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SCREENING OF ANTIBACTERIAL, CYTOTOXIC AND PESTICIDAL ACTIVITIES OF *ABROMA AUGUSTUM* (L.) SEEDS EXTRACT

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ABSTRACT
Abroma augustum is an important medicinal plant in Bangladesh, rightly called as Ulatkambal in Bengali and Devil's cotton in English. Present study was done to assess the antibacterial, toxicity, and pesticidal activities of *A. augustum* seeds extract. The antibacterial activity of *A. augustum* was evaluated using disc diffusion method against some pathogenic bacteria. Serial dilution technique was used to determine the potency of antibacterial activity. Minimum inhibitory concentration (MIC) of *A. augustum* was also studied against tested organisms. Toxicity of *A. augustum* was determined using brine shrimp lethality bioassay method. Pesticidal activity of *A. augustum* extract was tested towards *Sitophilus oryzae* adults. In case of antibacterial screening of *A. augustum* showed highest 27 mm diameter inhibition zone against *Salmonella typhii* at the concentration of 200µg/disc. Highest MIC value was found 250 mg/ml while the lowest MIC value was found 100 mg/ml in the selected bacteria. In toxicity activity test, LC_{50} (lethal concentration, 50%) of the extract against brine shrimp nauplii was 150µg/ml after 12h hour. In pesticidal activity, highest mortality and repellency was 70.33% and 60.3%, respectively towards *S. oryzae* adults. From the above results it can be concluded that the plant extract has moderate antibacterial activity against the tested bacteria, moderate toxicity and high pesticidal activity. The findings of the present research may be helpful for identify biological agents and drug designing for antibacterial activity.

1. INTRODUCTION

From the very beginning of human civilization plants have been using for the treatment of several ailments as they are abound source of phytochemical. *Abroma augustum* is an evergreen medicinal shrub belongs to the family Sterculiaceae and commonly known as Ulatkambal in Bengali and Devil's cotton in English. It generally grows in Asian sub-continent including Bangladesh (Miah et al. 2020). In the other parts of world the trend of using medicinal plant for research were also seen. Several

contains are found in this medicinal plant like secondary metabolites and alkaloids including triterpenes, steroids , benzohydrofurans megastigmanes and their glycosides and phenylethanoid glycosides and effective against a some bacteria and fungi (Gupta et al. 2011). Different types of disease are treated by the use of the several parts of this plant such as diabetes, leucorrhoea, scabies, gonorrhea, cough, leukoderma, jaundice, headache with sinusitis nerve stimulant, weakness stomachache, dermatitis, , hypertension, uterine disorder and so on (Al-bari et al. 2006). At present human life is going through the terrible threatening of infections caused by microorganisms which are pathogenic and is becoming a crucial cause of and mortality and morbidity worldwide specially in developing countries where immuno compromised patients are found (Rahmatullah et al. 2010).

The different antimicrobial and phytochemical constituents of medicinal plants are using for the treatment of microbial infections as an alternatives to chemically synthetic drugs. There are vast amount of reports on antimicrobial and cytotoxic activities of medicinal plants including *Pterospermum canescens, Pterospermum acerifolium, Hermannia incana, Polygonum hydropiper* and so on (Saikot et al. 2012). Miah et al. (2020) observed altered expression of apoptosis regulatory genes in EAC cells by *Abroma augusta* bark extract.

Abroma augustum is a potential medicinal plant may be considered as a potent antimicrobial and cytotoxic agent. *Abroma augustum* also have a very rich source of compounds which show the antioxidant activity. It can be an eminent naturalistic source for the new drugs establishment (Sunitha et al. 2018). By the supplementation of some foods such as vegetables, fruits, vegetables, seeds and nuts antioxidants are manufactured into the human body (Sunitha et al. 2018; Satyanarayana and Eswaraiah 2010). Many plant derived medicines are rich in, and, which possess strong antioxidant activity, thus protecting the body can get protection from such kind of degenerative diseases by the consumption of plants derived medicine which enriched with flavonoids, tannins and phenolic compound (Khanzadi 2012; Maha et al. 2015). The plant re-designated a massive natural source for the new drugs establishment (Sunitha et al. 2018). Very little information is available concerning the antibacterial activity of the studied plants. To the best of our knowledge, the activity of methanol extracts of *A. auguatum* has never been studied; methanol extracts from *A. absinthium* and *H. officinalis* were analyzed only by the disk-diffusion method, which provides qualitative and not always reproducible data and methanol extracts of *A. crithmifolia* and *A. sylvestris* seeds was tested against a limited range of bacteria (Karaalp et al. 2009; Sarker et al. 2003). Sunitha et al. (2018) showed some phytochemical and antioxidant properties on leaf extract of *Abroma augusta* in Malaysia. The pharmacognostical and phytochemical blueprint of *Abroma augusta* stem bark is reported by Hazra et al. (2021).

