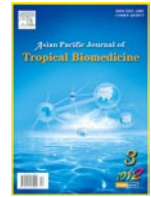




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Antimicrobial and cytotoxic activities of *Abroma augusta* Lnn. leaves extract

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ABSTRACT

Objective: To evaluate the antimicrobial and cytotoxic activity of acetone extract of leaves of *Abroma augusta*. **Methods:** Disc diffusion method was used to demonstrate antibacterial and antifungal activities. Cytotoxicity was determined against brine shrimp nauplii. In addition, minimum inhibitory concentration (MIC) was determined using serial dilution technique to determine antibacterial potency. **Results:** The extract showed significant antibacterial activities against three gram-positive (*Bacillus subtilis*, *Bacillus megaterium* and *Staphylococcus aureus*) and four gram-negative (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei* and *Salmonella typhi*) bacteria. The antifungal activity was found strong against five fungi (*Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Rhizopus oryzae* and *Aspergillus fumigatus*). In cytotoxicity determination, LC₅₀ of the extract against brine shrimp nauplii was 7.06 μg/ml. **Conclusions:** The *Abroma* leaves extract may be consider as a potent antimicrobial and cytotoxic agent for further advance research.

1. Introduction

Abroma augusta L. is an impotent medicinal plant belonging to the family of Sterculiaceae. The whole plant has been found to contain several alkaloids and secondary metabolites including steroids, triterpenes, flavonoids, megastigmanes, benzohydrofurans and their glycosides and phenylethanoid glycosides and very effective against a few bacteria and fungi [1]. Different parts of the plant are useful in treating diabetes, stomachache, dermatitis, leucorrhoea, scabies, gonorrhoea, cough, leukoderma, jaundice, nerve stimulant, weakness, hypertension, uterine disorders, rheumatic pain of joints and headache with sinusitis [2]. It is also used in dermatitis, anti-inflammatory and analgesics. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality

in immunocompromised patients in developing countries [3]. To overcome this problem many works have been done which aimed at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs [4]. There are many reports on antimicrobial and cytotoxic activities of several medicinal plants including *Polygonum hydropiper* [5], *Pterospermum canescens* [6], *Pterospermum acerifolium* [7], *Hermannia incana* [8]. But there is no sufficient report on antimicrobial and cytotoxic activity on this valuable plant. The present investigation was undertaken to study the antimicrobial and cytotoxic activity of *Abroma augusta*.

2. Materials and methods

2.1 Materials

2.1.1 Plant materials

The leaves of *Abroma augusta* were collected during July 2009 from Rajshahi University Campus, Rajshahi, Bangladesh and were identified by Md. Shahed Alam, Senior Technical Officer, Herbarium Museum, Department

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of Botany, University of Rajshahi, Bangladesh, where its voucher specimen (Herbarium No. AC205) was deposited for reference.

2.1.2 Chemicals and reagents

All the chemicals and reagents were used throughout the investigation of reagent grade.

2.1.2 Organisms

Antibacterial activity and minimum inhibitory concentration (MIC) were determined against three gram-positive bacteria (*Bacillus subtilis*, *Bacillus megaterium* and *Staphylococcus aureus*) and four gram-negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi* and *Shigella sonnei*). Antifungal screening was carried out against five fungi (*Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Rhizopus oryzae* and *Aspergillus fumigatus*). Cytotoxicity was determined against brine shrimp nauplii (*Artemia salina*). Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea water (3.8% sodium chloride solution) for 48h. These organisms were collected from the Microbiology Laboratory, Department of Microbiology and Institute of Nutrition and Food Sciences (INFS), University of Dhaka, International Centre for Diarrhoea Diseases Research Bangladesh (ICDDR), Dhaka, Bangladesh.

2.1.3 Media

Nutrient agar media (Difco laboratories) pH 7.2, nutrient broth media (Difco Laboratories) pH 6.8, Sabouraud dextrose agar media (Biolife Vole Monza) pH 5.6 and artificial seawater (3.8% sodium chloride solution) pH 8.4 were used for antibacterial screening, MIC determination, antifungal screening and cytotoxicity determination, respectively.

