Comparative Phytochemical and Antibacterial Studies on the bark of *Alstonia scholaris* R.Br. and *Alstonia macrophylla* Wall. ex G.Don

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ABSTRACT

Comparative phytochemical and antibacterial activities of bark of *Alstonia scholaris* and *Alstonia macrophylla* were investigated. The successive different solvent extracts showed the presence of alkaloids, phenolics, saponins and tannins were found in both the species. The antibacterial activities of bark of *A. scholaris* and *A. macrophylla* in successive different solvent were tested against gram +ve and gram –ve organisms. The chloroform extracts of *A. macrophylla* showed broader spectrum of antibacterial activity when compared with *A. scholaris*. However. *Alstonia scholaris* is widely used medicinal plant.

Keywords: Comparative, bark, *Alstonia* species, phytochemical, antibacterial
Editor: Mueen Ahmed KK, Phcog.Net

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INTRODUCTION

Green plants represent a reservoir of effective chemotherapeutics and can provide valuable source of natural antimicrobials (1, 2). Plant species which have one or more of its organs containing substances that can be used for therapeutic purpose are called medicinal plants (3).Plants have been used as medicinal agents from the earliest day of mans existence (4, 5) and has made it necessary to study them in details in order to discriminate the kinds employed for different purposes (6). In particular, the antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food formation, pharmaceuticals, alternative medicine and natural therapies (7).

Now a day, infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and Governments all over the world, especially with the current increasing trends of multidrug resistance among emerging –reemerging bacterial pathogens to the available modern drug or antibiotics (8, 9). It is therefore very necessary that the search for newer antibiotic serves to be a continued process. Plants are the cheapest and safer alternative source of antimicrobials (10, 11). Therefore, with increasing acceptance of traditional medicine as an alternative form of health care, the screening of plants for active compounds has become very important.

Alstonia scholaris R. Br. (Apocynaceae), popularly known as "Saptaparni" or "Devil's tree", and *A. macrophylla,* are used in the traditional systems of medicine. However, the former one is widely recognized medicinal plant.

The bark of *A. scholaris* is bitter, tonic, astringent, expectorant, alterative, anthelmintic, emmenagogue and galactogogue. It has proved valuable in fever, chronic diarrhea and in advanced stages of dysentery (12-15). Whereas the bark of *Alstonia macrophylla* is used for the same purpose as that of *Alstonia scholaris* (16). Decoction of the stem bark is effective in stomach ache, skin diseases and urinary infections (17). Moreover, phytochemistry on these two species studied earlier (18-21). The genus *Alstonia* has been the subject of antimicrobial activities (22, 23). This work, however, is designed to evaluate the comparative account of antibacterial activity of related species.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

The fresh barks of *A. scholaris* R. Br. were collected in the month of October 2005 from forest area of Aurangabad

district (M.S.) and that of *Alsotia macrophylla* from the Botanical Garden of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The plant species were identified with the help of Flora of Marathwada (24) and voucher specimens have 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 successively with 250 ml of each of the following solvents. Petroleum ether, chloroform, acetone and methanol (25). The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40°C) 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 (26).

Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods (27-30). The positive tests were noted as weak (+), moderate (++), strong (+++) and absent (-).

TEST CULTURE

The test bacteria used for the screening antimicrobial activity were *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus* MTCC 106 *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* MTCC 2488, *Klebsiella planticola* and *Bacillus megaterium*. The cultures were obtained from Microbial Type culture Collection (MTCC), IMTEC, Chandigarh, India. Cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4°C. All cultures were checked for viability and purity by regular plating. Test cultures were prepared by transferring a loop full of bacteria from stock culture nutrient broth and incubated at 37±1°C for 24 hours.

SCREENING FOR ANTIBACTERIAL PROPERTIES

The antibacterial activities of the successive bark extracts of petroleum ether, chloroform, acetone and methanol of both the plant species were tested by Agar well diffusion method (31). The culture plates were prepared by pouring 20 ml of sterile nutrient agar. 1ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (6 mm) was used to make wells in each plate for extracts. These plates were labeled and 100µl of each plant extracts (at concentration of 100mg/ml) was added aseptically into the well. Then the plates were incubated for 24 h at 37°C during which the activity was evidenced by the presence of zone of inhibition surrounding the well. Ampicillin (40 µg/ml) was used as standard antibiotics. 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.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the bark of both species of *Alstonia* are shown in Table 1. It indicates the presence of alkaloids, phenolics, saponins and tannins. The presence of steroids and terpenoids are indicated only in *Alstonia scholaris* while cardiac glycosides and flavonoids are found alone in *A. macrophylla*. However there were complete absence of anthraquinones and leucoanthocyanins in both species.

