

Antimicrobial Analysis of *Polygonum barbatum* Leaf Extracts

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ABSTRACT

Polygonum barbatum, known as *atalari* (in Tamil) or *ratu kimbulwenna* (in Sinhala), are perennial herbs of the family *polygonaceae* have been widely used anciently to treat various ailments including infections, fever, pain and tumor. In this study bioassays were carried out to analyse antibacterial activity by using leaves of *Polygonum barbatum*. Crude extracts were prepared from leaves by sequential extraction method, first by dichloro methane (DCM) then by ethyl acetate (EA) and finally by ethanol (EtOH). These were tested against gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* by agar well diffusion method. Ethanolic extracts showed a higher inhibition zones for all tested pathogens when compared with other extracts DCM and EA. Antibiotic streptomycin was used as a control for this analysis. The results revealed that the antibacterial activities of ethanolic extract of this plant was comparable to those of selected chemical antibiotic and suggesting its potential as alternative to the commercially available antibiotics in the treatment of infections caused by these microorganisms.

KEYWORDS: Antibacterial activity, Phytochemicals, Sequential extraction, *Polygonum barbatum*

Introduction

Medicinal plants have many traditional claims including the treatment of ailments of infectious origin. In the evaluation of traditional claims scientific research is important. In the present study an attempt was made to examine the sensitivity of pathogens to dichloro methane, ethyl acetate and ethanol extracts of *P. barbatum*.

The common names of *Polygonum* sp include joint weed, knotgrass and smartweed. Stem of *P. barbatum* is cylindrical, hollow, thickened at the nodes and leaves are long and tapering at both ends (Figure 1). Flowers are present in short, slender pedicels and perianth is white or purplish colour. *P. barbatum* is an annual herb

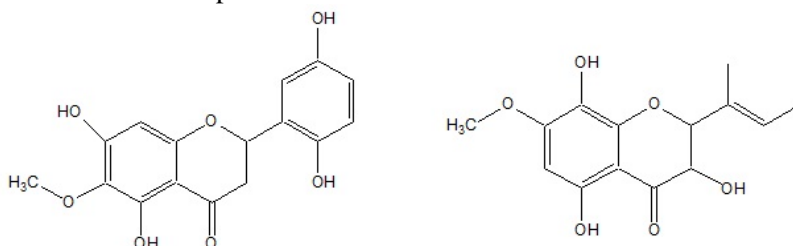
distributed throughout the hotter places of Sri Lanka, India, Thailand, Bangladesh and South East Asia. This is mainly found in marshy and aquatic places, by the sides of the river, seasonally flooded roadsides and in small ponds and it has been used traditionally in Siddha system of medicines to treat various diseases. Jeyaweera (2006) reported that the leaf powder of *P.barbatum* possess wound healing property, decoction of leaves used to wash off ulcers in China, seeds are used to relief colic in Malabar and Canara, roots are used as astringent in Brazil and seeds have aromatic, purgative and emetic action. Jeyaweera (2006) also reported that *P.barbatum* contains protein (15.7%), fat (2.1%), fiber (20.7), carbohydrate (50.4%) and minerals (11.1%).



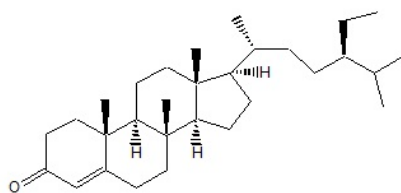
Figure 1: *Polygonum barbatum*

P.barbatum is abundantly present in Vavuniya tank as aquatic weeds. The effect of antibacterial activity of the leaf extract by direct extraction method, using 95% EtOH which had already been reported in the past by Queen Rosary Sheela *et al.* (2011). Therefore the aim of this study was to find the antibacterial activity of leaf extract by sequential extraction method using different solvents of varying polarity. Presents of secondary metabolites are the major components for the antimicrobial activities of plant extracts. Major phytochemical constituents found in *P. barbatum* are flavanone (Queen Rosary Sheela *et al.*, 2011), sitosterol, viscozulenenic acid and acetophenone (Mazid *et al.*, 2011).

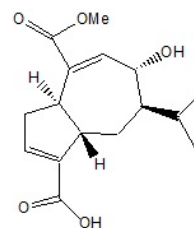
The structures of these compounds are as follows.



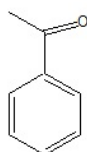
Flavanones



Sitosterone



Viscozulenenic acid



Acetophenone

Materials and Methods

Preparation of Leaf Powder

Fresh leaves of *P.barbatum* were collected from Vavuniya Tank, Vavuniya in May 2012 and they were washed with tap water to remove unwanted particles. Leaves were dried in shade and ground well into fine powder in an electric grinder and stored in air sealed polythene bags at 4°C in refrigerator before extraction.

