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Evaluation of Antimicrobial, Antifungal, and Cytotoxic Properties of *Abroma augusta* Linn.

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAM designed the study, wrote the protocol, and wrote the final draft of the manuscript. Authors ZI and MJH performed the extraction, fractionation and biological studies. Authors ABMHH, MKEZ and SZ revised the manuscript. Author DT performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Objective: This present study has been undertaken to evaluate the antimicrobial, antifungal and cytotoxic properties of four different fractionates such as petroleum ether, chloroform, ethyl acetate and dia-ion resin adsorbed fractions of *Abroma augusta* leaf extract.
Methodology: The antimicrobial and antifungal properties have been characterized by disc diffusion method. The cytotoxicity property was determined against brine shrimp nauplii.
Results and Conclusion: Among the four fractionates, the dia-ion resin adsorbed fraction showed the highest activity towards the *S. aureus*, *E. coli* bacteria and the fungus *C. albicans* with the zone inhibition of 12~13 mm which was comparable with that of the standard Kanamycin. The chloroform, petroleum ether, and ethyl acetate fraction was exhibited least activity toward the bacteria and

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fungus with the zone inhibition of 6~9 mm. Similarly, the dia-ion resin adsorbed fractions showed the highest antifungal activity with the zone inhibition of 15 mm. From the results of cytotoxicity test, it was observed that the chloroform and ethyl acetate fractions were found be the highest active on 6, 12, 18, 24, and 30h of exposures in which almost all the nauplii were dead. From the corresponding LD_{50} values, the chloroform and ethyl acetate fractions were found to be the highest toxic against *Artemia salina* nauplii of 75.019 and 65.553 ppm, respectively. It has been predicted that the *Abroma augusta* leaves might be considered as a potent cytotoxic agent for further advanced research.

Keywords: Abroma augusta; antimicrobial; antifungal; cytotoxicity; brine shrimp.

1. INTRODUCTION

Abroma augusta Linn. belonging to the family "Sterculiaceae" commonly known as Devil's cotton has been used as remedy for the treatment of various types of disorders. In Bangladesh *A. augusta* is very much familiar with name "Ulatkambal". It is one of the widely found plants all over in India and Australia [1,2]. The root of *A. augusta* are used as a uterine tonic, an emmenogogue, dysmenorrhoea, amenorrhoea, strerility and other menstrual diseases. The different solvent fractions showed the presence of tannins, glycosides, steroids and alkaloids. The whole plant contains several alkaloids and secondary metabolites including steroids, triterpenes, flavonoids, megastigmanes, benzohydrofurans and their glycosides and phenylethanoid glycosides [3]. The leaves of *A. augusta* contain octacosanol, taraxerol, β-sitosterol acetatelupeol, an aliphatic alcohol and mixture of long chain fatty diols. Different parts of *A. augusta* are useful in treating diabetes, stomachache, dermatitis, leucorrhoea, scabies, gonorrhea, cough, leukoderma, jaundice, nerve stimulant, weakness, hypertension, uterine disorders, dermatitis, inflammation, rheumatic pain of joints and headache with sinusitis [3]. The plant is reported to have hypolipidemic effect, however root bark is reported to contain antifertility agent [2]. The ethanolic extract of roots of *A. augusta* also exhibit the hypoglycemic effect in alloxan induced diabetic rats [4,5]. The petroleum extracts of roots of *A. augusta* is used for its anti-inflammatory activity [6]. The n-hexane extract of seeds of *A. augusta* is used as antifungal and phytotoxic activity [7]. From the point of view, it has been observed that a number of phytoconstituents and/or drugs are exclusively derived from the plant *A. augusta* such as betaine, β-sitosterol, maslinic acid, α-amyrin, protocalechuic acid, vanillic acid, caffeic acid etc [1,8]. In spite of being widely used in traditional systems of medicines, there is a repot published on antimicrobial, antifungal and cytotoxic properties of acetone fraction of the plant *A. augusta* [3]. In this context, the objective of the present study is to evaluate the antimicrobial, antifungal and cytotoxic properties on four different extracts such as petroleum ether, chloroform, ethyl acetate and dia-ion resin adsorbed fraction of the medicinal plant *A. augusta* using some known pathogenic bacteria and fungal.

