

Original Research Article

**Evaluation of cytotoxicity and antibacterial activities of
methanolic extract of *Antidesma bunius*(Linn.) (Family
Euphorbiaceae) leaf.**

ABSTRACT

Aims: The aim of this study was to find out the cytotoxicity and antibacterial activity of the methanolic extract of leaf of *A. bunius* and its ethyl acetate, N-hexane, dichloromethane partitionate.

Place and Duration of Study: The study was carried out in August 2017 in the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

Materials and Methods: Cytotoxicity activity was determined against brine shrimp nauplii by using the brine shrimp lethality bioassay. Vincristine Sulphate is used as standard or positive control. The antibacterial activity was evaluated using the disk diffusion technique. Here Kanamycin was used as standard.

Results: Crude methanolic extract and its different fractions demonstrated a significant cytotoxic activity. Especially, dichloromethane soluble fraction displayed considerable toxicity toward brine shrimps. The LC_{50} values of dichloromethane soluble fraction was 0.673 $\mu\text{g/ml}$, n-hexane soluble fraction was 0.867 $\mu\text{g/ml}$, ethyl acetate soluble fraction was 0.918 $\mu\text{g/ml}$ and crude methanolic extract was 1.031 $\mu\text{g/ml}$. The LC_{50} value of standard vincristine sulphate was 0.051 $\mu\text{g/ml}$. The n-hexane soluble fraction exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 0 mm to 19 mm. The maximum zone of inhibition produced by n-hexane soluble fraction was found to be 19 mm against *E.coli DH5 α* .

Conclusion: Observing the studies, it can be concluded that dichloromethane soluble fraction of *A. bunius* leaves possess good cytotoxic activity and n-hexane soluble fraction possess good antibacterial activity. Hence, further studies are suggested to identify the exact bioactive compounds that could be accounted for its pharmacological effects.

Key words: Antibacterial activity, Cytotoxicity activity, *A. bunius*, Ethyl acetate fraction, N-hexane fraction, Dichloromethane fraction, Methanolic extract.

INTRODUCTION

The plant kingdom comprises many species of plants containing substances of medicinal value, which are yet to be explored. Nowadays, various types of plants are constantly being screened for their possible medicinal value [1-3]. For thousands of years, plant, mineral and animal products were the main sources of drugs, the use of medicinal products with therapeutic properties is as ancient as

human civilization [4]. Nowadays people are being bombarded with thousands of unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution. Medicinal plants are plants that have a recognized medical use. They range from those used in the production of mainstream pharmaceutical products to plants used in natural medicine preparations. Natural medicine is one of the oldest forms of medical treatment in human history and could be considered one of the forerunners of the modern pharmaceutical trade. Plants that have medical uses can be found growing in many settings all over the world [5]. *Antidesma buni* (Family-Euphorbiaceae) is traditionally used as sudorific and in the treatment of snakebite, in Asia [6]. Also this plant contain a widespread of pharmacological activity including cytotoxicity, anti-diabetic, antioxidant, antiradical and pesticide agent etc. [7-14].

442. MATERIALS AND METHODS

45 **2.1 Plant material:** *Antidesma buni* fresh leaves were collected from Chittagong in the month of
46 August, 2016 and identified by an expert taxonomist. A voucher specimen was submitted to the
47 national herbarium, Mirpur, Dhaka, Bangladesh. Accession number: DACB 43490.

48 **2.1.1 Plant materials extraction and fractionation:** About 400g of dried and powders materials
49 obtained from the plant were soaked in 1.7 liter of methanol for about 14 days at room temperature
50 with occasional shaking. After 14 days, the solution were filtered using cotton filter and Whitman's
51 filter paper number 1. The filtrates were concentrated to afford solid masses by using a rotary
52 evaporator [15-16]. The concentrated methanolic extract leaves was fractionated by modified
53 Kupchan method [17] and the resultant fractions were dichloromethane, ethyl acetate, n-hexane and
54 aqueous soluble fractions.

55

563. **CYTOTOXICITY EVALUATION:** The brine shrimp lethality bioassay is widely used for probable
57 cytotoxic action or this technique was applied for the determination of cytotoxic activity of the plant
58 extracts [18-20]. 4mg of each of the extractives were dissolved in DMSO and solutions of various
59 concentrations such as 400 µg/ml to 0.781 µg/ml were obtained by serial dilution technique. Standard
60 Vincristine Sulfate was used as the positive control and DMSO was used as the control respectively
61 [21-23]. Nextten matured shrimps were taken to each of the experimental vials and the control vial.
62 The number of the nauplii that died after 24 hours was counted and the LC₅₀ was calculated from the
63 regression equation, obtained from the logarithm of sample concentration versus percentage mortality
64 of the shrimp nauplii.

654. **ANTIBACTERIAL TEST:** The antibacterial activity of the extractives was determined by disc
 66 diffusion technique [24-25] against thirteen bacteria. The bacterial strains used for the experiment
 67 were collected from the University of Jahangirnagar, Savar, Dhaka, Bangladesh. The extracts were
 68 dissolved separately in N-hexane, dichloromethane, ethyl acetate, and methanol as required and
 69 applied to sterile filter paper at 400 µg/disc and cautiously dried to evaporate the remaining solvent.
 70 Standard antibiotics, kanamycin (30 mg/disc) was used as positive control. All extract of the collected
 71 plants were tested against six Gram-positive and seven Gram negative bacteria (*Bacillus subtilis*,
 72 *Staphylococcus epidermis*, *Bacillus megaterium*, *Sarcinalutea*, *Staphylococcus aureus*, *Enterococcus*
 73 *faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella gs-B (N)*, *E.coli*
 74 *DH5 α*, *Proteus mirabilis*, *Vibrio cholera*). The antibacterial activities of the extracts were ascertained
 75 by measuring the respective zone of inhibition in millimeters.

