

ANTIMICROBIAL SCREENING OF *POLYGONUM BARBATUM* LEAF EXTRACTX. QUEEN ROSARY SHEELA^{1,*} V. ALEX RAMANI¹¹PG & Research Department of Chemistry, St. Joseph's College, Trichy 620 002, Tamil Nadu, India. Email: sheela910@gmail.com

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ABSTRACT

The antimicrobial screening of ethanolic leaf extract of *Polygonum barbatum* was assessed on ten micro organisms which include four gram positive, four gram negative bacteria and two funguses. The ethanolic leaf extract showed considerable activity on both the bacteria and fungi with Inhibition Zone Diameter (IZD) ranging from 14.17 mm to 31.45 mm. The minimum inhibitory concentrations range between 12 µg/ml to 16 µg/ml.

Keywords: *Polygonum barbatum*, Antimicrobial screening, Paper disc diffusion method.

INTRODUCTION

For a long period of time, plants have been valuable sources of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies¹. The plant kingdom is a treasure house of potential drugs and there has been an increasing awareness about their importance of medicinal plants². They are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. Different plants have been used as a source of inspiration in the development of novel drug³. Plants derived medicines are widely used because they are relatively safer than the synthetic alternatives, they are easily available and cheaper⁴. Many plants species have been evaluated for their antimicrobial activity in the past twenty year⁵. And since then efficacy of many medicinal plants in the treatment of many diseases have been put to test in many laboratories⁶.

Polygonum barbatum Linn (Family: Polygonaceae) a stout, annual herb, with erect stem, distributed throughout the hotter parts of India, particularly in wet places. The seeds are employed in Malabar and Canara to relieve the griping pains of colic. In Patna, the roots are used as an astringent and cooling remedy. In china, decoction of the leaves and stalks are said to be used as a stimulating wash for ulcers⁷⁻¹⁰. It has been reported that dichloromethane extract of *Polygonum barbatum* showed brine shrimp toxicity and spasmodic activity. The methanolic extract of *Polygonum barbatum* was found to possess cholinergic activity¹¹. The antinociceptive, anti-inflammatory and diuretic properties were also studied by Abdul Mazid and others¹².

MATERIALS AND METHODS

Plant material

The leaves of *Polygonum barbatum* were collected along the beds of Cauvery river, near Trichy, Tamil Nadu in February 2008. The plant was identified and authenticated by Dr.G.V.S.Moorthy, Joint Director, Botanical Survey of India (BIS), Agriculture University campus, Coimbatore, India. The voucher specimen number was BIS/SC/5/23/08-09/Tech-1614, and the specimen was deposited at herbarium.

Preparation of plant extract

Leaves were air dried and powdered mechanically. About 100 g of the powder were extracted with 95% ethanol by hot percolation method using soxhlet extractor for 4 h. The extract obtained was filtered and evaporated on water bath to get crude extract. The yield of ethanolic extract was found to be 30%. This extract was used for the antimicrobial studies.

Antimicrobial activity

Microorganism used

The in vitro antibacterial (*Staphylococcus aureus* ATCC 6538 p, *Streptococcus pyogenes* ATCC 14289, *Proteus vulgans* ATCC 6380, *Staphylococcus mutans* ATCC 25175, *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 6539, *Shigella flexneri* ATCC 29508, *Pseudomonas aeruginosa* ATCC 9027) and antifungal (*Aspergillus niger*

ATCC 9029 and *Candida albicans* ATCC 2091) activities of extract was evaluated by paper disc diffusion method. The microbes were obtained from microbial type collection center, Chandigarh, India. The minimum inhibitory concentrations (MIC) of extract were also determined by agar streak dilution method.

Paper disc diffusion method

The sterilized (autoclaved at 121 °C for 15 min) medium (40-50 °C) was inoculated (1 ml/100 ml of medium) with the suspension (10⁵ cfu/ml) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the extract (25, 50 and 100 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre incubated for 1 h at room temperature and incubated at 37 °C for 28 h and 48 h for antibacterial and antifungal activities, respectively. Ciprofloxacin (50 µg/disc) and ketoconazole (50 µg/disc) was used as standard for antibacterial and antifungal activities respectively¹³.