2. EXPERIMENTAL METHODS AND MATERIALS

2.1 Plant Collection and Extraction Process

The *Abroma augusta* plant leaves were collected from the uncultivated adjacent areas of Rajshahi University campus, Bangladesh. The collected leaves were washed thoroughly in water then dried in open air for a week at 35–40°C. The dried leaves were chopped and pulverized in electric grinder. The ground leaves of *A. augusta* were exhaustively extracted with methanol (MeOH, Analytical Grade) in soxhlet extractor. The resulting juicy extract was filtered through Whatman paper and concentrated under reduced pressure at 45°C using the Buchi Rotavapor (R–200). The obtained material was then called crude methanolic extract. The process was done for several times to increase the crude extract. The crude extract was divided into two parts: one part was kept as stock crude in refrigerator and other part was used to obtain water soluble component. The crude was taken in a reagent bottle (2.5L) and triturate with water; then it was filtered and water soluble triturate was separated out. It is known that the antioxidant compounds are water soluble; therefore, dia-ion resin column was used to separate the antioxidants components from the water soluble triturate.

The water soluble portion was passed through the dia-ion resin until the white color of the resin was turned to brown. The adsorbed components were then eluted with methanol (Merck KGaA, Germany). This process was repeatedly done to separate all the antioxidants components from the remaining water soluble part of the *A. august* extract. The methanol was removed from the elute using Rotavapor at 45°C and water was removed from the condensed eluted part by freeze dryer. This dried material was then used to fractionate into three fractions by triturating with petroleum ether, chloroform, ethyl acetate (Fluka, India) and by dissolving the residue in methanol. Finally, petroleum ether, chloroform and ethyl acetate triturate were collected and were subjected to the further evaluation of the antimicrobial, antifungal and cytotoxic properties.

2.2 Antimicrobial and Antifungal Activity Test

The antimicrobial and antifungal activity was determined by standard disc diffusion method by measuring the zone of inhibition and was compared to that of the standard disc [9, 10]. Two pathogenic bacteria (*Staphylococcus aureus, Escherichia coli*) and one fugal (*Candida albicans*) were used in this test. The nutrient agar media were dispensed to a number of clean test tubes, each containing 5 mL of the prepared slants. The test tubes were plugged with cotton and sterilized in an autoclave at 121°C and 15 lbs/sq-inch pressure for 15 min. After sterilization, the test tubes were kept in an inclined position for solidification. These were then incubated at 37.5°C to ensure the sterilization. Finally, the slants were streaked with pure culture of the test organisms in the laminar air flow and incubated at 37.5° for 24 hrs. The test plates were prepared by pouring nutrient agar in 15.0 mL in clean test tubes and plugged with cotton. The test tubes were sterilized by autoclaving and allowed to cool at about 50°C. The media in the test tubes were incubated with fresh culture. Bacteria were agitated to ensure uniform dispersion of organisms into the media. Finally, the media were poured into sterile petri discs in aseptic condition. The petri discs were rotated several times, first clockwise and then anticlockwise to assure homogeneous distribution of the test organisms. Thus the plates were ready for sensitivity test and stored it in a refrigerator at 4°C.

2.3 Preparation of Sample and Standard Discs

The sample solution was prepared in methanol in such a manner that 10 µL contained 200 µg of the bacteria and fungus. 20 µL of the test solution was applied on a disc and thus the disc containing 400 µg of the sample prepared. These discs were left for few min in aseptic condition for complete removal of the solvent. The standard discs were used as positive control to ensure the activity of the standard antibiotic against the test organisms as well as for comparison of the response produced by the known antibacterial agent. In this study, Kanamycin (K-30) containing 30 µg/disc of antibiotic was used as standard disc for comparison. The sample impregnated discs and standard antibiotic discs were placed gently on solidified agar plates seeded with the organisms to ensure contact with the media. The plates were kept in a refrigerator 4°C for 24h and then incubated at 37.5°C for 24h.

