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1 ***Original Research Article***

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

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6

7 **ABSTRACT**

8 This present study has been undertaken to evaluate the antimicrobial, antifungal and
9 cytotoxic properties of four different fractionates such as petroleum ether, chloroform, ethyl
10 acetate and dia-ion resin adsorbed fractions of *Abroma augusta* leaf extract. Among the four
11 fractionates, the dia-ion resin adsorbed fraction showed the highest activity with the zone
12 inhibition of 12~13 mm which was comparable with that of the standard Kanamycin. The
13 chloroform, petroleum ether, and ethyl acetate fraction was exhibited week activity with the
14 zone inhibition of 6~9 mm. Similarly, the dia-ion resin adsorbed fractions showed the highest
15 antifungal activity with the zone inhibition of 15 mm. From the results of cytotoxicity test, it
16 was observed that the chloroform and ethyl acetate fractions were found be the highest active
17 on 6, 12, 18, 24, and 30h of exposures in which almost all the nauplii were dead. From the
18 corresponding LD_{50} values, the chloroform and ethyl acetate fractions were found to be the
19 highest toxic of 75.019 and 65.553 ppm, respectively. It has been predicted that the *Abroma*
20 *augusta* leaves may be consider to be a potent cytotoxic agent for further advanced research.

21

22 **Keywords:** *Abroma augusta,* antimicrobial, antifungal, cytotoxicity, Brine shrimp.

23

24 **1. INTRODUCTION**

25 *Abroma augusta* Linn. belonging to the family "Sterculiaceae" commonly known as Devil's
26 cotton has been used as remedy for the treatment of various types of disorders. In Bangladesh
27 *A. augusta* is very much familiar with name "Ulatkambal". It is one of the widely found
28 plants all over in India and Australia [1,2]. The root of *A. augusta* are used as a uterine tonic,
29 an emmenogogue, dysmenorrhoea, amenorrhoea, sterility and other menstrual diseases. The
30 different solvent fractions showed the presence of tannins, glycosides, steroids and alkaloids.
31 The whole plant contains several alkaloids and secondary metabolites including steroids,
32 triterpenes, flavonoids, megastigmanes, benzohydrofurans and their glycosides and
33 phenylethanoid glycosides [3]. The leaves of *A. augusta* contain octacosanol, taraxerol, β-

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34 sitosterol acetatelupeol, an aliphatic alcohol and mixture of long chain fatty diols. Different
35 parts of *A. augusta* are useful in treating diabetes, stomachache, dermatitis, leucorrhoea,
36 scabies, gonorrhea, cough, leukoderma, jaundice, nerve stimulant, weakness, hypertension,
37 uterine disorders, dermatitis, inflammation, rheumatic pain of joints and headache with
38 sinusitis [3]. The plant is reported to have hypolipidemic effect, however root bark is reported
39 to contain antifertility agent [2]. The ethanolic extract of roots of *A. augusta* also exhibit the
40 hypoglycemic effect in alloxan induced diabetic rats [4,5]. The petroleum extracts of roots of
41 *A. augusta* is used for its anti-inflammatory activity [6]. The n-hexane extract of seeds of *A.*
42 *augusta* is used as antifungal and phytotoxic activity [7]. From the point of view, it has been
43 observed that a number of drugs are exclusively derived from the plant *A. augusta*. In spite of
44 being widely used in traditional systems of medicines, not any reports are published on
45 antimicrobial, antifungal and cytotoxic properties of the plant *A. augusta*. In this context, the
46 objective of the present study is to evaluate the antimicrobial, antifungal and cytotoxic
47 properties using some known pathogenic bacteria and fungal.
48
49 **2. EXPERIMENTAL METHODS AND MATERIALS**
50 **2.1 Plant collection and extraction process**
51 The *Abroma augusta* plant leaves were collected from the uncultivated adjacent areas of
52 Rajshahi University campus, Bangladesh. The collected leaves were washed thoroughly in
53 water then dried in open air for a week at 35–40°C. The dried leaves were chopped and
54 pulverized in electric grinder. The ground leaves of *A. augusta* were exhaustively extracted
55 with methanol (MeOH, Analytical Grade) in soxhlets apparatus. The resulting juicy extract
56 was filtered through Whatman paper and concentrated under reduced pressure at 45°C using
57 the Buchi Rotavapor (R–200). The obtained material was then called crude methanolic
58 extract. The process was done for several times to increase the crude extract. The crude
59 extract was divided into two parts: one part was kept as stock crude in refrigerator and other
60 part was used to obtain water soluble component. The crude was taken in a reagent bottle
61 (2.5L) and triturate with water; then it was filtered and water soluble triturate was separated
62 out. It is known that the antioxidant compounds are water soluble; therefore, dia-ion resin
63 column was used to separate the antioxidants components from the water soluble triturate.
64 The water soluble portion was passed through the dia-ion resin until the white color of the
65 resin was turned to brown. The adsorbed components were then eluted with methanol
66 (Merck, Germany). This process was repeatedly done to separate all the antioxidants
67 components from the remaining water soluble part of the *A. august* extract. The methanol was

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68 removed from the elute using Rotavapor at 45°C and water was removed from the condensed
69 eluted part by freeze dryer. This dried material was then used to fractionate into three
70 fractions by triturating with petroleum ether, chloroform, ethylacetate and by dissolving the
71 residue in methanol. Finally, petroleum ether, chloroform and ethyl acetate triturate were
72 collected and were subjected to the further evaluation of the antimicrobial, antifungal and
73 cytotoxic properties.

