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Short Communication

Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R. Br.

Khyade, M. S.* and Vaikos, N. P.

Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004 (MS), India.

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Phytochemical and antibacterial activity of leaves of *Alstonia scholaris* R.Br. was investigated. The different solvent extracts showed the presence of Iridoids, alkaloids, coumarins, flavonoids, leucoanthocyanins, reducing sugars, simple phenolics, steroids, saponins and tannins. Antibacterial activity was tested against both Gram +ve and -ve organisms. The methanol leaves extract exhibited broad-spectrum antibacterial activity against tested organisms. Maximum activity was exhibited against *Bacillus subtilis* followed by *Escherichia coli* and *Staphylococcus aureus*. Chloroform and acetone leaf extracts exhibited lesser activity, while petroleum ether extract showed no inhibition.

Key words: *Alstonia scholaris*, leaves, phytochemical, antibacterial.

INTRODUCTION

Finding healing powers in plant is an ancient idea (Cowen, 1999). The increasing interests on traditional ethnomedicine may lead to discovery of novel therapeutic agents. Many of the plant species have been documented pharmacologically and clinically in the world, which are endowed phytochemicals with marked activity on human pathogenic bacteria (Anonymous, 1976; Ray and Majumdar, 1976; Farnsworth, 1988; Rastogi and Mehrotra, 1991, 1993; Asolkar et al., 1992; Cox, 1994; Rastogi, 1998; Perry and Metzer, 1998; Khan et al., 2002). Moreover, different national and international pharmaceutical companies are utilizing such plant based formulations in treatment of various diseases and disorders worldwide (Singh and Gautam, 1997; Satyavati et al., 1987; Jain, 1991; Kirtikar and Basu, 1935). Hence there is a dire need to study the antimicrobial properties of herbs, which will be helpful in the treatment of several diseases caused by microorganisms.

The present study was carried out on the phytochemical and antibacterial activity of leaf of *Alstonia scholaris*, which is popularly known as the “Saptaparni” or ‘Devil’s tree’. It is widely distributed in dried forests of India, Western Himalayas, Western Ghats and in the Southern region. It is a well known remedy for the treatment of various types of disorders in the ayurvedic, homoeopathic and folklore system of medicine in India (Nadkarni, 1976; Joshi, 2000). Moreover, a lot of work has been done on its phytochemistry. Chatterjee et al. (1965) studied the alkaloids in the leaves of *A. scholaris*. The new indole alkaloid, alstonamine and a sitsirikine type indole alkaloid, rhazimanine, have been isolated from the leaves of *A. scholaris* by Atta-ur-Rahman and Alvi (1987). Hadi and Bremner (2001) tested *A. scholaris* for antimalarial properties of alkaloids obtained from young plants leaves. Patrick et al. (2005) isolated different types of alkaloids from the leaves of the Philippine medicinal plant, *A. scholaris.* The present research is focused on antibacterial activity of *A. scholaris*.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *A. scholaris* were collected in the month of October (2005) 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 (Naik, 1998) 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 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following

*Corresponding author. E-mail: mskhyade@rediffmail.com.

Table 1. Phytochemical components of different solvent leaves extracts of *A. scholaris*.

Phytochemical constituents	Petroleum ether	Chloroform	Acetone	Methanol
Acubins/Iridoids	--	--	+	++
Alkaloids	--	+++	--	+++
Anthraquinone	--	--	--	--
Cardiac glycoside	--	--	--	--
Coumarins	--	--	--	++
Flavonoids	--	--	++	++
Leucoanthocyanins	--	--	--	--
Phlobatannin	--	--	--	++
Reducing sugars	--	--	--	++
Simple phenolics	--	--	--	+++
Steroids	++	--	--	--
Saponins	--	--	++	+++
Tannins	--	--	++	+++
Terpenoid	--	--	--	--

solvents; petroleum ether, chloroform, acetone and methanol (Vogel, 1988). 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 (Beyer and Walter, 1997).

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 (Harborne, 1984; Trease and Evans, 1987; Ajaiyeoba, 2000; Edeoga et al., 2005). 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 subtilis*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Corynobacterium glutamicum*, *Klebsiella planticola* and *Bacillus megaterium*. 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 (Kavanagh, 1972). 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 (6 mm) was used to make wells in each plate for extracts. These plates were labeled and 100 µl 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.

RESULTS AND DISCUSSION

The results of qualitative screening of phytochemical components in leaves of *A. scholaris* revealed the presence of acubins/iridoids, alkaloids, coumarins, flavonoids, leucoanthocyanins phlobatannins, reducing sugars, simple phenolics, steroids saponins and tannins (Table 1).

Results obtained for the antibacterial tests performed on different solvent extracts of *A. scholaris* are presented (Table 2). Among the extracts tested, methanol extracts showed broader spectrum of activity, being active to both Gram-positive and Gram-negative organisms compared to chloroform and acetone, while petroleum ether showed no inhibition. Activities of the various extracts were comparable to those of standard antibacterial agent ampicillin and DMSO as control. Of all the bacteria tested the Gram-positive were slightly more susceptible to the extracts than the Gram-negative bacteria. 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 (Marjorie, 1999).

Demonstration of antibacterial activity of *A. scholaris* against test bacteria is an indication that the possibility of sourcing alternative antibiotic substances in this plant for the development of newer antibacterial agents. Bacteria used in this study are associated with different type of infections including wounds, burns, typhoid fever, cough, urinary infection and skin infections.

Table 2. Antibacterial efficacy of different solvent extracts of *A. scholaris* leaves.

