BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded and Social Sciences Citation Index
Bangladesh J Pharmacol 2010; 5: 41-44
ISSN: 1991-0088

Antibacterial and antifungal properties of the methanol extract from the stem of *Argyreia argentea*

Md. Atiar Rahman[1], Taslima Ahsan[1] and Saiful Islam[2]

[1]*Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong 4331, Bangladesh;*
[2]*Bangladesh Council for Scientific and Industrial Research, Chittagong, Bangladesh.*

Article Info

Received: 2 April 2010
Accepted: 31 May 2010
Available Online: 8 June 2010

DOI: 10.3329/bjp.v5i1.4700

Cite this article:
Tahman MA, Ahsan T, Islam S. Antibacterial and antifungal properties of the methanol extract from the stem of *Argyreia argentea.* Bangladesh J Pharmacol. 2010; 5: 41-44.

Abstract

Antibacterial properties of methanol extract of *Argyreia argentea* stem was studied on Gram positive and Gram negative bacteria by disc diffusion method. The extract showed zone of inhibition against Gram positive bacteria *(Bacillus cereus, B. subtilis, B. megaterium and Staphylococcus aureus)* and Gram negative bacteria [*E. Coli, Salmonella typhae, S. paratyphae, Pseudomonous sp. (I), Pseudomonous sp. (II) and Shigella sonnei*]. In addition, the extract was found effective against some fungi like *Aspergillus flavous, Fusarium equiseti, Altenaria alternate, Aspergillus niger, Colletotrichum corphori*.

Introduction

*Argyreia argentea (*family Convolvulaceae) is an evergreen shrub that is mainly found in different districts of Bangladesh (Uddin, 2006). It is widely used by the tribal communities of Chittagong Hill tracts (locally known to Chakma as *bitarak rupar tola ludi*) for the treatment of various diseases (boils, gastric, tumor, marasmus, paralysis and spermaforrhoea). However, a few scientific evaluations of this plant have been documented. This study interests to evaluate the antibacterial and antifungal activity of *A. argentea* methanol extract.

Materials and Methods

Collection of plant

The stems of *A. argentea* were collected from Chittagong Hill tracts, Bangladesh, in January 2009. The plant was taxonomically identified and authenticated by Bangladesh National Herbarium, Mirpur, Dhaka. The specimen is preserved in Bangladesh National Herbarium under the Plant Accession No. 34198.

Preparation of crude extract

The fresh stems of *A. argentea* were washed with distilled water immediately after collection. The collected stems were chopped into small pieces, air dried at room temperature for about 20 days and ground into powder to store in an airtight container. 790 g powder was macerated in 8 L pure methanol (99% Anal-R) for 7 days at room temperature with occasional stirring. Methanol extract, 7 days later, was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure below 50°C through rotatory vacuum evaporator. The concentrated extracts were collected in an eggplant flask and allow to air dry for complete evaporation of methanol. The whole process was repeated three times and finally, 15 g greenish colored, concentrated stem extract was obtained (1.9% w/w) which was kept in refrigerator to 4°C.

Bacterial strains

Gram positive (*Bacillus cereus, B. subtilis, B. megaterium* and *Staphylococcus aureus*) and Gram negative (*E. Coli, Salmonella typhae, S. paratyphae, Pseudomonous* sp. (I),

Pseudomonous sp. (II) and *Shigella sonnei*) bacterial species were used.

Fungal strains

Three human pathogens (*Aspergillus fumigates, A. flavous* and *A. niger*) and three plant pathogens (*Fusarium equiseti, Altenaria alternate* and *Colletotrichum corphori*) were used.

Preparation of stem extracts solution

A measured amount of 200 mg *A. argentea* stem extract was dissolved in 2 mL of methanol to give a solution of known concentration (100 pg/pL). Methanol was chosen as solvent because, in addition to the complete dissolution of the crude extracts, it has no inhibitory effect on the cultures.

Preparation of sample discs

The sample discs of about 4 mm in diameter were cut by punching machine from Whatman No. 1 filter paper. The discs were taken in a petri dish and sterilized by autoclave, dried in oven at 180°C.

Standard antibiotic disc

Kanamycin antibiotic disc (Oxoid, England,) with concentrations of 30 pg/disc was used as standard to compare with the sample.