74

75 **2.2 Antimicrobial and antifungal activity test**

76 The antimicrobial and antifungal activity was determined by standard disc diffusion method
77 by measuring the zone of inhibition and was compared to that of the standard disc [8, 9]. Two
78 pathogenic bacteria (*Staphylococcus aureus, Escherichia coli*) and one fugal (*Candida*
79 *albicans*) were used in this test. The nutrient agar media were dispensed to a number of clean
80 test tubes, each containing 5 mL of the prepared slants. The test tubes were plugged with
81 cotton and sterilized in an autoclave at 121°C and 15 lbs/sq-inch pressure for 15 min. After
82 sterilization, the test tubes were kept in an inclined position for solidification. These were
83 then incubated at 37.5°C to ensure the sterilization. Finally, the slants were streaked with
84 pure culture of the test organisms in the laminar air flow and incubated at 37.5° for 24 hrs.
85 The test plates were prepared by pouring nutrient agar in 15.0 ml in clean test tubes and
86 plugged with cotton. The test tubes were sterilized by autoclaving and allowed to cool at
87 about 50°C. The media in the test tubes were incubated with fresh culture. Bacteria were
88 agitated to ensure uniform dispersion of organisms into the media. Finally, the media were
89 poured into sterile petri discs in aseptic condition. The petri discs were rotated several times,
90 first clockwise and then anticlockwise to assure homogeneous distribution of the test
91 organisms. Thus the plates were ready for sensitivity test and stored it in a refrigerator at 4°C.

92

93 **2.3 Preparation of sample and standard discs**

94 The sample solution was prepared in methanol in such a manner that 10 µL contained 200 µg
95 of the bacteria and fungal. 20 µL of the test solution was applied on a disc and thus the disc
96 containing 400 µg of the antibiotic prepared. These discs were left for few min in aseptic
97 condition for complete removal of the solvent. The standard discs were used as positive
98 control to ensure the activity of the standard antibiotic against the test organisms as well as
99 for comparison of the response produced by the known antibacterial agent. In this study,
100 Kanamycin (K-30) containing 30 µg/disc of antibiotic was used as standard disc for

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101 comparison. The sample impregnated discs and standard antibiotic discs were placed gently
102 on solidified agar plates seeded with the organisms to ensure contact with the media. The
103 plates were kept in a refrigerator 4°C for 24h and then incubated at 37.5°C for 24h.

104

105 **2.4 Cytotoxicity activity test**

106 **2.4.1 Brine shrimp lethality bioassay**

107 Brine shrimp lethality bioassay was used for the probable cytotoxic activity according to the
108 method described here [10–12]. The eggs of Brine Shrimp (*Artemia salina*) were collected
109 from the aquarium shop of Kalabagan, Dhaka, Bangladesh and hatched in a small artificially
110 partitioned tank with constant oxygen supply at temperature around 37°C. The artificial sea
111 water contains 3.8% of sodium chloride was made by dissolving 38 g sodium chloride in
112 1000 mL distilled water. The P^H of the brine water was maintained at 8 ~ 9 using $NaHCO_3$.
113 In the two partitioned tank, the eggs were hatched in the darkened side whereas the other part
114 of the tank was put under sunlight. With the help of light illumination, the larvae (nauplii)
115 were attracted to one side of the tank and were easily collected from the non-hatched eggs.
116 One day old mature nauplii were used for the experiment. The extracts (petroleum ether,
117 chloroform, ethyl acetate and dia-ion resin adsorbed) dissolved in DMSO were added into
118 each vial to obtain final concentration of 800, 400, 200, 100, 50 and 25 ppm. Each
119 concentration was tested in triplicate. The controls were prepared in same manner except that
120 DMSO was used instead of the extracts. 30 shrimp nauplii were used as negative control
121 group. When the nauplii in the control showed a rapid mortality, then the test was considered
122 to be invalid due to reasons other than the cytotoxicity of the test compounds. After 24h, the
123 number of survivors were counted by magnifying glass and next, the percentage of death and
124 LD_{50} was calculated by probit analysis [13]. The mortality percentage was corrected by using
125 the Abbott's formula [14].

