ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antibacterial Efficacy of *Alstonia Scholaris* (L.) R. Br. Stem Bark Extracts

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ABSTRACT

Three extracts of the stem bark of *Alstonia scholaris* (L.) R. Br. were evaluated for *in vitro* antibacterial activity against two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and four Gram negative (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria. The disc diffusion method was used for this study. Ethyl acetate and n-butanol fractions showed significant inhibitory effect against the selected bacteria. Phytochemical screening of different extracts revealed the presence of alkaloids, flavonoids, terpenoids, tannins and saponins.

Keywords: *Alstonia scholaris*, Phytochemicals, Antibacterial, Disc diffusion, Broth dilution.

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ISSN: 0975-8585

INTRODUCTION

Plants have been always a treasure of medicines. Forest is referred to as God's own pharmacy. They are used for treating many health problems since a long time. Among more than 250,000 species of higher plants, only 5-10% has been chemically investigated [1]. World Health Organization has estimated that about 80% of people on earth rely chiefly on traditional medicines for their primary health care needs. It can safely be presumed that a major part of traditional therapy involve the use of plant extracts or their active principles [2].

The plant of *Alstonia scholaris* (L.) R. Br. belongs to family Apocynaceae and is also known as Devil's tree or Dita Bark tree. The plant grows throughout India, in deciduous and evergreen forests and also in plains. The plant is known to possess a lot of medicinal properties in folk medicine. *Alstonia scholaris* is an antimalarial drug used in the marketed Ayurveda preparation Ayush-64, NRDC, India. The milky juice of the plant is applied on wounds, ulcers and rheumatic pains. Tincture of the bark and juice of the leaves act as powerful galactogogue in certain cases. The drug is also used in case of snake bite [3]. *Alstonia scholaris* is known to possess *in vitro* antioxidant, free radical scavenging [4], analgesic, anti-inflammatory and anti-ulcerogenic activities [5]. Besides it also possesses anti-anxiety and anti-depressant activities [6]. The bark extracts of *Alstonia scholaris* possess immunostimulating effect [7]. The plant is known to possess anticancer activity on skin carcinogenesis in mice and cytotoxic activity to HeLa cells [8]. The combination of *Alstonia scholaris* and berberine hydrochloride, a topoisomerase inhibitor showed enhanced chemomodulatory activity in Ehrlich ascites carcinoma-bearing mice [9]. The leaves of *A. scholaris* possess broncho-vasodilatory activity [10]. Acetone extract of *A. scholaris* possesses schizonticidal properties. Methanolic crude extract possesses Anti-diarrhoeal and spasmolytic activity [11]. The alkaloid fraction of the leaves showed anti-tussive, anti-asthmatic and expectorant activities and is proved to be a valuable lead material for respiratory diseases drug development [12].

Bacterial infections are continuously causing significant morbidity and mortality worldwide which caused by treatment failure or treatment option restrictions because of the prevalence of antibiotic-resistant isolates. The use of antibiotics is often accompanied by side effects and often development of resistant strains. Resistance to antimicrobial agents may arise because of many factors in which inappropriate or inadequate antibiotic therapy is an important factor. Besides a high prevalence of disease coupled with lack of capacity to initiate prevention programs or surveillance is also a major cause [13]. There is a need of continuous searching of novel antibacterial drugs which are non-toxic and cause no or less side effects. In this search, the plant extracts and further the compounds purified from them are showing strong potential of being future antimicrobial agents.

The aim of the present study is to determine the antibacterial activity of various extracts of *Alstonia scholaris* stem bark which is having traditional claims for treatment of several diseases.

ISSN: 0975-8585

MATERIALS AND METHODS

Plant material

Alstonia scholaris (L.) R. Br. stem bark was collected from Vidisha, Madhya Pradesh, India and authenticated by Dr. P. N. Shrivastava, Professor, Botany Department, S. S. L. Jain P. G. College, Vidisha (M. P.), India. After proper identification, a voucher specimen of the plant was deposited in the herbarium of Pest Control and Ayurvedic Drug Research Laboratory, S. S. L. Jain P. G. College, Vidisha (M. P.). The freshly collected bark was washed with running tap water and shade-dried at room temperature. The dried bark was powdered using a manual mill.

Preparation of extracts

Powdered bark of *A. scholaris* was extracted with 90% ethanol in a soxhlet apparatus at 40^0C. Extraction was done for 48 hours. The extract was filtered through a Buchner funnel with Whatman filter paper no. 1. The filtrate was evaporated to dryness using rotary evaporator at 40^0C to get the crude extract. The ethanol crude extract was stored at 4^0C in airtight bottles until further use.

To get the fractions, ethanol crude extract of the plant was dissolved in water and partitioned subsequently with equal volumes of petroleum ether (60^0-80^0C), ethyl acetate and n-butanol. All solvents used were of analytical grade (Merck, Germany). All the fractions were filtered through Whatman filter paper no. 1, evaporated to dryness and percentage yields were calculated. The ethanol extract and its fractions were further used for the study and screening against selected bacterial strains [14].