Assay for antibacterial activity

Antibacterial activity of plant extract was determined by disc diffusion method (Bauer et al., 1966). All the test bacterial species were collected from the Research Laboratories of the Department of Microbiology, University of Chittagong. Dried filter paper discs (4 nm in diameter) impregnated that controls the diffusion of molecules through agar gel. The plates were then incubated at 37°C for 24 hours to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called "zone of inhibition". The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Standard antifungal disc

Grisofulvin (Glaxosmithkline, Chittagong) 100 pg/mL was used as standard to compare the tested results under identical conditions.

Assay for antifungal activity

The poisoned food technique (Grover and Moore, 1962) was used to screen for antifungal activity of plant extract. Potato Dextrose Agar (PDA) was used as a culture medium. Each extract was dissolved in methanol and then mixed with sterilized PDA to obtain a final concentration of 2 mg/mL. From this 20 mL medium of each extract was poured into separate sterilized petri plate and allowed it to solidify. Inoculation was done at the center of each plate with a 5 mm mycelium block for each fungus. Mycelium block was prepared with the help of cork borer from the growing area of a five day old culture of the test fungi PDA. The blocks were placed at the center of each petriplate in an inverted position to get greater contact of the mycelium with the culture medium. The inoculated plate was incubated at 25°C. The experiment was repeated for three times. Proper control (PDA) without extracts was also maintained. After five days of incubation the diameter of fungal colony was measured in mm.

The percentage of inhibition of mycelial growth of the test fungus was calculated by the following formula:-

$$I = \frac{(C-T)}{C} \times 100$$

Where, I = Percentage of inhibition; C = Diameter of the fungal colony in control; T = Diameter of the fungal colony in treatment.

Results

Table I showed that 1,000 pg/disc of extract exhibited 13, 14, 10 and 15 mm zone of inhibition against Gram positive bacteria *Bacillus cereus*, *B. subtilis*, *B. megaterium* and *Staphylococcus aureus* respectively, and 14, 13, 10, 14, 12 and 12 mm zone of inhibition against Gram negative bacteria namely *E. coli, Salmonela typhi, Salmnela paratyphi* (Figure 1A), *Pseudomonous sp. (I)* (Figure 1B), *Pseudomonous sp. (II)* (Figure 1C) and *Shigella sonnei* (Figure 1D), respectively. On the other hand, standard antibiotic kanamycin (30 µg/disc) showed more significant antibacterial activity against all tested Gram positive and Gram negative bacteria showing the larger zone of inhibition in every case. This results indicate that *A. argentea* stem extract has promising antibacterial activity. In the assay of antifungal activity (Table II), *A. argentea* stem extract inhibited the mycelia growth of *Aspergillus flavous, Fusarium equiseti* (Figure 2B), *Altenaria alternate* (Figure 2A) and *Colletotrichum corphori* with the %inhibition of 44.4, 66.7, 44.4 and 75.6%, respectively, whereas no inhibition was observed against *Aspergillus niger* (Figure 2C) and *Aspergillus fumigates*.

Discussion

Plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack. Some of these compounds are toxic to animals,

Table I

Diameter of zone of inhibition against bacteria

Bacteria	Diameter of zone of inhibition (mm)	
	Kanamycin (30 pg/disc) (mm)	*A. argentea* extract (1,000 pg/disc) (mm)
Gram positive bacteria		
Bacillus cereus	28	13
Bacillus subtilis	32	14
Bacillus megatarium	26	10
Staphylococcus aureus	30	15
Gram negative bacteria		
E. Coli	30	14
Salmonella typhae	30	13
Salmonella para typhae	30	10
Pseudomonous Sp. (I)	29	14
Pseudomonous Sp (II)	30	12
Shigella sonnei	28	12

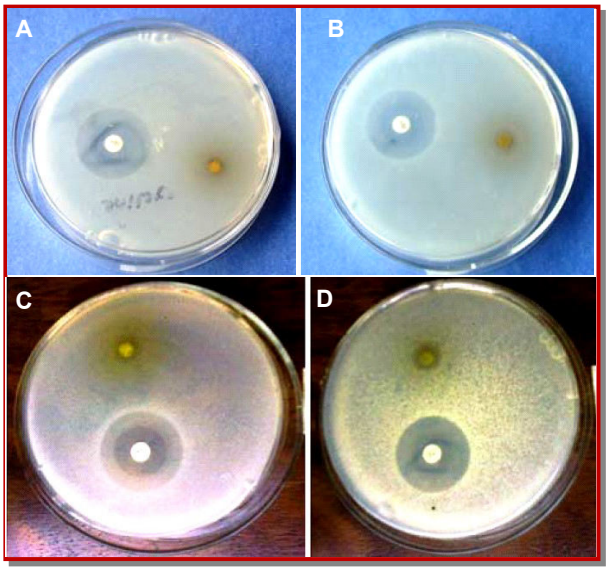

Figure 1: Zone of inhibition showed by the *A. argentea* extract (A-C) against the bacterial strains (A) *Salmonella para typhae;* (B) *Pseudomonous Sp. (I);* (C) *Pseudomonas Sp. (II)* and (D) *Shigella sonnei* (inhibition zone in presence of kanamycin)