Minimum inhibitory concentration (MIC)

MIC of the extract was determined by agar streak dilution method. A stock solution of the extract (25, 50 and 100 µg/ml) in dimethyl formamide was prepared and graded quantities of the extract were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40-50 °C) containing extract was poured into a Petridis to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 10⁵ cfu/ml and applied to plates with serially diluted extract in dimethyl formamide and incubated at 37 °C for 24 h and 48 h for bacteria and fungi respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate¹⁴.

RESULTS AND DISCUSSION

The ethanolic extract of *polygonum barbatum* showed considerable antibacterial activity against four gram positive, four gram negative bacteria and two funguses. For bacteria, the IZD ranges between 14.17 mm to 30.34 mm and for fungi it ranges between 14.42 mm to 31.45 mm.

The extract was more active against *Aspergillus niger* with IZD of 31.45 mm and it has less activity against *Salmonella typhi* with IZD of 23.22 mm.

The potency of the crude extract was comparable to those of antibiotics which are pure substances. Ciprofloxacin was used as standard against the bacterial strains; it shows the IZD ranging from 34.16 mm to 36.29 mm and Ketoconazole was used as standard against the fungal strains and the IZD ranges from 36.45 mm to 38.37 mm.

The result of MIC determination indicates the values ranges from 12-16 µg/ml. The lowest MIC value, 12 µg/ml was obtained on *Escherichia coli* and *Pseudomonas aeruginosa*. The highest MIC value, 16 µg/ml was recorded for *Salmonella typhi* and *Proteus vulgans* (Table1).

Table 1: Antimicrobial activities of *Polygonum barbatum* leaf extract on different microbes and their corresponding IZD and MIC

Sl. No	Micro organisms	Zone of inhibition (mm)				
		Standards ^a	25µg/ Disc	50µg/ Disc	100µg/ Disc	MIC µg/ml
1	<i>Staphylococcus aureus</i> ATCC 6538 p	34.16±	16.39±	19.26±	28.22±	15
		0.05	0.1	0.15	0.1	
2	<i>Streptococcus pyogenes</i> ATCC 14289	36.29±	18.24±	20.25±	27.43±	13
		0.15	0.24	0.13	0.07	
3	<i>Proteus vulgans</i> ATCC 6380	34.25±	15.25±	19.31±	27.23±	16
		0.09	0.11	0.05	0.08	
4	<i>Staphylococcus mutans</i> ATCC 25175	34.25±	14.17±	17.33±	25.41±	15
		0.03	0.06	0.05	0.09	
5	<i>Escherichia coli</i> ATCC 8739	36.25±	19.38±	23.33±	30.07±	12
		0.08	0.04	0.17	0.12	
6	<i>Salmonella typhi</i> ATCC 6539	34.41±	15.11±	19.25±	23.22±	16
		0.1	0.13	0.09	0.06	
7	<i>Shigella flexneri</i> ATCC 29508	34.37±	16.44±	22.37±	25.33±	15
		0.1	0.11	0.18	0.16	
8	<i>Pseudomonas aeruginosa</i> ATCC 9027	34.30±	19.29±	24.21±	30.34±	12
		0.06	0.06	0.11	0.18	
9	<i>Aspergillus niger</i> ATCC 9029	38.37±	16.27±	23.35±	31.45±	15
		0.1	0.11	0.12	0.07	
10	<i>Candida albicans</i> ATCC 2091	36.45±	14.42±	17.28±	23.26±	15
		0.07	0.08	0.08	0.08	

^aStandards (Ciprofloxacin for bacteria and Ketoconazole for fungus)

CONCLUSION

The results of the above research shows that the ethanolic extract of *polygonum barbatum* is a broad spectrum agent which can be used against gram positive, gram negative and also fungi.

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