularia daemia* showed the presence of phytochemical constituents such as alkaloids, terpenoids, tannins, saponins, flavonoids, and glycosides. The above results indicates that, the leaves of plant investigated are rich in alkaloids, terpenoids, tannins, saponins, flavonoids, and also showed the presence of glycosides. Chloroform, ethyl acetate and methanol extracts showed the presence of saponins. All extracts have showed the absence of anthroquinones. The leaf extracts were tested against Gram positive *Bacillus subtilis* and Gram negative *Pseudomonas fluorescences* and also tested for antifungal activity against *Candida albicans* showed the inhibition of growth. Water, ethyl acetate and methanol were found to be highly sensitive against Gram positive *Bacillus subtilis* and Gram negative *Pseudomonas fluorescences* (with zone of inhibition above 14mm means highly sensitive). Ethyl acetate extract was showed more antimicrobial activity than standard antibiotics Streptomycin and Chloramphenicol. The maximum antifungal activities were observed in methanol extract (13mm) other than chloroform extract (8mm)and hexane extract (10mm) The inhibitory effect of these extracts confirmed the potential use of the plant in the treatments of antimicrobial induced ailments.

S.No	Chemical composition	Water extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Hexane extract
1	Alkaloids	+	-	-	+	-
2	Tannins	+	-	-	+	-
3	Anthroquinones	-	-	-	-	-
4	Glycosides	-	+	-	-	-
5	Saponins	-	+	+	+	-
6	Flavonoids	+	-	+	-	+
7	Terpenoids	+	+	+	-	-

Table 1: Phytochemical screening of *Pergularia daemia* leaf extracts.

(+) Present; (-) Absent

Extracts	Bacterial strains		Fungal strain
	<i>Bacillus subtilis</i> (Gram +)	<i>Pseudomonas fluorescences</i> (Gram -)	<i>Candida albicans</i>
Water extract	+++	++	+
Chloroform extract	-	-	-
Ethyl acetate extract	+++	+++	-
Methanol extract	+++	++	++
Hexane extract	++	-	+
Streptomycin	+++	+++	-
Chloramphenicol	++	++	-
Fluconazole	-	-	+

Table 2: Antimicrobial activity of leaf extracts of *Pergularia daemia*

(+) slightly active; (++) moderately active; (+++) highly active; (-) inactive.

CONCLUSION

This study revealed the presence of many secondary metabolites in the leaves of *Pergularia daemia*. It has further confirmed that the plant extracts could be used for the treatment of various infections including stomach infections and dental caries. The results lend credence to the folkloric use of this plant in treating microbial infection and shows that *Pergularia daemia* could be exploited for new potent antimicrobial agents.

REFERENCES

1. Srivastava, J., J. Lambert and N. Vietmeyer, 1996. Medicinal plants: An expanding role in development. World Bank Technical Paper. No.320.
2. Uniyal, S.K., K.N Singh, P. Jamwal and B. Lal, 2006. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J. Ethnobiol. Ethnomed., 2:1-14.
3. Balandrin, M.F., J.A. Klocke, E.S. Wurtele and W.H. Bollinger, 1985. Natural plant chemicals: Sources of Industrial and Medicinal materials. Science, 22: 1154 -1160.
4. Hebbar SS, Harsha VH, Shripathi V, Hegde GR. 2004. Ethnomedicine of Dharward district in Karnataka, India-plants used in oral health care. J Ethnopharmacol 2004; 94:261-266.

5. Dokosi OB. Herbs of Ghana. Ghana university press. Accra; 1998. p. 313-314.
6. Bruce TBF. Personal communications, phytotherapist. Accra, Ghana; 1998&2000.
7. Sureshkumar SV, Mishra SH. Hepatoprotective effect of extracts of *Pergularia daemia* forsk. J Ethnopharmacol 2006; 107:164-168.
8. Golam sadik, Gafur MA, Shah Alam Bhuiyan M, Khurshid Alam AHM, Helal U Biswas M, Parvez Hassan, Abdul Manna, Omar Faruk Khan M, Chowdhury AKA. Antifertility Activity of *Pergularia daemia*. The science2001:1(1): 22-24.
9. Wahi AK, Ravi J. Hemalatha S, Singh PN. Anti diabetic activity of *Daemia extens*. Journal of Natural Remedies 2002; 2(1):80-83.
10. Sathish CJ, Sharma RA, Jain R, Macalo N, Capasso F, Vijayvergia R, Mittal C. Ethno pharmacological evaluation of *Pergularia daemia* (forsk.) chivo. Phytotherapy research1998; 12:378-30.
11. Anjaneyulu ASN, Raju DVS, Srinivasa Rao S. Chemical evaluation of *Pergularia daemia*. Indian journal of Chemistry 1998; 37B: 318-320.
12. P.L. Garrod, P.H. Lambert, A. O. Grady, Antibiotics and Chemotherapy. Church – Hill. Livingstone Press, London.,(1981) 385.
13. G.E. Trease, W.C. Evans, Pharmacognosy. 11thEdn. Brailliar Tiridel and Macmillian Publishers, London. (1989).
14. J. B. Harbone, Phytochemical Methods 3rd Edn. Chapmaan and Hall, Ltd., London. (1973) 135 – 203.