2.2 Methods

2.2.1 Plant material extraction and fractionation

The leaves were cut, air-dried powdered in a grinding machine and stored in an airtight polybag. Powdered dried leaves (300g) of the plant were extracted (cold) with acetone (1.2 Liter) in flat bottom conical flask, through occasional shaking and stirring for 10 days [9]. The contents were pressed through the markin cloth to get maximum amount of extract. The whole mixture was then filtered by Whatman filter paper No. 41 and the remaining filtrate was dried [5] in vacuo to afford a blackish mass. The output extracts and fractions were collected to glass vials and preserved in a refrigerator at 4°C.

2.2.2 Antibacterial screening

Antibacterial screening was performed by disc diffusion method [5, 10] against three gram-positive and four gram-negative bacteria at the concentration of 300 µg/disc,

which is a qualitative to semi quantitative test. Briefly 20 ml quantities of nutrient agar were plated in petri dish with 0.1 ml of a 10² dilution of each bacterial culture. Filter paper discs (6 mm in diameter) impregnated with various concentrations of plant extracts were placed on test organism-seeded plates. Acetone was used to dissolve the extract and was completely evaporated before application on test organism seeded plates. Blank disc impregnated with solvent acetone followed by during off was used as negative control. The activity was determined after 18 hours of incubation at 37°C. The diameters of zone of inhibition produced by the extract were then compared with the standard antibiotic kanamycin 30 µg/disc.

2.2.3 Minimum inhibitory concentration (MIC) measurements

Serial tube dilution technique [5, 11] was used to determine of MIC of the extracts against three gram-positive and four gram-negative bacteria. The plant extract (1.0 mg) was dissolved in 2 ml distilled water (2 drops tween-80 was added to facilitate dissolution) to obtain stock solution. After preparing the suspensions of test organisms (10⁷ organisms per ml), 1 drop of suspension (0.02 ml) was added to each broth dilution. After 18 hours incubation at 37°C, the tubes were then examined for the growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Growth was observed in those tubes where the concentration of the extract was below the inhibitory level and the broth medium was observed turbid (cloudy). Distilled water with 2 drops of tween-80 and kanamycin were used as negative and positive control, respectively.

2.2.4 Antifungal screening

The antifungal activity of the extract was tested by disc diffusion method [5, 11] against the five pathogenic fungi at the concentrations of 300 µg/disc for each. Here 20 ml quantities of Sabouraud dextrose were plated in petri dish. Blank disc impregnated with solvent acetone followed by drying off was used as negative control. The activity was determined after 72 hours of incubation at room temperature (32°C). The diameter of zone of inhibition produced by the extract was then compared with the standard antibiotic kanamycin 30 µg/disc.

2.2.5 Cytotoxicity assay

Cytotoxicity of *Abroma* leaves was screened against *Artemia salina* in a one day in vivo according to published protocol [12, 13]. Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea water (3.8% sodium chloride solution) for 48 hrs in 25°C. Dissolution for extract was performed in artificial sea water using DMSO. Serially diluted test solutions (0.5, 1, 2, 5, 10, 20 and 40 µg/ml) were added to the sea water (5 ml) containing 10 nauplii. After incubation for 24 hrs at 25°C, the numbers of survivors was counted. From this data, the percentage of mortality of the

nauplii was calculated for each concentration and the LC50 values were determined using probit analysis described by Finney [14]. Each sample was used in triplicate for the determination of the LC50 (50% lethal concentrations, μ g/ml). Gallic acid and vincristine sulfate were used as standards in this bioassay.

2.2.6 Statistical analysis

All the above assays were conducted in triplicate and repeated threes for consistency of results and statistical purpose. The data were expressed as mean \pm SE and analyzed by one way analysis of variance (ANOVA) followed by Dunnett 't' test using SPSS software of 10 version. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Antibacterial activity

The results representing antibacterial activity of acetone extract of leave presented in Table 1. The highest activity of plant extract was 27.0 mm diameter of zone inhibition found against *B. megaterium* (gram-positive) followed by 26.0 mm diameter of zone inhibition against *S. typhi* (gram-negative)

at the concentration of 300 μ g/disc. On the contrary, the lowest activity of plant extract was 21.0 mm diameter of zone inhibition observed against *S. dysenteriae* at the concentration of 300 μ g/disc.

