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Research Article

ANTIBACTERIAL ACTIVITY OF TRUNK BARK OF ALSTONIA SCHOLARIS

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ABSTRACT

The *in vitro* antibacterial activity of methanol, aqueous and total alkaloid extracts from the trunk bark of *Alstonia scholaris* (L.) R.Br (Apocynaceae), growing in north-east India was evaluated against two Gram-positive bacteria including *Bacillus subtilis* and *Streptococcus pyogens* and four Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonous aeruginosa* and *Proteus mirabilis* using disk diffusion method. All extracts showed varying degrees of inhibitory activity against all bacteria. Aqueous extract was found very active against both Gram-positive and Gram-negative bacteria. The extracts showed antibacterial activity against two Gram-positive bacteria and one Gram-negative in a dose dependent manner other two extract was found less active in comparison to aqueous extract. Methanol extract was found active against both Gram-positive and Gram-negative bacteria without showing dose dependency. Total alkaloidal extract was found only active against gram-negative bacteria. Comparatively Gram-positive bacteria were more sensitive to the extracts, except total alkaloid extract.

Keywords: Plant product, *Alstonia scholaris*, antibacterial activity, zone of inhibition.

INTRODUCTION

The use of higher plants and their preparation to treat infectious and non-infectious disease is an age old practices and are the only method available in the past. Though the use of natural sources like plant material for curing diverse forms of ailments leads to human civilization, the scientific analysis of different natural sources for their possible medicinal potency is comparatively recent origin. (Sikinner, 1955).[1] The emergence and spread of antibiotic resistance microorganisms triggered this type of plant investigations. (Cowan, 1999).[2] Hence the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective agents. (Rios, 2005).[3]

Alstonia scholaris (L.) R.Br. (Apocynaceae) is an evergreen tropical tree native to Indian sub-continent and South East Asia having grayish rough bark and milky sap rich in poisonous alkaloid. The bark also called dita bark is traditionally used by many ethnic group of North-East India and also other part of the world as a source remedy against bacterial infection, malarial fever, toothache, rheumatism, snake bite, dysentery, bowl disorder etc. And latex is used in treating coughs, through sores and fever.[4,5,6]

Among the several genera of Alstonia, only scholaris species has been studied for antimicrobial potency. M.R. Khan et al, 2003 evaluated the antibacterial activity of the crude methanolic extracts of the leaves, stem and root barks of *Alstonia scholaris* along with *Leea tetramera* on partitioning (petrol, dichloromethane, ethyl acetate, and butanol).[7] And they reported improved and broader spectrum of antibacterial activity. However, there is no report on antibacterial activity of aqueous extract of this plant. The present work therefore, attempts to evaluate the comparative antibacterial activity of the aqueous and crude alkaloidal extract with methanolic extract.

MATERIALS AND METHODS

Plant Material

Healthy and disease free plant is selected in the Botanical garden of Dibrugarh University, Dibrugarh, Assam (India). From the selected plant bark was collected by scrapping the trunk using neat and clean knife during the month of June 2008 and collected material was stored at room temperature in low humidity condition. The plant material was dried in shade and grinded in mechanical grinder to make course powder. The course powder was stored in plastic bags at room temperature.[8]

Preparation of extracts

Preparation of Methanolic and a2ueous extract

The powdered plant material was extracted with methanol and distilled water by cold maceration. Powdered plant materials (20 g) were macerated with 100 mL of methanol separately at room temperature for 2 days with frequent shaking. After 2 days the extract were filtered by using Whatmann No 1 filter paper and to the marc part again 100 mL of methanol was added allowed to stand for next 2 days at same condition for second maceration (Re-maceration) and after 2 days, again filtered similarly. The combined filtrates (extracts) were evaporated to dryness and stored. Similarly plant material is also macerated with distilled water. These extracts were named as methanolic extract (ME) and aqueous extract (AE).[8]

Preparation of total alkaloidal extract

Before extraction of total alkaloids, powdered bark (50 g) was mixed sufficiently with 20% alcoholic granulated and dried at room temperature. This granulated bark was extracted with benzene for 6 hrs. Extracts was shake with three successive portions of 25mL of 5% sulfuric acid and decolorized by heating with activated charcoal. Hot solution was then filtered using Whatmann No 1 filter paper. Then the combined extract was acidified with ammonium solution (pH 8.5) and liberated alkaloid was extracted with three successive portion of 20 mL chloroform and chloroform extracts was combined. Chloroform was distilled off to get the total alkaloidal residue which was then dried. Extracted total alkaloid was named as total alkaloid extract (TAE).[9]

Preparation of test solution

Test solution of four different concentrations (6.25, 12.5,25 and 50 mg/mL) were prepared by dissolving the extract in normal saline.