Organisms	Gram stain + / -	Dose (mg/ml)	Petroleum ether	Chloroform	Acetone	Methanol	DMSO	Ampicillin
S. aureus	+	A	0	0	0	17	0	23
		B	0	0	0	14		
B. subtilis	+	A	0	15	0	17	0	21
		B	0	11	0	12		
B. megaterium	+	A	0	0	0	15	0	25
		B	0	0	0	12		
M. luteus MTCC 106	+	A	0	13	0	16	0	30
		B	0	10	0	13		
E. coli	-	A	0	17	14	17	0	17
		B	0	14	11	13		
S. typhi	-	A	0	14	10	13	0	19
		B	0	11	0	10		
P. aeruginosa MTCC2488	-	A	0	11	10	12	0	16
		B	0	9	0	9		
K. planticola	-	A	0	0	0	11	0	21
		B	0	0	0	0		

A = 100 mg/ml (100 µl/well); B = 50 mg/ml (100 µl/well); antibiotic = 20 g/ml (100 µl/well); 0 = no inhibition.
Values are diameter of zone of inhibition (mm).

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REFERENCES

Ajaiyeoba EO (2000). Phytochemical and antimicrobial studies of *Gynandropeis gynandra and Buchholzia coriaceae* extracts. Afr. J. Biomed. Res. 3(3): 161-165.

Anonymous (1976). Indian material medica, Vol.1, pp. 283-284, Population Prakashan, New Delhi, India.

Asolkar LV, Kakkar KK, Chakra OJ (1992). Second supplement to glossary of Indian medicinal plants with active principles, part 1(A-K). XIVII+414, Pub. and Inf. Div. (CSIR), New Delhi.

Atta-ur-Rahman, Alvi KA (1987). Indole alkaloid from *Alstonia scholaris,* Phytochemistry, 26(7): 2139-2142.

Beyer H, Walter W (1997). Organic chemistry, 4th ed. Published by Jerry March, pp. 146-148.

Chatterjee A, Mukherjee B, Ray AB, Das B (1965). The alkaloid of the leaves of *Alstonia scholaris* R. Br. Tetrahedron Lett. 41: 3633-3637.

Cowen MM (1999). Plant Product as Antimicrobial Agents Clinical Microbiol. Rev. 2(4): 564-582

Cox PA (1994). The ethnobotanical approach to drug discovery: strengths and limitations. In, ethnobotany and the search for the new drugs. John Wiley and Sons. England. pp. 25-36.

Edeoga HO, Okwu DE, Mbacbie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 4(7): 685-688.

Farnsworth NR (1988). Sereening plants for new medicines. Wilson EO (Ed.). Biodiversity, pp.83-97, National Academy Press, Washington.

Hadi S, Bremner JB (2001). Initial studies on Alkaloids from Lombok Medicinal Plants. Molecules, 6: 117-129.

Harborne JB (1984). Phytochemical methods, London Chapman Hall.

Jain SK (1991). Dictionary of Indian Folk medicine and ethnobotany, pp. XII+311, Deep publication, New Delhi.

Joshi SG (2000). Medicinal plants, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi.

Kavanagh F (1972). Analytical microbiology Part II. New York Academic Press, p. 126.

Khan AV, Parveen G, Alam MM, Singh VK (2002). Ethnomedicinal uses of Neem in rural areas of Uttar Pradesh, India. Ethnomed and Pharmacog, (Sci. Tech. Pub. USA). II Rec. Prog. Med. Plants pp. 7: 319-326.

Kirtikar KR, Basu BD (1935). Indian Medicinal Plants. Vol 3, p. 1841. Allahabad. Lalit Mohan Publication.

Marjorie MC (1999). Plant products as antimicriobial agents. Clin. Microbiol. Rev. 12(4): 564-582

Nadkarni AK (1976). Indian Materia medica, Popular Prakashan, Bombay.

Naik VN (1998). Flora of Marathwada Vol. I, Amrut Prakashan, Aurangabad MS, India, pp. 528-529.

Patrick A, Macabeo G, Karsten Krohn Dietmor Ghl,e Roger, Read W, Joseph JB, Geoffrey Cordell Scott A, Franzblau G, Alicia MA (2005). Indole alkaloids from the leaves of Phillipine *Alstonia scholaris*, Phytochemistry, 66(10): 1158-1162.

Perry LM, Metzer J (1998). Medicinal plants of east and south Asia, attributed properties and uses. Massachisetts and London: MIT press Cambridge.

Rastogi RP, Mehrotra BN (1991). Compendium of Indian Medicinal Plants, Vol. III 1: 497, CSIR Publication, India.

Rastogi RP, Mehrotra BN (1993). Compendium of Indian Medicinal Plants, Vol. IV 1: 497 CSIR Publication, India.

Rastogi RP (1998). Compendium of Indian Medicinal Plants, Vol. 1, 497, CSIR Publication, India.

Ray PG, Majumdar SK (1976). Antimicrobial activity of some Indian Plants. Econ. Bot. 30(4): 317-329.

Satyavati GV, Gupta AK, Tanabu N (1987). Medicinal plants of India Vol.2 pp. XI+557, CSIR Publication, Indian Council of Medical Research, Cambridge printing worker, N. Delhi.

Singh SH, Gautam M (1997). Bioresources of Med. and Aro. Plants of India. Their conservation and related issues, Kurukshetra 56(3): 9-13.

Trease E, Evans WC (1987). Pharmacognosy, Billiare Tindall, London.

Vogel AI (1988). In: Elementary practical organic chemistry (Second edition), Orient Longman Limited, pp. 45-168.