126

127 $$P_t = \frac{(P_o - P_c)}{(100 - P_c)} \times 100 \quad (1)$$

128

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

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134 ## 3. RESULTS AND DISCUSSION
135 ### 3.1 Antimicrobial and antifungal activity
136 The antibacterial and antifungal activities of the four different fractions were examined and
137 the results are given in the Table 1. It has been observed that among the four fractions tested,
138 the dia-ion resin adsorbed fraction showed broader spectrum of activity, being active to both
139 the *S. aureus*, *E. coli* bacteria and *C. albicans* fungus with the inhibition zone of 12, 13 and
140 15 mm, respectively. The ethyl acetate fraction showed the inhibition zone (8~9 mm) against
141 *E. coli* and *S. aureus* bacteria and 11 mm against *C. albicans* fungal. The chloroform and
142 petroleum ether fractions showed mild activity against the tested organism with the inhibition
143 zone of 6~8 mm. From the results of our finding, it is obvious that the dia-ion resin adsorbed
144 fraction have the highest antimicrobial and antifungal activity. The highest activity of the dia-
145 ion resin adsorbed fraction may be due to the higher amount of polyphenolic compounds
146 which inhibit the microbial growth [15].
147
148 ### 3.2 Cytotoxicity activity test
149 The brine shrimp lethality assay has been used extensively in the primary screening of the
150 crude extracts as well as the isolated compounds to evaluate cytotoxic, phototoxic, pesticidal,
151 and many other activities towards brine shrimp that could provide an indication of possible
152 cytotoxic properties of the test material [16]. In this present study, the brine shrimp lethality
153 test was used to assess cytotoxic potential of the petroleum ether, chloroform, ethyl acetate,
154 and dia-ion resin adsorbed fractionates of *Abroma augusta*. The lethality of the different
155 fractions of *Abroma augusta* was determined on *A. salina* after 6-30-hour exposure. The
156 results are presented in Table 2. From the mortality percentage of brine shrimp, the probits
157 were calculated for each concentration of the fractions and plotted against the corresponding
158 log concentrations. From this plot, the LD_{50} values were calculated and the values are
159 presented in Table 3. The results of brine shrimp lethality test has been expressed as: the
160 fraction would not be toxic with the value of $LD_{50}>1000$ ppm, would show weak toxicity
161 with the value of LD_{50} 500-1000 ppm, mighty be toxic with the value of LD_{50} 100-500 ppm
162 and would be very toxic with the value $LD_{50}<100$ ppm [17]. Among all the four fractions, it
163 has been observed that the chloroform and ethyl acetate fractions displayed significant
164 toxicity and different mortality rate towards shrimp nauplii. The mortality rate of nauplii was
165 found to be increased with concentration of each of the fractions. The chloroform and ethyl
166 acetate fractions showed the highest level of toxicity with the LD_{50} values of 75.01 and 65.55

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167 μg/mL, respectively at 30h exposure. On the other hand, the petroleum ether and dia-ion resin
168 adsorbed fraction showed the toxicity with LD_{50} values of 407.11 and 268.02 μg/mL,
169 respectively at 30h exposure. The inhibitory effect of the extract might be due to the toxic
170 components present in the active fraction that possess ovicidal and larvicidal properties. The
171 metabolites either affected the embryonic development or slay the eggs [18]. Therefore, the
172 toxicity effects of the plant extract articulate that it can be selected for further cell line assay
173 because there is a correlation between cytotoxicity and activity against the brine shrimp
174 nauplii [19].
175

176
4. CONCLUSION

177 The antimicrobial, antifungal and cytotoxic properties of *Abroma augusta* leaf extract have
178 been evaluated on *S. aureus, E. coli*, *C. albicans*, and *A. salina*, respectively. Among the four
179 fractionates, dia-ion resin adsorbed fraction showed the activity with the zone inhibition of
180 12~13 mm that was comparable with the standard Kanamycin. The chloroform, petroleum
181 ether, and ethyl acetate fractions exhibited week activity with the zone of inhibition 6~9 mm.
182 In the case of antifungal activity test, the dia-ion resin adsorbed fractions showed the highest
183 antifungal activity with the zone inhibition of 15mm. From the results of cytotoxicity test, it
184 was observed that the chloroform and ethyl acetate fractions were found be the highest active
185 on 6, 12, 18, 24, and 30h of exposures in which almost all the nauplii were dead. From the
186 corresponding LD_{50} values, the chloroform and ethyl acetate fractions were found to be the
187 highest toxic of 75.019 and 65.553 ppm, respectively. It has been predicted that the *Abroma*
188 *augusta* leaves may be considered to be a potent cytotoxic agent for further advanced
189 research.
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Conflict of interest

193 We declare that we have no conflict of interest.
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249 **Table 1. Antimicrobial and antifungal activity of four different fractions such as**
250 **petroleum ether, chloroform, ethyl acetate and dia-ion resin adsorbed fractionate of *A.***
251 ***augusta*.**
252

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

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258 **Table 2. Cytotoxic activity of four different fractionates of *Abroma augusta* on *Artemia salina* nauplii after**
259 **6-30h exposure in which 30 nauplii were used.**
260

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

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264 **Table 3. LD_{50}, 95% confidence limits and regression equations of *Abroma augusta* extracts against *A.***
265 ***salina* nauplii.**
266

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

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