Phytochemical screening

Phytochemical screening of the plant extract and different fractions was carried out qualitatively for the presence of alkaloids, flavonoids, tannins, terpenoids, saponins and steroids according to standard procedures [15].

Antibacterial screening

Test microorganisms

Bacterial cultures of *Bacillus subtilis* (MTCC441) and *Staphylococcus aureus* (MTCC3160) were used as Gram-positive bacteria and the bacterial cultures of *Escherichia coli* (MTCC739), *Salmonella typhi* (MTCC531), *Pseudomonas aeruginosa* (MTCC741) and *Klebsiella pneumoniae* (ATCC15380) were used as Gram-negative bacteria.

In vitro determination of antibacterial activity

The determination of zone of inhibitions of the test samples was done by disc diffusion method [16]. 100 µl of bacterial suspension (10^6 cfu/ml) was applied uniformly on the surface of Mueller-Hinton agar (HiMedia) plate. The dried plant extracts were dissolved

separately in 5% dimethylsulphoxide (DMSO) to reach a final concentration of 100 mg/ml. Sterilized discs (6 mm in diameter) impregnated with 20µl of extract (100 mg/ml) were arranged on the surface of inoculated plates and incubated at 37^0C for 24 hours. Along with this streptomycin disc (10µg) was studied for antimicrobial activity as positive control whereas the solvent DMSO (5%) was used as negative control. After incubation, the inhibition zones formed around the discs were measured with HiMedia zone scale. The study was performed in triplicate and the mean values are presented.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) of different extracts with respect to different test microorganisms were determined by broth dilution method. A two-fold serial dilutions (0-5 mg/ml) of the crude extracts were prepared in Mueller Hinton broth. 0.1 ml of standardized suspension of bacteria (10^6 cfu/ml) was added to each tube (containing crude extracts at a final concentration of 0-5 mg/ml). Appropriate antibiotic controls were also prepared. The tubes were incubated at 37^0C for 24 hours and observed for visible growth after vortexing the tubes gently. The lowest concentration of the test extract in a tube that failed to show any visible growth after incubation was considered as MIC [17].

RESULTS AND DISCUSSION

The present study carried out on the plant extracts revealed the medicinal activities. The percentage yield of various extracts of stem bark of *Alstonia scholaris* are shown in **Table 1**. The yield of 90% ethanol extract of *A. scholaris* stem bark was 10.2% (w/w) of the plant material applied for extraction while the yields of petroleum ether, ethyl acetate and n-butanol fractions were 2.5%, 0.9% and 3.2% (w/w) respectively.

Table 1 - Percentage yield of various extracts of *A. scholaris* stem bark

Extract	% yield (w/w)
90% ethanol	10.2
Petroleum ether fraction	2.5
Ethyl acetate fraction	0.9
n-butanol fraction	3.2

The phytochemicals present in the stem bark of *A. scholaris* are represented in **Table 2**. Phytochemical screening revealed the presence of alkaloids, flavonoids and saponins in ethyl acetate and n-butanol fractions. Tannins were present in n-butanol fraction while terpenoids were present in petroleum ether and n-butanol fraction. Ethyl acetate and n-butanol fractions were found to be phytochemically more active than the petroleum ether fraction. The presence of phytochemicals flavonoids, cardio glycosides, saponins, oils and fats, terpenoids, alkaloids, steroids, tannins, phenolic compounds, amino acids and quinines have been reported in *Alstonia scholaris* [18,19]. The presence of active phytochemicals is the major cause of various activities of the plant extracts including antimicrobial activity.

ISSN: 0975-8585

Table 2 – Qualitative analysis of the phytochemicals present in *A. scholaris* stem bark

Phytochemicals	PE	EA	B
Alkaloids	-	+	+
Flavonoids	-	+	+
Tannins	-	-	+
Terpenoids	+	+	-
Saponins	-	+	+
Steroids	-	-	-

PE = Petroleum ether fraction, EA = Ethyl acetate fraction, B = n-Butanol fraction