Though, the plant has important medicinal values, there is no sufficient report to understanding the antibacterial, cytoxicity and pesticidal activities of *Abroma augustum* plant (Saikot et al. 2012). So, the present study was aimed to assess the antibacterial, toxic and pesticidal potentialities of *Abroma augustum* seed extract.

MATERIALS AND METHODS

Plant materials collection

Mature seeds *Abroma augusta* plant were collected from Rajshahi University Campus, Rajshahi, Bangladesh. Seeds of these plants were used as plant material for this present investigation.

Collection of organisms

For antibacterial activity investigation and MIC values examination bacteria were collected from Professor Joardar DNA and Chromosome Research Laboratory. The cytotoxicity test was carried out against brine shrimp nauplii (*Artima salina*). Brine shrimp nauplii eggs were collected and by hatching for 48 h in an artificial sea water. Moreover, pesticidal activity was tested against *S. oryzae* adults which were collected from the Department of Zoology, University of Rajshahi, Bangladesh.

Chemical and reagents used

The chemicals Methanol was ordered from Merck, Germany and the Kanamycin was purchased from Square Pharmaceuticals Ltd., Bangladesh. Reagent grade was used for all the reagents and chemical used in the investigation.

Media Preparation

For this investigation, we utilized LB media (Difco laboratories) with pH 7.2 was used for antibacterial screening, and artificial seawater (3.8% sodium chloride solution) with pH 8.4 was used for cytotoxic activity testing. Finally, a regular 19:1 ratio blend of rice and powdered brewer's yeast was used as a food medium to culture *S. oryzae* for pesticidal activity research.

Plant material extraction and fractionation

Collected plant seeds were air-dried, powdered and placed in an airtight polybag in a grinding machine. The dried powdered seeds (400 g) were extracted (cold) with methanol (1.25L) in a flat conical bottom flask by shaking and stirring periodically for 15 days (Denney et al. 2000). The material was through the labeling cloth to get the maximum quantity of extract. Whatman membrane paper No. 41 subsequently filtered the complete mixture and the residual filtrate became dried in a vacuum to provide a blackish mass (Hussain et al. 2010). The extract and the manufacturing fraction were collected in glass vials and afterwards stored at 4 °C inside a refrigerator.

Antibacterial screening

Antibacterial screening was performed by disc diffusion method against the tested bacteria at the concentration of 50-200µg/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 10^2 dilution of each bacterial culture. Filter paper discs impregnated with various concentrations of plant extracts were placed on test organism-seeded plates. The activity was determined after 18 hours of incubation at 37ºC.

Toxicity determination on *Brine shrimp* nauplii

For this test Brine shrimp nauplii were obtained by hatching brine shrimp eggs in saline water for 48 h in 25°C. The volumes of 0, 20, 40, 80, 100 and 150µg extract were added to the six test tubes which contain 20ml sea water with 10 nauplii and the numbers of survivors was counted. Each sample of the test was used in triplicate for the determination of the LC_{50}.

Assessment of pesticidal activity

Pesticidal activity of *Abroma augusta* extract was assessed against *S. oryzae and T. castenum* adults. The mortality test was performed at the concentrations of 1gm per 1ml methanol solution and 100µl, 200µl and 300µl methanol extract. Ten adult insects were release into each petri dish, kept in room temperature and mortality (%) was recorded at 12, 24 and 48h after treatment. For determining

repellency test, 1gm extract was dissolved in 1ml methanol to obtain the concentrations. Filter papers were cut into two half, and 100, 200 and 300 µl methanol extract from the stock solution was applied. Ten adult insects were release in treated papers and petri dishes were placed in the laboratory at room temperature. The insects present on each half of the paper strip were counted at 6, 12, 24, and 48h after treatment.

RESULTS

Antibacterial potency screening

Highest potency of plant extract was 27.0 mm zone of inhibition against *Salmonella typhi* (gram-negative) at the concentration of 200µg/disc followed by 21 mm zone of inhibition was found on *Eschericia coli* as the same concentration. On the other hands, lowest inhibition zone was 6.0 mm observed by seeds extract on *Pseudomonas sp.* at the concentration of 50µg/disc. Data presented in Table 1 and Figure 1.