Bacteria

The bacteria used in this study included two Gram-positive bacteria *Bacillus subtilis* (ATCC11774) and *Streptococcus pyogens* (ATCC19615) and four Gram-negative bacteria *Escherichia coli* (ATCC10536), *Klebsiella pneumoniae* (ATCC33495), *Pseudomonous aeruginosa* (ATCC10662) and *Proteus mirabilis* (ATCC12453). All the bacterial cultures were obtain from the Division of Microbiology of Dept. of Pharmaceutical Science, Dibrugarh University and maintained at usual laboratory conditions.

Table 1: In Vitro Antibacterial Activity of *Alstonia scholaris* extracts by disk diffusion method.

Extract	Concentration of Extract (mg/mL)	Zone of Inhibition (mm)					
		A	B	C	D	E	F
ME	6.25	--	7	--	--	--	7
	12.5	--	7	--	7	--	7
	25	--	7	--	--	--	--
	50	--	7	--	7	7	7
AE	6.25	11	8	--	--	--	7
	12.5	9	8	--	--	--	8
	25	8	10	--	--	--	9
	50	11	12	--	7	--	10
TAE	6.25	--	--	--	--	7	--
	12.5	--	--	--	--	7	--
	25	--	7	--	--	7	--
	50	--	7	--	7	7	--
NC	6.25	--	--	--	--	--	--
	12.5	--	--	--	--	--	--
	25	--	--	--	--	--	--
	50	--	--	--	--	--	--

ME=Methanolic extract, AE= aqueous extract, TAE= Total alkaloid extract, NC= Negative control. A= *Bacillus subtilis,* B= *Streptococcus pyogens*, C= *Escherichia coli*, D= *Klebsiella pneumoniae*, E= *Proteus mirabilis* and F= *Pseudomonous aeruginosa*

Antibacterial activity Disc diffusion method

The antibacterial activities of different extracts were evaluated by disc diffusion method. The turbidity of the bacterial culture in broth was adjusted with sterile saline (0.9%w/v) according to 0.5 McFarland turbidity standards, for preparation of the inoculum. Nutrient agar media was prepared and sterilized at 121^0C and 121 psi for 15 minutes in an autoclave. When the media cooled to approximately 45-50^0C , 15-20 mL of this media were poured into 9 cm pre-sterilized the inoculum was added to the molten agar media in the Petri dishes and the plates were swirled gently to disperse the bacteria homogenously. The plates were then allowed to solidify. Whatmann No 1 filter paper discs (0.6 mm), previously sterilized were impregnated with each test extract at four different concentrations (6.25, 12.5,25,50 mg/mL) and placed on the solidified surface of the media seeded with respective bacterial sample. Similarly, filter paper disc impregnated with vehicle sterile saline was used as control (NC). No standard antibiotic were employed. Then the Petri dishes were incubated in inverted position at 37^o C for 24 hrs in an incubator. After incubation the zones of inhibition around the disks were measured by means of a transparent ruler in mm. [10,11,12,13]

RESULTS AND DISCUSSION

The antibacterial activity of methanol and aqueous extract from *Alstonia scholaris* trunk bark against five bacterial strains was initially assessed by disk diffusion method. The result is shown in Table 1.

The different extract showed varying degree of antibacterial activity against Gram-positive and Gram-negative bacteria. Methanolic extract exhibited moderate inhibition against Gram-positive *Streptococcus pyogens* in a dose independent manner and weak inhibition against Gram-negative *Klebsiella pneumoniae* and *Pseudomonous aeruginosa* .Aqueous extract was found very active against both Gram-positive and Gram-negative bacteria. The extracts showed antibacterial activity against two Gram-positive bacteria in a dose dependent manner. Maximum inhibition was found against *Streptococcus pyogens* at a concentration of 50mg/mL extract. The activities decreased with decrease in concentration. Similar result was also found against *Bacillus subtilis*. Total alkaloid extract showed comparatively weak activity against all the tested bacteria i9n comparison to other two extract. Only *Proteus mirabilis* found inhibited by this extract in dose independent manner. No significant inhibitory activity was found against Gram-positive bacteria.

Gram-positive bacteria are found more susceptible to this plant extract especially against aqueous extract followed by methanolic extract and total alkaloid extract was found weakly active. Among the gram-negative *Pseudomonous aeruginosa* was found more susceptible to the extract as compared to other. As the extracts were effective against most Gram-positive and Gram-negative bacteria tested, thereby indicating a broad Spectrum of activity. The aqueous extract can be consider as best extract among the other having antibacterial activity. Though the activity was evaluated by disc diffusion method, it is not always reliable as in many cases diffusion is the main barrier showing activity

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