INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

ANTIMICROBIAL ACTIVITY OF *PHYLLANTHUS DEBILIS*

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Article Received on: 02/04/2011 Revised on: 10/05/2011 Approved for publication: 03/06/2011

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ABSTRACT
Methanolic extract of *Phyllanthus debilis*, family Euphorbiaceae was evaluated for antimicrobial activity using *Staphylococcus aureus* ATCC 29756, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 26513. The effect produced by methanolic extract was comparable to that of Ampicillin. The result revealed that the methanolic extract of the aerial parts of *Phyllanthus debilis* exhibited significant antibacterial activity against both gram positive and gram negative bacteria.
KEY WORDS: *Phyllanthus debilis,* Ampicillin, antibacterial activity

INTRODUCTION

Different species of *Phyllanthus* are considered to be very effective hepatoprotective agents in the Indian indigenous systems of medicine and are considered bitter, astringent, stomachic, diuretic, febrifuge, deobstruant and antiseptic. Various species of *Phyllanthus* are being sold in India under the trade name 'Bhuiamlki'[1]. Therefore *Phyllanthus debilis*, family Euphorbiaceae is taken up for screening of antibacterial activity.

MATERIALS AND METHODS

Preparation of Methanolic Extract

The aerial parts of *Phyllanthus debilis* were collected from Mangalore, Karnataka, India during June 2007. They were dried in shade. The shade dried powdered stem bark (2kg) were soaked in Methanol and kept aside for four days. After four days the methanol layer was decanted off. The Process was repeated for four times. The solvent of total extract was distilled off and concentrate was evaporated on a water bath to a syrupy consistency and evaporated to dryness (200g).

Tested microorganisms

Two Gram-positive bacteria, namely *Staphylococcus aureus* and *Bacillus subtilis*, two Gram-negative bacteria , *Escherichia coli* and *Pseudomonas aeruginosa* were included. Antimicrobial activity was tested using above microorganisms using ampicillin (10 µg/ml) as standard by cup plate agar diffusion method[2-4] and by determination of MIC using tube dilution technique[5].

Antimicrobial activity assay

Agar diffusion method

5 mg of methanol extract was dissolved in 5 ml of DMF (stock solution). From the stock solution, 2.5 ml was taken aseptically and made up to 5 ml which give a concentration of 500 µg/ml. 0.1 ml of the solution containing 50 µg of the sample transferred into the cup in the Petri dish. Each Petri dish was inoculated with one of the bacterial cultures suitably diluted to contain above 10^6 cells/ml by spreading 0.1 ml suspension of the organism with a sterile cotton swab. Each plate was divided into four portions and in each portion a cup of 6 mm diameter was cut with a cork borer. Two cups were filled with sample (Methanol extract) while the other two were filled with solvent 0.1 ml DMF and 0.1 ml ampicillin solution respectively.

All the plates were kept in the refrigerator for 30 minutes to allow the diffusion of sample in to the surrounding agar medium. The petri dishes were incubated at 37°C for 24 h. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with that of the diameter produced by standard ampicillin. The diameter of zone of inhibition is proportional to the antibacterial activity of the substance.

Determination of MIC by tube dilution technique

5 mg of the Methanolic extract was dissolved in 5 ml of DMF that gave 1000 µg/ml concentration. From the

above solution 1 ml was transferred to a test tube containing 1 ml of nutrient broth and the resultant concentration was half of the previous one. From the above test tube 1 ml was taken and transferred to another test tube containing 1 ml of nutrient broth. This was repeated up to six dilutions. 1 ml was discarded from the last test tube. The test tubes were closed with cotton plugs. Aseptic conditions were maintained throughout the process of sample transfer to the test tubes. Concentration of test compound in the test tubes are 500, 250, 125, 62.5, 31.25 and 15.62 µg/ml.

Following the above method one set of the test tubes were prepared and used to inoculate a different bacterial culture (10^7 cells/ml approx).The above steps were followed for the Methanolic extract. A positive control and negative control were also prepared to confirm the nutritive and sterility properties of the prepared medium respectively. All the tubes were incubated at 37˚C for 24 h. Presence or absence of growth of organisms was observed and MIC of the extract against each organism was calculated.

RESULTS

The Methanol extract of the aerial parts of *Phyllanthus debilis* were subjected to antibacterial studies. The results indicated that the Methanol extract showed activity against *B. subtilis, E. coli* and *S. aureus* (MIC 125 µg/ml). But the activity against *P.aeruginosa* was (500 µg/ml).

Thus the result revealed that the Methanol extract of the aerial parts of *Phyllanthus debilis* exhibited antibacterial activity against both gram positive and gram negative bacteria.

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Table -1. Antimicrobial activity methanol extract of *Phyllanthus debilis*

Name of the Compound	Diameter of Zone of Inhibition in mm*(mean ± SE)			
	S.aureus	***B.subtilis***	***P.aeruginosa***	***E.coli***
Extract	13±0.21	15±0.13	14±0.09	14±0.23
Ampicillin	17±0.14	19±0.23	18±0.06	17±0.22

*values are the mean of three replicates

Table-2 MIC methanol extract of *Phyllanthus debilis*

Name of the compound	Mininum inhibitory concentration (MIC)																							
	S.aureus						*B.subtilis*						*P.aeruginosa*						*E.coli*					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Methanolic Extract	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+
+ve control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-ve control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

" -" absence of growth,
"+" presence of growth.

Source of support: Nil, Conflict of interest: None Declared