2.4 Cytotoxicity Activity Test

2.4.1 Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was used for the probable cytotoxic activity according to the method described here [11–13]. The eggs of Brine Shrimp (*Artemia salina*) were collected from the aquarium shop of Kalabagan, Dhaka, Bangladesh and hatched in a small artificially partitioned tank with constant oxygen supply at temperature around 37°C. The artificial sea water contains 3.8% of sodium chloride was made by dissolving 38 g sodium chloride in 1000 mL distilled water. The pH of the brine water was maintained at 8 ~ 9 using $NaHCO_3$. In the two partitioned tank, the eggs were hatched in the darkened side whereas the other part of the tank was put under sunlight. With the help of light illumination, the larvae (nauplii) were attracted to one side of the tank and were easily collected from the non-hatched eggs. One day old mature nauplii were used for the experiment. The extracts (petroleum ether, chloroform, ethyl acetate and dia-ion resin adsorbed) dissolved in DMSO were added into each vial to obtain final concentration of 800, 400, 200, 100, 50 and 25 ppm. Each concentration was tested in triplicate. The controls were prepared in same manner except that DMSO was used instead of the extracts. 30 shrimp nauplii were used as negative control group. When the nauplii in the control showed a rapid mortality, then the test

was considered to be invalid due to reasons other than the cytotoxicity of the test compounds. After 24h, the number of survivors were counted by magnifying glass and next, the percentage of death and LD_{50} was calculated by probit analysis [14]. The mortality percentage was corrected by using the Abbott's formula [15].

$$P_t = \frac{(P_o - P_c)}{(100 - P_c)} \times 100 \quad (1)$$

Where, P_t = corrected mortality%, P_o = observed mortality %, and P_c = control mortality %.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial and Antifungal Activity

The antibacterial and antifungal activities of the four different fractions were examined and the results are given in the Table 1. It has been observed that among the four fractions tested, the dia-ion resin adsorbed fraction showed broader spectrum of activity, being active to both the *S. aureus*, *E. coli* bacteria and *C. albicans* fungus with the inhibition zone of 12, 13 and 15 mm, respectively. The ethyl acetate fraction showed the inhibition zone (8~9 mm) against *E. coli* and *S. aureus* bacteria and 11 mm against *C. albicans* fungal. The chloroform and petroleum ether fractions showed mild activity against the tested organism with the inhibition zone of 6~8 mm. However, the acetone extract of the *A. augusta* was reported to found the highest activity with the zone inhibition of 27 mm against *B. megaterium* which was little higher than that of our results [3]. From the results of our finding, it is obvious that the dia-ion resin adsorbed fraction have the highest antimicrobial and antifungal activity. The highest activity of the dia-ion resin adsorbed fraction may be due to the higher amount of polyphenolic compounds which inhibit the microbial growth [16].

3.2 Cytotoxicity Activity Test

The brine shrimp lethality assay has been used extensively in the primary screening of the crude extracts as well as the isolated compounds to evaluate cytotoxic, phototoxic, pesticidal, and many other activities towards brine shrimp that could provide an indication of possible cytotoxic properties of the test material [17]. In this present study, the brine shrimp lethality test was used to assess cytotoxic potential of the petroleum ether, chloroform, ethyl acetate, and dia-ion resin adsorbed fractionates of *Abroma augusta*. The lethality of the different fractions of *Abroma augusta* was determined on *A. salina* after 6-30-hour exposure. The results are presented in Table 2. From the mortality percentage of brine shrimp, the probits were calculated for each concentration of the fractions and plotted against the corresponding log concentrations. From this plot, the LD_{50} values were calculated and the values are presented in Table 3. The results of brine shrimp lethality test has been expressed as: the fraction would not be toxic with the value of LD_{50}>1000 ppm, would show weak toxicity with the value of LD_{50} 500-1000 ppm, mighty be toxic with the value of LD_{50} 100-500 ppm and would be very toxic with the value LD_{50}<100 ppm [18]. Among all the four fractions, it has been observed that the chloroform and ethyl acetate fractions displayed significant toxicity and different mortality rate towards shrimp nauplii. The mortality rate of nauplii was found to be increased with concentration of each of the fractions. The chloroform and ethyl acetate fractions showed the highest level of toxicity with the LD_{50} values of 75.01 and 65.55 μg/mL, respectively at 30h exposure. On the other hand, the petroleum ether and dia-ion resin adsorbed fraction showed the toxicity with LD_{50} values of

Table 1. Antimicrobial and antifungal activity of four different fractions such as petroleum ether, chloroform, ethyl acetate and dia-ion resin adsorbed fractionate of *A. augusta*.