Preparation of Leaf Extracts

Seventy grams of leaf powder was taken in a stoppered bottle. Two hundred and fifty mL of DCM was added and soaked for three days. Bottles were intermittently shaken. After three days the supernatant was collected and this procedure was repeated further twice to ensure the complete separation of all the constituents which were dissolved in DCM. Then the extracts were pooled together. Then the remaining powder sample was opened to air dry. A Similar procedure was adopted with ethyl acetate solvent and ethanol solvent. The solvent in the extract was evaporated to dryness under reduced pressure and reduced temperature (i.e. lower than the boiling point of the solvent) by using rotary evaporator.

Antibacterial Assay

In vitro anti-bacterial activity of extracts was studied against human pathogenic bacteria namely *P.aeruginosa*, *S.aureus*, *E.coli*, *B.subtilis* obtained from the Department of Microbiology of the Faculty of Medicine of the University of Jaffna. The antibacterial assay was performed by agar well diffusion method according to Patience (2006); 10⁶ cells/mL bacterial suspensions were prepared by tenfold

dilution method. Haemocytometer was used to count the bacterial cells. One mL of bacterial suspension was added into sterile petridish.

Then twenty mL of molten nutrient agar was added on to it and rotated slowly to ensure the uniform distribution of the microorganisms. Then it was allowed to solidify. Four wells were made in the plates with the help of eight mm cork borer. Test extract were dissolved in acetone and mother solvent. 100 μ L of the test solutions such as 10 mg/100 μ L, 30 mg/100 μ L, 50 mg/100 μ L, and streptomycin (1mg/1mL) were dispensed separately into the well with the help of micropipette. Streptomycin (1mg/1mL) was used as standard. These plates were incubated at 37°C and inhibition zones were recorded at regular time intervals (24h, 48h and 72h).

Results and Discussion

The results pointed out that the EtOH extract showed a higher inhibition zone for all tested pathogens than other two extracts (Figure 2). EtOH extract of *P.barbatum* showed a higher inhibition zone for all tested pathogens than that of standard antibiotic streptomycin. Antibacterial activity of DCM extract (Table 1) and EA extract (Table 2) showed a maximum inhibition zone of 11.5 mm for *P.aeruginosa* and 15 mm for *Bacillus w* respectively. 50mg/100 μ L of this extract inhibit the growth of *S.aureus*, *Bacillus*, *E.coli* and *P.aeruginosa* with the zone of inhibition of 26 mm, 24 mm, 25 mm and 21 mm respectively after 72 hours of incubation period (Table 3).

Streptomycin could not affect the growth of *E.coli* but the ethanol extract (Plate No. 01) of this plant inhibit the growth of this bacterial pathogen with a large inhibition zone of 21 mm after 48 hours of incubation. DCM and ethylacetate extracts of this plant showed minimum inhibition zones for these four bacterial pathogens. Time course analysis indicated that there was no considerable change in the inhibitory effect after 48 hours (Figure 3).

Ethanol extract of *P. barbatum* was the good extract which was obtained by a sequential extraction method of leaf extract first by DCM (Plate No. 5 and 6) and then by EA (Plate No. 7 and 8) finally by EtOH (Plate No. 1, 2, 3 and 4). The result of this study forms a good basis for selection of this plant for further phytochemical and pharmacological investigation for their use as possible antimicrobial agent for the treatment of various infections.

Plates illustrating the effect of different extracts of *P. barbatum* on the growth of tested pathogens are as follows. Here 10, 30, 50 and std indicates 10 mg/100 μ L, 30 mg/100 μ L, 50 mg/100 μ L of the crude extract and standard antibiotic respectively. In each plates dark circled area around well indicates the extent of inhibition of growth of a particular microorganism by a specific concentration of crude extract. Other than this area (whitish color) indicates the intense growth of bacteria.

