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Antibacterial and Preliminary Phytochemical Screening on the Leaves of *Alstonia macrophylla* Wall.ex G.Don

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Abstract: The aim of the present research was focused on the antibacterial and preliminary phytochemical properties of *Alstonia microphylla* Wall.ex G.Don. [Apocynaceae] via in vitro approach. Antibacterial activity was tested against, gram +ve and gram -ve organisms. The acetone leaves extract exhibited broad-spectrum antibacterial activity against tested organisms. Maximum activity was exhibited against *S. typhi* followed by *B. subtilis* and *K. planticola*. Chloroform and methanol leaves extract exhibited less activity, while petroleum ether showed negative inhibition. The different solvent extracts showed the presence of iridoids, alkaloids, flavonoids, simple phenolics, steroids, saponins, tannins and terpenoids.

Key words: *Alstonia macrophylla*, leaves, antibacterial, phytochemical

Introduction

Alstonia macrophylla Wall.ex G.Don (Apocynaceae) is an evergreen tree grows in the low hill rain forest of south East Asia and native to Malaysia [1]. Medicinally it is used for the same purpose as that of *Alstonia scholaris* [2]. The decoction of leaves and stem bark is widely used in stomachic, skin diseases and urinary infections [3].The leaves are known to have anticholeric and high vulnery effects and are greased with hot coconut oil for sprains, bruises and dislocated joints as poultice and useful as febrifuge [4]. Moreover the leaf vapours are also inhaled in fever by the tribal community- Shompen in Nicobar Islands [5, 6]. Various studies have been carried out with *A. macrophylla* on the antimicrobial, antipyretic and anti-inflammatory activities [1, 7, 8], alkaloids [9-13] and presence of tannin, flavonoids, sterols, triterpenes and reducing sugars [13]. However, this study reports the antibacterial activity of different solvent extracts using some more strains.

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Materials and Methods

Collection of plant materials

The fresh leaves of *Alstonia macrophylla* were collected in the month of October (2006) from the plant growing in Botanical Garden of Dr. Babasaheb Ambedkar Marathwada University Aurangabad (M.S.) India. The plant was identified with the help of Flora of Marathwada [14] and a voucher specimen has been deposited at the Botany department of the university. Plant samples were washed, shade dried at room temperature for 15 days.

Preparation of extracts and phytochemical screening

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 gm of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; Petroleum ether, chloroform, Acetone and Methanol [15]. The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator and were suspended in dimethyl sulphoxide (DMSO) for prior to use [16].

Some of the extracts were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods [17-19]. The positive tests were noted as weak (+), moderate (++), strong (+++) and absent (-).

Tested microorganisms

Various cultures of human pathogenic, gram positive and gram negative bacteria were used. These are *Staphylococcus aureus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Corynobacterium glutamicum and, Klebsella planticola.* The cultures were obtained from Microbial Type culture Collection (MTCC), IMTEC, Chandigarh, India. The microorganisms were repeatedly subcultured in order to obtain pure isolates. A loop full test organism was inoculated on nutrient broth and incubated for 24 h at 37±1ºC and maintained in sterile condition.

Screening for antibacterial properties

Antibacterial activities of plant extracts were tested by Agar well diffusion method [20]. The culture plates were prepared by pouring 20 ml of sterile nutrient agar.1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (8 mm) was used to make wells in each plate for extracts. These plates were labeled and 100µl of each plant extracts (at concentration of 50,100 mg/ml) was added aseptically into the well. Then the plates were incubated for 24 h at 37ºC during which the activity was evidenced by the presence of zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extracts when compared to the controls.

Result and Discussion

The results of preliminary phytochemical components in leaves of *Alstonia macrophylla* revealed the presence of acubins / Iridoids, alkaloids, flavonoids, simple phenolics, steroids saponins, tannins and terpenoids (Table 1).

Results obtained for the antibacterial tests performed on different solvent extracts of *Alstonia macrophylla* are presented (Table 2). Among the extracts tested, acetone extracts showed broader spectrum of activity, being active to both Gram-positive and Gram- negative organisms compared to chloroform and methanol, while petroleum ether showed negative inhibition. The acetone extract at 100mg/ml for example, 26 mm was recorded as diameter zone of inhibition against *S. typh*i. This was followed by 17 mm *B. subtilis*, 16 mm *M. luteus*, *K.planticola,*15 mm *B. megaterium, E. coli,* 13 mm *S. aureus* and 12 mm *P. aeruginosa* and *C. glutamicum* respectively.. Whereas at the same concentration the methanol extracts exerted highest activity against *S. typhi* with diameter 28 mm followed by 15 mm *S. aureus*,14mm *B. megaterium, E. coli*, 13 mm *M. luteus* and 11 mm *K. planticola*. The least activity 11 mm against *B. megaterium, E.coli* at 100mg/ml was recorded by chloroform extracts, while petroleum ether showed negative inhibition against all the tested organisms. Activities of the various extracts were comparable to those of standard antibacterial agent ampicillin and DMSO as control. The differences in the observed activities of the various extracts may be due to varying degree of solubility of the active constituents in the four solvents used. It has been documented that different solvents have diverse solubility capacities for different phytochemical constituents [21].

Table 1. Phytochemical constituents of leaves extracts of *Alstonia macrophylla*

Chemical constituents	Observation
Acubins / Iridoids	++
Alkaloids	
a) Dragendorff's reagent	+++
b) Mayer's reagent	+++
c) Wagner's reagent	+++
Anthraquinone	--
Cardiac glycoside	--
Coumarins	--
Flavonoids	+++
Leucoanthocyanins	--
Phlobatannin	++
Simple phenolics	++
Steroids	++
Saponins	+
Tannins	
Test – a true tannin	+++
Test – b pseudotannin	+
Terpenoid	+++

Table 2: Antibacterial efficacy of different solvent extracts of *Alstonia macrophylla* leaves

Organisms	Gram + / -	Dose (mg/ml)	Petroleum ether	Chloroform	Acetone	Methanol	DMSO	Ampicillin
Staphylococcus aureus	+	A	0	0	13	15	0	23
Bacillus subtilis	+	A	0	0	17	10	0	21
Bacillus megaterium	+	A	0	11	15	14	0	25
Micrococeus luteus MTCC 106	+	A	0	0	16	13	0	30
Escherichia coli	-	A	0	11	15	14	0	17
Salmonella typhi	-	A	0	0	26	28	0	19
Pseudomonas aeruginosa MTCC2488	-	A	0	0	12	0	0	16
Klebsiella planticola	-	A	0	0	16	11	0	21
Corinobacterium glutamicum	-	A	0	0	12	13	0	18

A-100mg/ml (100µl/well); Ampicillin (standard Antibiotic) -40µg/ml (100µl/well); 0 -no inhibition; Figures are diameter of zone of inhibition (in triplicates).

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