Fractions	Dose (μg/disc)	Zone of inhibition (mm)		
		Bacteria		Fungal
		S. aureus	*E. coli*	*C. albicans*
Petroleum ether	400	7	6	8
Chloroform	400	8	7	8
Ethyl acetate	400	9	8	11
Dia-ion resin adsorbed	400	12	13	15
Kanamycin (K-30)	30	22	22	22

407.11 and 268.02 μg/mL, respectively at 30h exposure. The inhibitory effect of the extract might be due to the toxic components present in the active fraction that possess ovicidal and larvicidal properties. The metabolites either affected the embryonic development or slay the eggs [19]. Therefore, the toxicity effects of the plant extract articulate that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii [20].

Table 2. Cytotoxic activity of four different fractionates of *Abroma augusta* on *Artemia salina* nauplii after 6-30h exposure in which 30 nauplii were used

Fractions	Concentration (ppm)	No. of nauplii killed after exposure (h)					Control
		6	12	18	24	30	
Petroleum ether	400	0	0	4	10	11	0
	200	0	0	2	5	9	0
	100	0	0	1	5	7	0
	50	0	0	1	2	5	0
	25	0	0	0	2	2	0
Chloroform	400	3	20	24	26	29	0
	200	1	5	12	15	21	0
	100	1	2	5	12	15	0
	50	1	3	5	9	11	0
	25	0	3	6	6	8	0
Ethyl acetate	400	4	12	21	26	30	0
	200	1	8	14	22	28	0
	100	2	7	8	12	14	0
	50	0	7	8	11	12	0
	25	0	3	6	8	8	0
Dia-ion resin	400	2	3	6	11	14	0
	200	0	0	3	7	11	0
	100	0	2	3	5	5	0
	50	0	0	3	3	4	0
	25	0	0	1	4	4	0

Table 3. LD_{50}, 95% confidence limits and regression equations of *Abroma augusta* extracts against *A. salina* nauplii. where X is the log dose and Y is the working probit of the analytical calculation

Fractions	Exposure (h)	Regression equation	χ^2 value for heterogeneity	LD_{50} μg/mL	95% confidence limits	
					Lower	Upper
Petroleum ether	12	-	-	-	-	-
	18	Y = 1.524 + 0.875 X	0.287	9300.042	397.99	217.31
	24	Y = 1.959 + 0.997 X	1.367	1123.295	442.77	2849.73
	30	Y = 1.745 + 1.247 X	4.640	407.113	256.24	646.80
Chloroform	12	Y = 1.171 + 1.461 X	14.467	416.878	112.07	1550.59
	18	Y = 1.821 + 1.362 X	10.697	215.466	94.08	493.44
	24	Y = 2.013 + 1.425 X	4.208	124.717	87.72	177.30
	30	Y = 1.901 + 1.652 X	3.658	75.019	54.66	102.96
Ethyl acetate	12	Y = 2.878 + 0.701 X	1.024	1071.242	166.43	6894.83
	18	Y = 2.466 + 1.098 X	2.405	202.310	119.60	342.20
	24	Y = 2.156 + 1.463 X	3.246	87.646	62.41	123.06
	30	Y = 1.431 + 1.965 X	6.967	65.553	58.63	111.23
Dia–ion resin	12	Y = 2.103 + 0.679 X	0.391	18384.830	53.44	6324.85
	18	Y = 1.528 + 1.104 X	4.758	1391.716	532.51	3637.19
	24	Y = 1.542 + 1.317 X	11.171	421.572	200.24	887.54
	30	Y = 1.247 + 1.545 X	10.903	268.025	155.47	462.05

4. CONCLUSION

The antimicrobial, antifungal and cytotoxic properties of *Abroma augusta* leaf extract have been evaluated on *S. aureus, E. coli*, *C. albicans*, and *A. salina*, respectively. Among the four fractionates, dia-ion resin adsorbed fraction showed the activity with the zone inhibition of 12~13 mm that was comparable with the standard Kanamycin. The chloroform, petroleum ether, and ethyl acetate fractions exhibited week activity with the zone of inhibition 6~9 mm. In the case of antifungal activity test, the dia-ion resin adsorbed fractions showed the highest antifungal activity with the zone inhibition of 15 mm. From the results of cytotoxicity test, it was observed that the chloroform and ethyl acetate fractions were found be the highest active on 6, 12, 18, 24, and 30h of exposures in which almost all the nauplii were dead. From the corresponding LD_{50} values, the chloroform and ethyl acetate fractions were found to be the highest toxic of 75.019 and 65.553 ppm, respectively. It has been predicted that the *Abroma augusta* leaves may be considered to be a potent cytotoxic agent for further advanced research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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