Plate 1: Effect of EtOH extract on the growth of *E. coli*



Plate 2: Effect of EtOH extract on the growth of *S. aureus*



Plate 3: Effect of EtOH extract on the growth of *Bacillus*

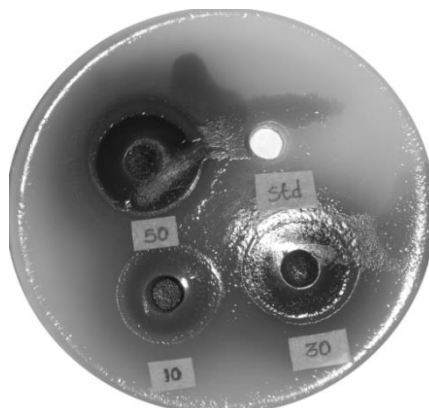


Plate 4: Effect of EtOH extract on the growth of *P. aeruginosa*

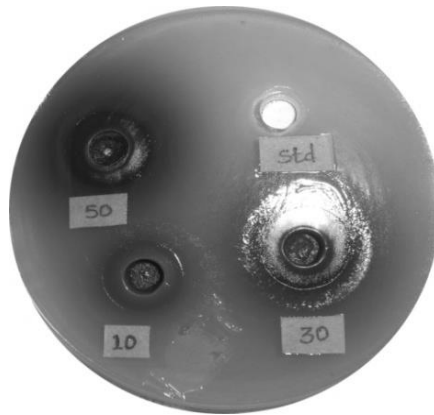


Plate 5: Effect of DCM extract on the growth of *Bacillus*



Plate 6: Effect of DCM extract on the growth of *S. aureus*

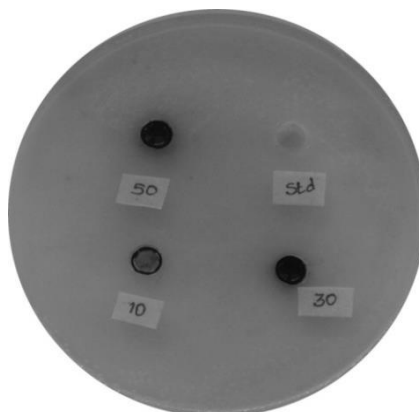


Plate 7: Effect of EA extract on the growth of *Bacillus*



Plate 8: Effect of EA extract on the growth of *S. aureus*

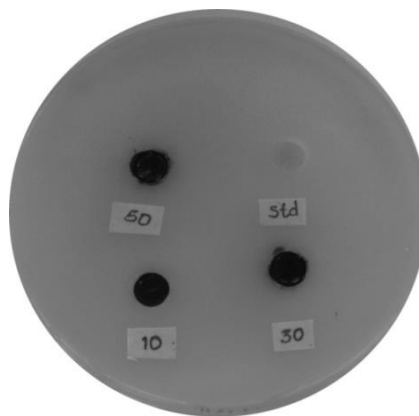


Table 1: Antibacterial activity of *P. barbatum* (DCM extract)

Concentration (mg/100µl)	Diameter of inhibition zone in mm											
	<i>S. aureus</i>			<i>Bacillus w</i>			<i>P. aeruginosa</i>			<i>E. coli</i>		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
10	-	-	-	9.0	9.5	9.5	10.0	10.5	10.0	-	-	-
30	9.5	10.0	9.5	9.0	9.0	9.0	10.5	10.5	10.0	-	-	-
50	10.0	10.0	10.0	10.0	10.5	10.0	11.5	11.0	11.0	9.0	9.5	9.0
Std	11.0	11.5	12	10.0	13	13	12.0	12	12	-	-	-

Table 2: Antibacterial activity of *P. barbatum* (EA extract)

Concentration (mg/100µl)	Diameter of inhibition zone in mm											
	<i>S. aureus</i>			<i>Bacillus w</i>			<i>P. aeruginosa</i>			<i>E. coli</i>		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
10	-	-	-	9.0	9.5	9.0	9.0	9.5	9.0	9.0	9.5	9.5
30	9.5	9.5	9.0	10.0	10.0	10.0	9.0	9.5	9.5	9.0	9.0	9.0
50	9.5	10.0	10.0	15.0	14.0	14.0	9.0	9.0	9.0	9.0	10.0	10.0
Std	15	15	15.5	14	14.5	14.5	13	13.5	13	-	-	-

Table 3: Antibacterial activity of *P. barbatum* (EtOH extract)

Concentration (mg/100µl)	Diameter of inhibition zone in mm											
	<i>S. aureus</i>			<i>Bacillus w</i>			<i>P. aeruginosa</i>			<i>E. coli</i>		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
10	16.0	18.0	18.0	14.0	20.0	20.0	17.0	17.0	17.0	16.0	16.5	16.5
30	18.0	23.0	23.0	20.0	21.0	21.0	20.0	24.0	24.0	18.0	18.5	18.5
50	18.5	25.0	26.0	22.0	24.0	24.0	23.0	24.0	25.0	19.0	21.0	21.0
Std	10	10.5	11	13	13.5	13.5	12.0	13	14	-	-	-

Note: Indicates that there was no antibacterial activity. Inhibition zones include the diameter of well (8mm)