3.2 Minimum inhibitory concentration (MIC) measurement

The Minimum inhibitory concentration (MIC) values of the extract against tested bacteria were shown in Table 2. The MIC values were 16, 8, 64, 32, 64, 32 and 16 μ g/ml respectively, against the tested organisms. The MIC values against the tested gram-positive bacteria ranged from 8 to 64 μ g/ml and against gram-negative bacteria from 16 to 64 μ g/ml. Antibacterial potency of plant extract against these bacteria expressed in MIC indicated the plant extract is more effective against gram-positive (lowest 8 μ g/ml) at lower concentration than that against gram-negative bacteria (lowest 16 μ g/ml).

3.3 Antifungal activity

The antifungal activities of acetone extract of the plant leaves (300 μ g/disc) and standard kanamycin (30 μ g/disc) were determined against five pathogenic fungi (Table 3). The highest activity was 30.0 mm diameter of zone inhibition

Table 1

Antibacterial activity of acetone extract of *Abroma augusta* leaves

Test organisms	Diameter of zone of inhibition (in mm)		ANOVA
	Acetone extract (300 μ g/disc)(M \pm SE)	Kanamycin (30 μ g/disc)(M \pm SE)	
Gram-positive			
<i>Bacillus subtilis</i>	25.0 \pm 0.2	33.0 \pm 1.0	$P < 0.05$
<i>Bacillus megaterium</i>	27.0 \pm 0.5	34.0 \pm 0.3	$P < 0.05$
<i>Staphylococcus aureus</i>	22.0 \pm 1.0	29.0 \pm 0.1	$P < 0.05$
Gram-negative			
<i>Escherichia coli</i>	24.0 \pm 0.1	31.0 \pm 0.2	$P < 0.05$
<i>Shigella dysenteriae</i>	21.0 \pm 0.6	30.0 \pm 0.0	$P < 0.05$
<i>Shigella sonnei</i>	24.0 \pm 0.0	32.0 \pm 0.7	$P < 0.05$
<i>Salmonella typhi</i>	26.0 \pm 0.5	32.0 \pm 0.0	$P < 0.05$

Note: The control disc used for solvent had no zone of inhibition; therefore, it has not been presented.

Table 2

Minimum Inhibitory Concentrations (MIC) of acetone extract of *Abroma augusta* leaves

Test organisms	MIC values (in μ g/ml)		ANOVA
	Acetone extract(M \pm SE)	Kanamycin (M \pm SE)	
Gram-positive			
<i>Bacillus subtilis</i>	16.0 \pm 0.0	2.0 \pm 0.0	$P < 0.05$
<i>Bacillus megaterium</i>	8.0 \pm 0.7	2.0 \pm 0.0	$P < 0.05$
<i>Staphylococcus aureus</i>	64.0 \pm 0.9	16.0 \pm 0.0	$P < 0.05$
Gram-negative			
<i>Bacillus subtilis</i>	32.0 \pm 0.4	8.0 \pm 0.0	$P < 0.05$
<i>Bacillus megaterium</i>	64.0 \pm 0.0	8.0 \pm 0.0	$P < 0.05$
<i>Staphylococcus aureus</i>	32.0 \pm 0.0	4.0 \pm 0.0	$P < 0.05$
<i>Bacillus subtilis</i>	16.0 \pm 0.0	2.0 \pm 0.0	$P < 0.05$

observed against *Aspergillus niger* followed by 29.0 mm diameter of zone inhibition against *Aspergillus flavus* and *Rhizopus oryzae* at the concentration of 300 μg/disc. On the other hand, the lowest activity was 24.0 mm diameter of zone inhibition found against *Aspergillus fumigatus* at the concentration of 300 μg/disc.

3.4 Cytotoxicity assay

The cytotoxicity values of the extracts against tested brine shrimp nauplii were shown in Table 4. In cytotoxicity assay with brine shrimp nauplii, the LC50 value of the acetone extract of leaves of the plant was 7.06 μg/ml. The cytotoxicity of the plant extract was compared with cytotoxicity of standard gallic acid and vincristine sulfate. LC50 value of standard gallic acid and vincristine sulfate was 10.18 and 5.03 respectively. No mortality was found in the control group.