Table 1: Antibacterial effects of methanol extract of seeds against several bacterial strains

Concentrations (µg/disc)	**Zone of inhibition (in mm)**		
	Pseudomonas sp.	*Escherichia coli*	*Salmonella typhi*
50	6	6	6
100	6	6	8
150	8	12	14
200	10	21	27

Notes: Zone size <10 mm = Resistance (R), Zone size 10-15 mm = Intermediate resistance (IR), Zone size >15 mm = Sensitive (S)

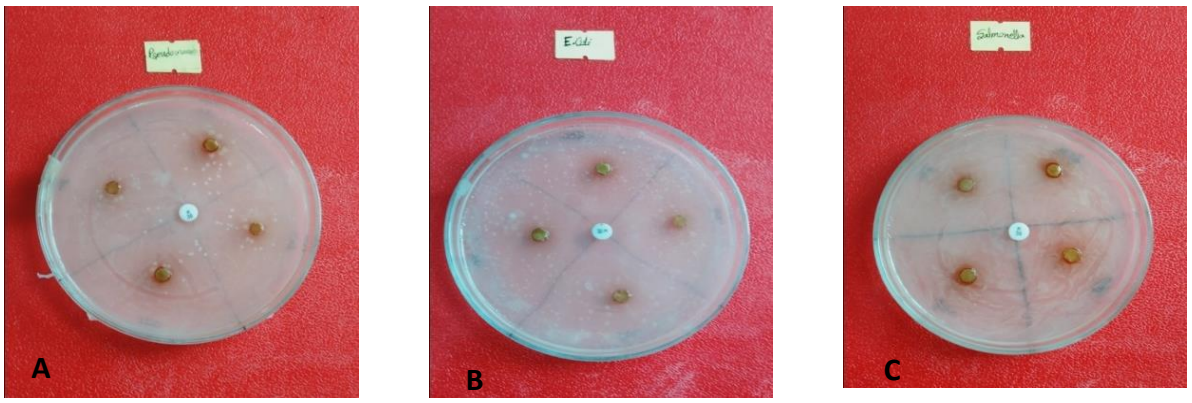

Figure 1: Antibacterial activity of seeds extract against the tested bacteria; (A) *Pseudomonas sp.*, (B) *Escherichia coli* and (C) *Salmomella typhi*

Measurement of Minimum Inhibitory Concentration (MIC)

MIC values of the extract against the tested bacteria were 16, 8, 64, 32, 64, 32 and 16 µg/ml and it was different in ranges for gram positive and negative bacteria.

Toxicity bioassay on brine shrimp nauplii

LC_{50} value was 150µg/ml by seed extract. LC_{50} value was found at the concentration of 150µg after 12 hour. The highest mortality percentage was found at the concentration of 150µg after 24hour and the lowest mortality percentage was found at the concentration 20µg after 6 hour. Detailed data are presented in Figure 2 and 3.

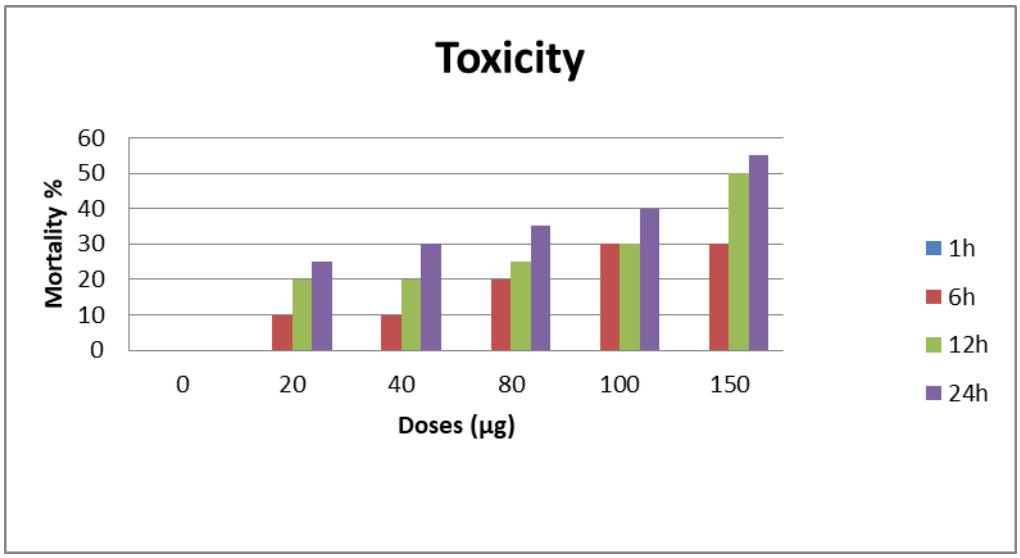

Figure 2: Toxicity of *Abroma augusta* seeds extract on brine shrimp nauplii

Figure 3: *A. augusta* seeds extract on brine shrimp nauplii in the different concentrations.

