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Research Article

ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF STEM BARK OF *HOLOPTELEA INTEGRIFOLIA* ROXB (PLANCH)

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ABSTRACT
The petroleum ether, benzene, chloroform, methanol and aqueous extracts of the stem bark of *Holoptelea integrifolia* were evaluated for the antibacterial activity against various microorganisms viz *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. Disc diffusion method was adapted for the assessment of *in vitro* antibacterial activity. The antibacterial activity of different extracts of *Holoptelea integrifolia* at various concentrations were evaluated where zone of inhibition was compared with the standard drug i.e. ampicillin. Chloroform extract was found to be very effective against all the test microorganisms used; petroleum ether extract was only effective against *P. aeruginosa*; benzene extract was effective against *E.coli* and *B.subtilis*; methanol extract was effective against *E.coli* and aqueous extract was effective against *S.aureus* and *E.coli*, respectively when compared to standard drug ampicillin. The minimum inhibitory concentration for chloroform extract was found to be 50,300,25 and 100 µg/ml against S.aureus, B. subtilis, E. coli and P. aeruginosa; for petroleum ether extract was 100 µg/ml (P. aeruginosa); for benzene extract was 100 µg/ml (E.coli) and 25 µg/ml (B. subtilis); for methanol extract was 100 µg/ml (E.coli) and for aqueous extract was 50 µg/ml (S. aureus) and 25 µg/ml (E. coli) respectively suggesting the antibacterial activity of Holoptelea*integrifolia*.

KEYWORDS: *Holoptelea integrifolia*, antibacterial activity, zone of inhibition, minimum inhibitory concentration, stem bark.

INTRODUCTION

The history of Ayurvedic medicine goes back to at least three thousand years, in which centuries after centuries rolled by witnessing varied fluctuations in human life. In Ayurvedic science, visible or invisible minute animals that affect on living and non-living things of biosphere are described very efficiently. Under the heading 'Krimi', as they have been described worms, insects, bacteria, viruses and parasites. In Ayurvedic system of medicine, a large number of plants are being used for treating diseases of bacterial origin and good results have been obtained with some of them. In the same context, one such drug is stem bark of *Holoptelea integrifolia.*

Holoptelea integrifolia (Roxb) Planch (Ulmaceae), is commonly known as Indian elm, kanju. It is a large deciduous tree, commonly found throughout the greater part of India up to an altitude of 660 m, lower ranges of Himalaya from Jammu to Oudh, Rohilkhand, forest of Dehra Dun, Saharanpur, Orissa, Chota Nagpur, Bihar, West Bengal, Hill of Deccan, Eastern slopes of Western Ghats and North Circas[1,2]. In traditional system of medicine, bark and leaves are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism[3].The phytoconstituents isolated so far from stem bark are holoptelin A and B[4],2-aminonapthaquinone, friedelin,epifriedelin, β-sitosterol and its β-D-glucose[5,6]. Since there is no report on antibacterial activity of *Holoptelea integrifolia*, an attempt was made to evaluate the antibacterial activity

of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark by agar disc diffusion method.

MATERIALS AND METHODS

Plant Material

Stem bark of *Holoptelea integrifolia* Roxb. (Planch) from various parts of Tirupati were collected and authenticated by Mr.Madhava Chetty (S V University, Tirupati, Andhra Pradesh). A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore. The stem bark was dried under normal environmental conditions. The dried stem bark were powdered and stored in a closed container for further use.

Preparation of Extracts

The dried stem bark powder were coarsely powdered and subjected to successive extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, methanol and distilled water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 0.26, 0.45, 0.21, 4.77 and 7.85 % w/w respectively with reference to the air dried plant material.

5 mg of the extract was weighed and dissolved in 5ml of DMSO which was labeled as stock 1. From stock 1, further dilution were made so as to get 10, 25, 50, 100, 200, 300, 400 and 500 µg/ml concentrations by using DMSO as solvent.

Microorganisms Used

All the microbial cultures, used for antimicrobial screening were procured from National centre for Industrial Microorganisms (NCIM), Pune, India and from The Oxford College of Science, Bangalore. The bacterial culture was maintained on Muller Hinton agar slants which were stored at 4^{o}C.

Antibacterial Activity

Determination of Minimum Inhibitory Concentration (MIC)

The extract were screened for their antibacterial activity *in vitro* by disc diffusion method[7] using S.aureus, *B. subtilis, E. coli* and *P. aeruginosa* as test organism. Agar cultures of the test microorganisms were prepared. Three to five similar colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated for 24 h at 37^{o}C and suspension was checked to provide approximately 10^{10} colony forming units per ml. 0.1 ml of organism's suspension were spread evenly on the agar plates. For screening, sterile 3 mm diameter disc (Whatman filter paper No. 1) were impregnated with different concentration till saturation, dried and placed in inoculated plates of Muller Hinton agar medium. DMSO solvent was used as negative control. The plates were incubated at 37^{o}C for 24 h. After incubation for 24 h, the results were recorded by measuring the zones of inhibition surrounding the disc and the lowest concentration of each extract which is showing inhibition of growth of bacteria was determined as MIC. Ampicillin (50 µg/ml) was used as standard for bacteria.

RESULTS AND DISCUSSION

The antibacterial activity of *Holoptelea integrifolia* stem bark extracts was studied by employing disc diffusion method against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. The results of minimum inhibitory concentration and zone of inhibition are given in Table 1 and Table 2.

It is clear from the Table 1 and 2, chloroform extract was found to be very effective against all the microorganisms used; petroleum ether extract was only effective against *P. aeruginosa*; benzene extract was effective against *E.coli* and *B.subtilis*; methanol extract was effective against *E.coli* and aqueous extract was effective against S.aureus, B. subtilis, E. coli and P. aeruginosa; for petroleum ether extract was 100 µg/ml (P.aeruginosa); for benzene extract was 100 µg/ml (E.coli) and 25 µg/ml (B.subtilis); for methanol extract was 100 µg/ml (E.coli) and for aqueous extract was 50 µg/ml (S.aureus) and 25 µg/ml (E.coli) respectively suggesting the antibacterial activity of *Holoptelea integrifolia*. Work is under progress to reveal the chemical nature of the active constituents responsible for the antibacterial activity.

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Table 1: MIC values of different extracts of stem bark of *Holoptelea integrifolia*

Microorganism used	MIC with concentration of extract [µg/ml]				
	Petroleum ether	Benzene	Chloroform	Methanol	Aqueous
Staphylococcus aureus	-	500	50	-	50
Bacillus subtilis	400	25	300	-	500
Escherichia coli	500	100	25	100	25
Pseudomonas aeruginosa	100	500	100	-	-

Table 2: Zone of inhibition values (mm) in MIC of different extracts of *Holoptelea integrifolia*

Microorganism used	Zone of inhibition (mm) of extracts and standard					
	Petroleum ether	Benzene	Chloroform	Methanol	Aqueous	Ampicillin
Staphylococcus aureus	-	10	10	-	10	10
Bacillus subtilis	9	9	9	-	9	9
Escherichia coli	9	9	9	9	11	9
Pseudomonas aeruginosa	9	9	9	-	-	9

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