90% ethanol extract of *A. scholaris* stem bark and its ethyl acetate and n-butanol fractions were tested for *in vitro* antibacterial activity against selected bacterial strains. The result of the activity is mentioned in **Table 3**. Ethyl acetate and n-butanol fractions were found to have better antibacterial activity against the tested microorganisms at the given concentration than the unfractionated ethanol extract. The antibacterial activities of the two fractions are comparable to the standard antibiotic streptomycin (10µg). Ethanol extract showed no activity against *E. coli* and *P. aeruginosa* at given concentration while its ethyl acetate and n-butanol fractions showed good activity against these bacteria. A comparative chart of the three extracts along with the standard antibiotic streptomycin is given in **Figure 1**. Madan *et al* [20] reported significant antimicrobial activity of petroleum ether extract and dichloromethane-ether-methanol mixture (1:1:1) of *A. scholaris* bark. Antimicrobial activity of *A. scholaris* flower extracts has been reported by Thankamani *et al* [21] The leaves extracts of *A. scholaris* were found to have potent antimicrobial activity against a wide range of microorganisms [22]. Therefore it is observed that not only stem bark but leaves and flowers of this plant also possess potent antibacterial activity against the selected Gram-positive and Gram-negative bacteria. The values of minimum inhibitory concentration (MIC) of different extracts of stem bark against the selected bacteria are given in **Table 4**. The MIC ranged between 0.625-5 mg/ml however in some cases the MIC was above 5 mg/ml (ethanolic extract against *E. coli* and *P. aeruginosa*). Ethyl acetate and n-butanol fractions showed promising activity against the selected bacteria. These results emphasise the medicinal use of this plant in folklore medicine. Further the isolation and detailed study of the active principles of this plant which are responsible for the medicinal properties is recommended so that larger population may get the medical benefits and that to in a scientific manner.

Table 3 – *In vitro* antibacterial activity of *Alstonia scholaris* stem bark*.

	Gram stain +/-	E	EA	B	DMSO (5%) (20µl)	St (10µg)
B. subtilis	+	8	10	14	0	13
S. aureus	+	9	11	13	0	12
E. coli	-	0	10	12	0	14
S. typhi	-	6	9	11	0	13
P. aeruginosa	-	0	11	15	0	12
K. pneumoniae	-	8	10	14	0	11

*Values are of inhibition zone (mm) and an average of triplicate, E=90% ethanol extract, EA=Ethyl acetate fraction, B=n-Butanol fraction, St=Streptomycin, DMSO=Dimethyl sulphoxide

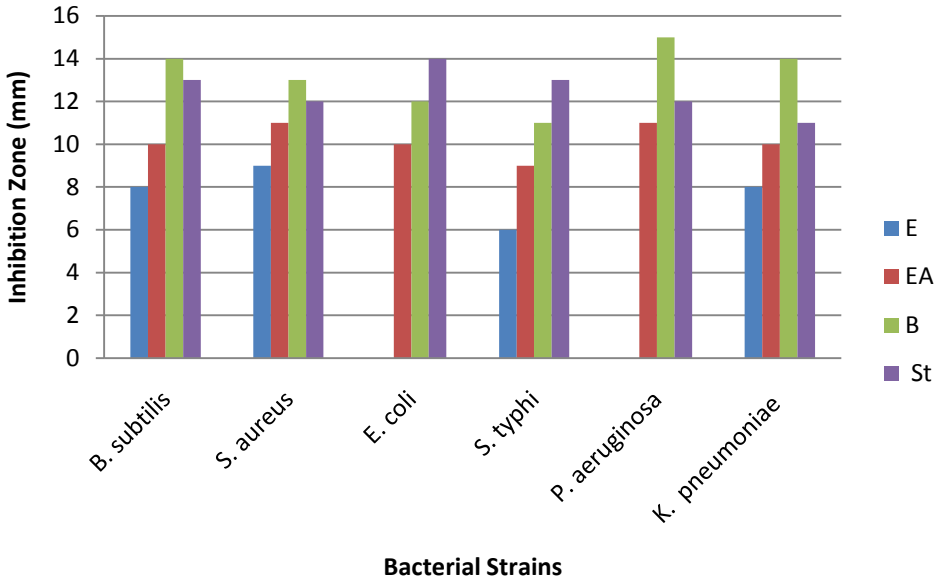
ISSN: 0975-8585

Table 4 – Minimum inhibitory concentration of *A. scholaris* stem bark extracts against microorganisms*

Organism	E (mg/ml)	EA (mg/ml)	B (mg/ml)
B. subtilis	2.5	0.625	0.625
S. aureus	2.5	0.625	1.25
E. coli	>5	1.25	1.25
S. typhi	5	1.25	0.625
P. aeruginosa	>5	1.25	0.625
K. pneumoniae	5	2.5	2.5

* Values are mean of three replicates. E=90% ethanol extract, EA=ethyl acetate fraction, B=n-butanol fraction

Figure 1: Antibacterial activity of *Alstonia scholaris*



E- 90% ethanol extract, EA- ethyl acetate fraction, B- n-butanol fraction, St- streptomycin

CONCLUSION

From the present study it can be concluded that 90% ethanol extract of *A. scholaris* stem bark and its ethyl acetate and n-butanol fractions showed promising antibacterial activity against the selected bacteria however the fractions are much more active against the bacteria than the unfractionated ethanol extract. Further the work is in progress to isolate the active principles present in ethyl acetate and n-butanol fractions responsible for potent antibacterial activity.

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