Pesticidal activity

Sitophilus oryzae was investigated for pest effect capabilities at different concentrations of methanol extract of seeds and time periods. Mortality and repellency percentages of the tested pests have been given in the table.

Mortality

The highest mortality of *Sitophilus oryzae* was 65.33 % found at the concentration of 300mg/ml after 24 hours of treatment but no mortality was recorded after 6 hours treatment of 100mg/ml extract. Data represented in Figure 4 and 5.

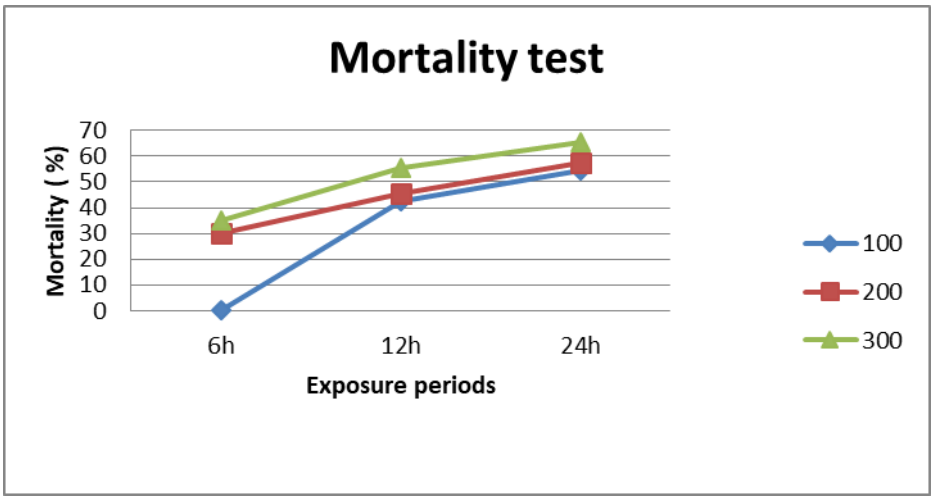

Figure 4: Mortality (%) of *Sitophilus oryzae* adults

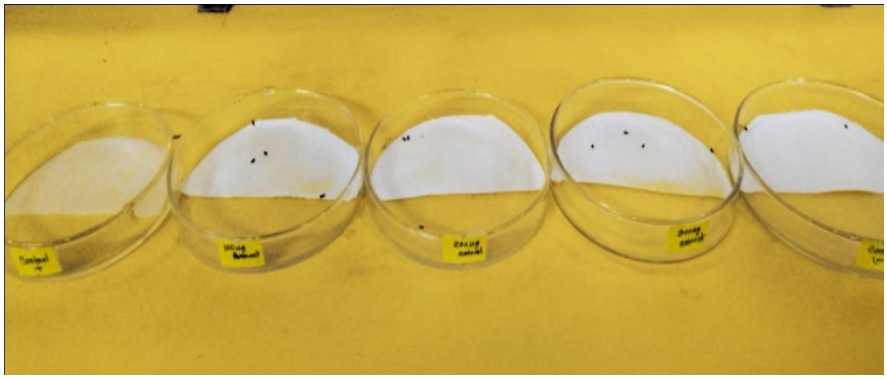

Figure 5: Mortality (%) of *Sitophilus oryzae* adults.

Repellency

The highest repellency was 60.3% (concentration of 300mg/ml after 24 hours) and lowest repellency was 10.6% found at 100mg/ml extract (after 6 hours). Data are given in Figure 6 and 7.