4. Discussion

The findings presented in this investigation were the screening of an important medicinal plant leaves extract as antimicrobial and cytotoxic agents. In the present investigation, 300 μg/disc acetone extract of the plant leaves showed more significant antibacterial activity against the gram positive bacteria (average of 24.6 mm diameter zone of inhibition) than the gram-negative bacteria (average of 23.75 mm diameter zone of inhibition), comparison to reference standard kanamycin 30 μg/disc. But the negative control disc used for solvent had no activity against the tested bacteria (both gram-positive and gram-negative). The finding of zone of inhibition was found higher than a study reported by other researchers [15]. Antibacterial potency

of the plant extract against these bacteria express in MIC as presented in Table 2 also indicated the plant extract is effective against gram-positive bacteria than that gram-negative bacterium. Khan *et al.* [10] reported similar result for *Amorphophallus campanulatus* tuberous roots extract. Many researchers reported antibacterial activity of different medicinal plant extracts against some pathogenic bacteria [16–19] which supported our present findings.

The antifungal activity of the leaves extract activity was also very strong (highest 30.0 mm diameter of zone inhibition) against the tested pathogenic fungi. There are many reports on antifungal activities [16, 19, 20] which supports our present findings. The leaves of *Abroma augusta* plant contains taraxerol and its acetate, β-sitosterol, lupeol, an aliphatic alcohol (C32H66O), octacosanol, and probably a mixture of long chain fatty acid which are very effective against a few bacteria and fungi [1]. Overall, the acetone extract of *Abroma augusta* leaves showed strong antifungal activity than that of antibacterial activity.

From the findings (LC50 was 7.06 μg/ml) of brine shrimp lethality bioassay, the plant extract did not show strong mortality in vitro toxicity compared to positive control [21] and no mortality was found in the negative control. Rahmatullah *et al.* [2] observed the lethality (LC50 was 12.68 mg/ml) of methanol extract of *Abroma augusta* leaves using *Artemia salina*. Since many scientists have shown a correlation between cytotoxicity and activity against the brine shrimps nauplii using extracts or isolated compounds from terrestrial plants [10, 13, 22].

These antibacterial, antifungal and cytotoxic experiments are probably first reported for the leaves extracts of *Abroma augusta*. Further, remarkable antimicrobial and cytotoxic activities found by the experiment support the claims of traditional medicine. It was concluded that this findings can be source of antibiotic substances for possible treatment

Table 3

Antifungal activity of acetone extract of *Abroma augusta* leaves

Test organism	Diameter of zone of inhibition (in mm)		
	Acetone extract (300 μg/disc)(M±SE)	Kanamycin (30 μg/disc)(M±SE)	ANOVA
<i>Aspergillus flavus</i>	29.0±0.3	31.0±0.0	<i>P</i> <0.05
<i>Aspergillus niger</i>	30.0±0.2	32.0±0.1	<i>P</i> <0.05
<i>Candida albicans</i>	28.0±0.4	30.0±0.2	<i>P</i> <0.05
<i>Rhizopus oryzae</i>	29.0±0.0	31.0±0.0	<i>P</i> <0.05
<i>Aspergillus fumigatus</i>	24.0±0.9	29.0±0.6	<i>P</i> <0.05

Note: The control disc used for solvent had no zone of inhibition; therefore, it has not been presented.

Table 4

Cytotoxicity of acetone extract of *Abroma augusta* leaves

Sample	LC ₅₀ (μg/ml)	95% confidence limits (μg/ml)	Regeneration equation	X ² value
Plant extract	7.06	5.77–10.17	Y=2.57+2.74X	3.40
Gallic acid and	10.18	7.16–36.53	Y=4.06+0.79X	0.04
Vincristine sulfate	5.03	3.20–7.89	Y=3.17+2.59X	0.32

Note: The control group had no mortality; therefore, it has not been presented.

of microbial infections and the cytotoxicity result reveals that *Abroma augusta* leaves might be considered as a non-toxic. However, to isolate these active phytochemicals and determine their activities are in progress.

Conflict of interest statement

We declare that we have no conflict of interest.

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