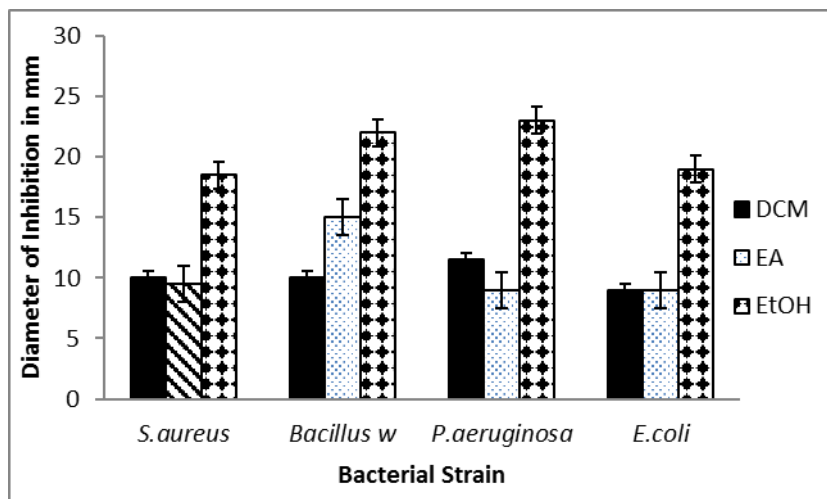


Figure 2: Effect of EtOH, EA and DCM extracts (50 mg/100 μ L) on tested pathogens after 24 hrs of incubation

Here the EtOH extract showed higher inhibition zone than the other ones.

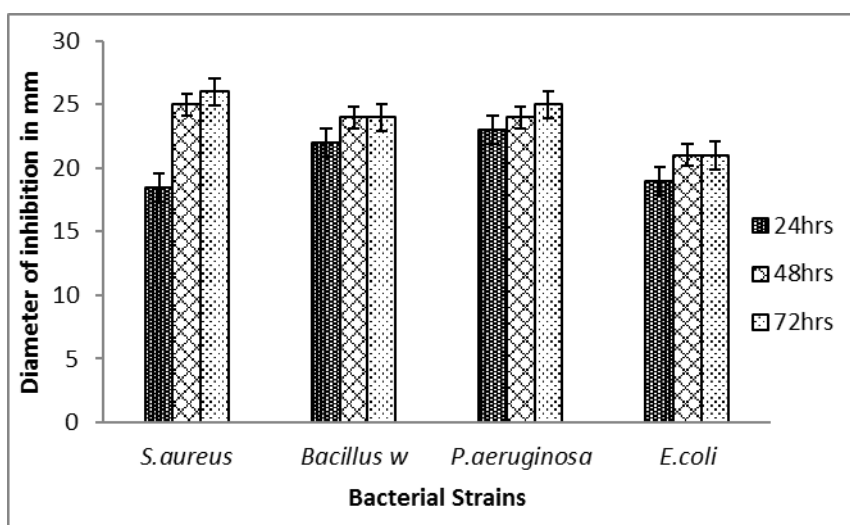


Figure 3: Effect of EtOH extract (50 mg/100 μ L) on tested pathogens at different incubation period

Incubation zones increases with increasing incubation periods. But commonly higher inhibition zone can be observed at 48 hours of incubation.

Queen Rosary Sheela and Ramani (2011) analysed the antimicrobial activity of *P. barbatum* leaf extract by hot extraction method by using 95% EtOH. They used paper disk diffusion method to analyse antimicrobial activity and this is a direct extraction method, they used only one solvent to separate the phytochemical compounds. They also indicate the EtOH extract showed a higher inhibition zones

for Gram positive and Gram negative bacteria. Therefore there is a need to analyse hot/ direct extraction or cold/ sequential extraction method is a better extraction method by maintaining the experimental conditions as the same for both.

Mazid *et al.* (2011) reported that the antitumor activity of methanol (MeOH) extract of sun dried, ground aerial parts of *P. barbatum*. Here they first obtained the crude extract by using MeOH then the obtained 90% aqueous MeOH extract and subjected to solvent partitioning with petroleum ether (PE). The resulting aq. MeOH extract was further partitioned with chloroform and finally by EA. In their study PE extract of *P. barbatum* showed a higher inhibition zone than the other solvents.

Conclusion

In conclusion the crude extract of ethanol has higher antibacterial activity and this confirmed the historical use of *P. barbatum* as an antibacterial agent. Here the antibacterial activity was analysed to the crude EtOH extract. In the crude extract there is a possibility for the presence of other compounds, some will increase/ decrease/ having no activity against bacteria. Therefore there is a need to separate the compounds in this extract and the individual compounds may have greater antibacterial activity than the crude extract. Therefore EtOH extract could be used as initial material for further bioassay guided isolation and purification to find the compound responsible for antibacterial activity.

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