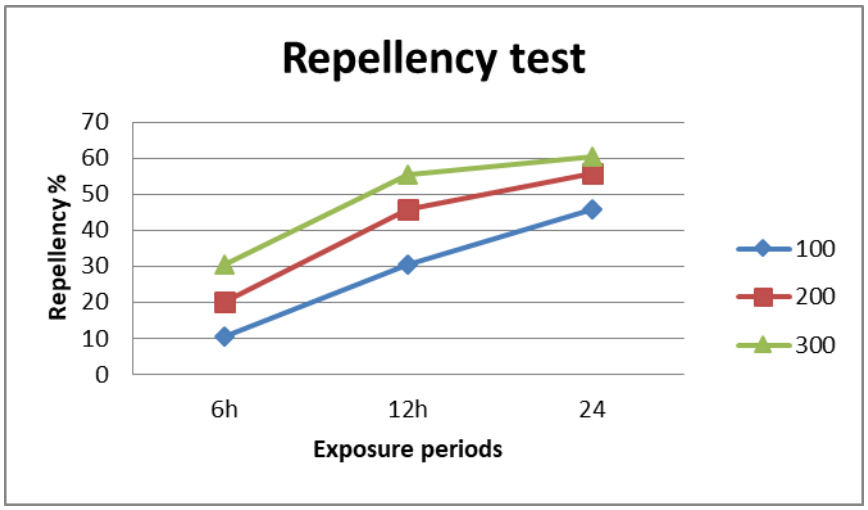

Figure 6: Repellency (%) of *Sitophilus oryzae* adults

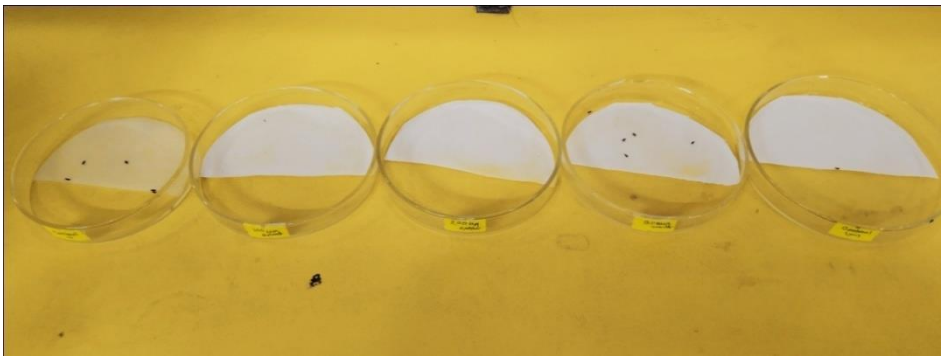

Figure 7: Repellency (%) of *Sitophilus oryzae* adult

DISCUSSION

In this present investigation, antibacterial, cytotoxic and antioxidant activities were investigated. In the present experiment, 150µg/disc methanol extract of *Abroma augusta* seed showed average of 23.75 mm diameter zone of inhibition against the tested bacteria, compared with kanamycin (30 µg/disc). Negative control did not show any activity. Kulsum et al. (2019) found significant antimicrobial activity on *Abroma augusta* seed extracts. Similar results were reported by previous researchers (Maha et al. 2015; Georgiev et al. 2009; Alam et al. 2007). Plant extract also expresses MIC potency on some bacteria. Wibowo et al. (2018) reported similar result for tuberous extract of *Amorphophallus campanulatus*. Other reports on antibacterial capability of plant extracts on the same bacteria were observed (Renzullo 2004; Wibowo et al. 2018; Hasan and Rahman 2011). These findings supported our present results. Overall, the methanol extract of *Abroma augusta* seed showed moderate antibacterial activity.

This investigation showed LC_{50} at 7.06 µg/ml of seed extract on brine shrimp lethality test. The seeds extract showed less mortality compared to positive control (OCDE 1997). No mortally was recorded in negative control. Rahmatullah et al. (2010) observed LC_{50} by 12.68 mg/ml of methanol extract of *Abroma augusta* leaves on *Artemia salina*. *Arcangelisia flava* and *Artemisia vulgaris* showed high toxicity against brine shrimp larvae (Clemen-Pascual et al. 2022; Judzentiene and Budiene 2021). This results support our present findings.

In this investigation, *Abroma augusta* seed extract showed moderate pesticidal activities towards *S. oryzaea and T. castaneum* adults with 65.3% mortality. Similar result was reported by Rahman et al. (2007) in *Sapindus mukorossi* against *Sitophilus oryzae*, and Roy et al. (2005) in *Blumea lacera* against *Sitiphilus oryzae* with 60.3% repellency at the present investigation. These quite similar results were reported in *Blumea lacera* against *Sitiphilus oryzae*, and *Vitex negundo* against several species of stored products pests (Akter et al. 2015). The results of our investigation are primarily proved but more investigation is needed for confirming the detection of antibacterial, cytotoxic, and pesticidal activities for drug designing in near future.

CONCLUSION

Abroma augusta seeds extract showed moderate antibacterial activity of methanol
extract of seeds against selected bacteria. In the development of *Brine shrimp* nuplli the initial stage of embryonic expansion, and possibly the hatching period are most responsive to plant extract toxicity but numerous disturbances caused with plant seeds extract tend to lower numbers and larvae quality during in the developmental period. The LC_{50} value of the plant extract was 150µg/ml found at the

concentration of 150µg after 12 hour. The seeds extract showed significant pesticidal activity. The present research work may help for further investigation of screening of antibacterial, toxicity and pesticidal activities.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper

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