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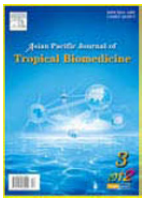

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Antimicrobial and cytotoxic activities of *Abroma augusta* Lnn. leaves extract

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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, LC_{50} 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 glycosidesand 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, gonorrhea, 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, 21] 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.

2.1.2Chemicals 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 (Artima 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 (ICDDRB), 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℃.

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℃. 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℃, 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℃). 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℃. 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℃, the numbers of survivors was counted. From this data, the percentage of mortality of the