Table II

***In vitro* antifungal activities of *A. argentea* extract**

Fungus	% inhibition of fungal mycelial growth	
	A. *argentea* extract (2 mg/ mL)	Grisofulvin (100 pg/mL)
Human pathogen		
Aspergillus fumigatus	0	0
Aspergillus flavous	44.4	66.7
Aspergillus niger	0	22.2
Plant pathogen		
Fusarium equiseti	66.7	72.2
Altenaria alternata	44.4	66.7
Colletotrichum corphori	75.6	66.7

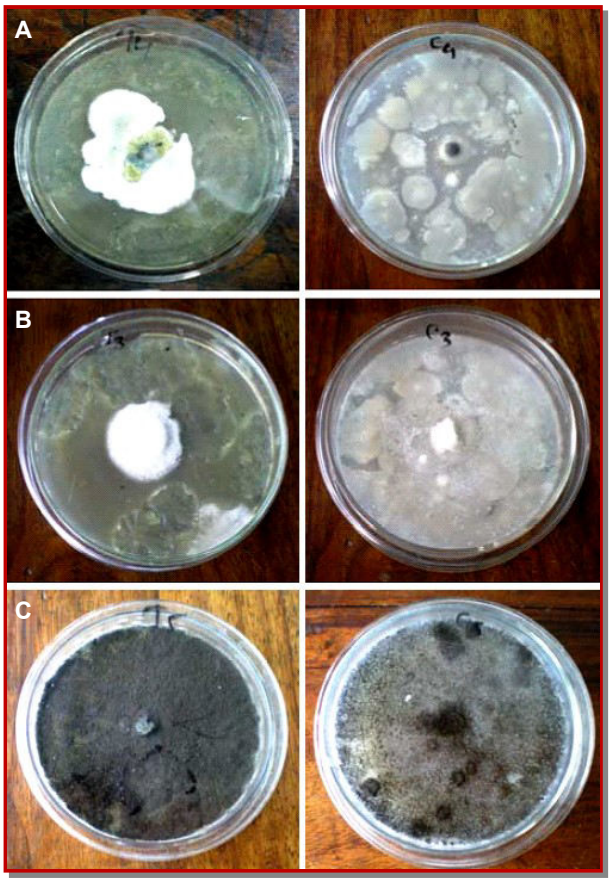

Figure 2: Percentage of fungal growth inhibition showed by the *A. argentea* stem extract against the fungal strains (A) *Altenaria alternate;* (B) *Fusarium equiseti;* (C) *Aspergillus niger.* Left column is treated and right column is control

but others may not be toxic. Indeed, many of these compounds have been used in the form of whole plants or plant extracts for food or medical applications in human (Wallace, 2004) because plants are the natural reservoir of many antimicrobial, anti-cancer agents, analgesics, anti-diarrheal, antifungal as well as various therapeutic activities (Lucy and DaSilva, 1999). Acceptance of medicines from such plant origin as an alternative form of healthcare is increasing because they

are serving as promising sources of novel antibiotic prototypes (Rabe and Van Staden, 1997; Koduru et al., 2006). Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity (Okeke et al., 2001; Ebi and Ofoefule, 1997).

In the current study, the results of testing the crude extracts for antimicrobial activities against 10 bacterial and 6 fungal species might be due to the presence of some sorts of bioactive or inhibitory compounds or factors in the extract or synergism by the existence of some compounds or factors in the extract of *A. argentea.*

Conclusion

This demonstrates that the methanol extract of *A. argentea* stem extract exhibits antibacterial and antifungal effect in experimental models which therefore offer a scientific basis for using this plant as a good source of traditional microbiological references.

Acknowledgement

The authors wish to pay thankful gratitude to Bangladesh Council for Scientific and Industrial Research Laboratories, Chittagong for their continuous support in progress of this study.

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Author Info
Md. Atiar Rahman (Principal contact)
e-mail: atiarh@yahoo.com