The antibacterial activities of *A. scholaris* and *A. macrophylla in* petroleum ether, chloroform, acetone and methanol extracts against bacteria examined in the current study and their potency were qualitatively assessed by the presence or absence of inhibition zones and zone diameter (Table 2). The results showed that the extracts of *A. scholaris* mediated some degree of activity against bacteria. Except, *S. aureus* and *S. typhi* other strains like *M. luteus, B.subtilis, B. megaterium, K. planticola E. coli* and *P. aeruginosa* are inhibited by acetone extracts only, while other extracts such as petroleum ether, chloroform and methanol showed negative inhibition at test concentration.

The antibacterial activity of *A. macrophylla* extracts were the most prominent in activity against all bacteria tested at test concentration (table 2). Except petroleum ether extracts, others such as chloroform, acetone and methanol showed broader spectrum of activity. However, chloroform extracts of *A. macrophylla* exhibited significant antibacterial activities against all the bacteria tested but, particularly, to *B. subtilis, S.aureus* and *E. coli* with a diameter same or greater than standard antibiotic ampicillin.

Based on these results, it is possible to conclude that of two species of *Alstonia*. *A. macrophylla* exhibited broader range of antibacterial activity to varying degrees, as it is a less known medicinal plant in the Indian literature. Particularly, the chloroform extracts of *A. macrophylla*

Table-1: Phytochemical constituents of bark extracts of *A.scholaris* and *A. macrophylla*

	Alstonia scholaris				*Alstonia macrophylla*			
Phytochemicals	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Alkaloids	—	+++	—	+++	—	+++	—	+++
Anthraquinones	—	—	—	—	—	—	—	—
Cardiac glycosides	—	—	—	—	—	—	++	+++
Coumarins	—	—	—	—	—	—	—	—
Flavonoids	—	—	—	—	—	++	++	+++
Leucoanthocyanins	—	—	—	—	—	—	—	—
Simple phenolics	—	—	+	++	—	++	++	+++
Steroids	—	—	—	++	—	—	—	—
Saponins	—	—	+	++	—	++	++	+++
Tannins	—	—	+	++	—	+	++	+++
Terpenoids	—	++	++	++	—	—	—	—

Note: - A - Pet ether, B - Chloroform, C - Acetone, D - Methanol, (+) - weak, (++) - moderate, (+++) - strong and (-) - absent.

Table-2: Antibacterial activity of bark extracts of *A. scholaris* and *A. macrophylla*

Organisms	**Gram stain + / –**	**Inhibition zone in diameters (mm / sensitive strains)**									
		A. scholaris				***A. macrophylla***				**DMSO**	**Ampicillin**
		A	**B**	**C**	**D**	**A**	**B**	**C**	**D**		
Staphylococcus aureus	+	—	—	—	—	—	24	16	15	—	23
Bacillus subtilis	+	—	—	11	—	—	32	16	18	—	21
Bacillus megaterium	+	—	—	11	—	—	10	15	15	—	25
Micrococcus luteus	+	—	—	13	—	—	27	15	17	—	30
Escherichia coli	–	—	—	10	—	—	18	15	17	—	17
Salmonella typhi	–	—	—	—	—	—	11	13	10	—	19
Pseudomonas aeruginosa	–	—	—	10	—	—	—	14	14	—	16
Klebsiella planticola	–	—	—	11	—	—	22	18	15	—	21

Note: - A - Petroleum ether, B - Chloroform, C - Acetone, D - Methanol, — no inhibition, Figures are diameter of zone of inhibition.

showed significant antibacterial activities and could be used as antimicrobial agents in new drugs for therapy.

ACKNOWLEDGEMENT

The authors wish to thank Head of Botany Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing the necessary laboratory facilities.

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