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775. RESULTS AND DISCUSSION

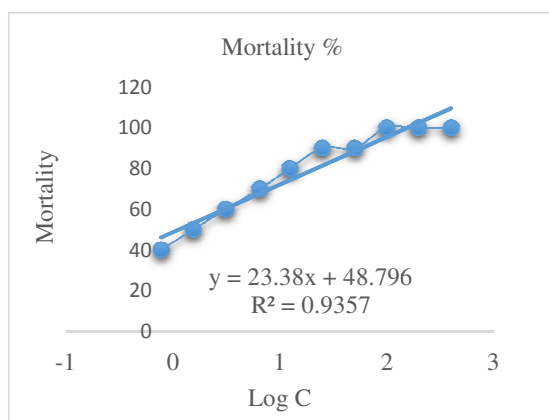
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79 In the present study, this article describes the cytotoxicity and antibacterial activities of plant *A.*
 80 *bunius* and this plant used in different diseases.

81

82 **5.1 Results of the cytotoxicity test:** Crude methanolic extract and n-hexane fraction, ethyl
 83 acetate fraction, dichloromethane fraction of the plant were tested. Brine shrimp lethality bioassay
 84 using the following the procedure of Meyer [18]. This method was applied for the determination of
 85 toxic property of the extractive. The LC₅₀ values for standard vincristine sulphate, methanolic extract,
 86 ethyl acetate fraction, n-hexane fraction, dichloromethane fraction were found to be 0.051 µg/ml,
 87 1.031 µg/ml, 0.918 µg/ml, 0.867 µg/ml, and 0.673 µg/ml respectively which indicates that the plant
 88 has potent cytotoxic effect.

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91 **Figure 1. Vincristine Sulphate**

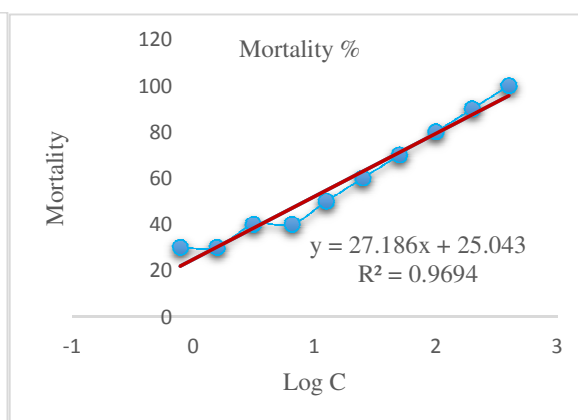


Figure 2. Methanolic extract of A. bunius

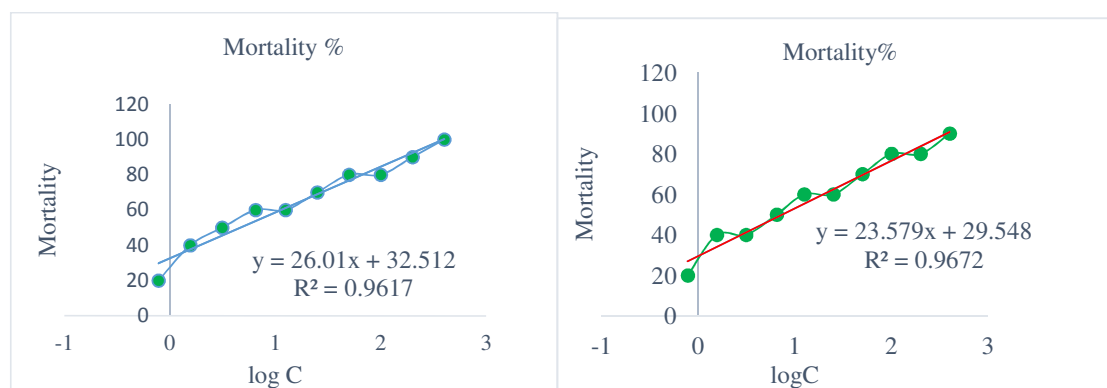


Figure 3. Dichloromethane fraction Figure 4. N-hexane fraction

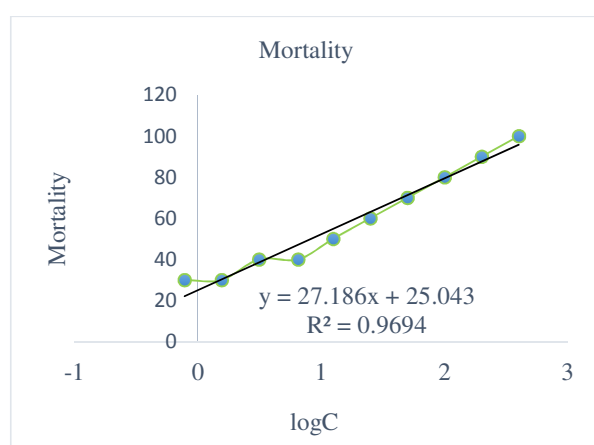


Figure 5. Ethyl acetate fraction

Figure 1, 2, 3, 4 and 5: Determination of LC_{50} values for standard Vincristine Sulphate and methanolic extract, dichloromethane fraction, n-hexane fraction, ethyl acetate fraction of leaves *A. bunius* from linear correlation between logarithms of concentration versus percentage of mortality.

5.1.1 Results of the antibacterial test: The methanolic extract of the leaves of *A. bunius* (ME) and its different partitionates- ethyl acetate (EA), n-hexane (NH), dichloromethane (DCM) soluble partitionate of the methanolic extract of whole plant were subjected to antibacterial screening with a concentration of 400 $\mu\text{g}/\text{disc}$ in every case. The results are given in the following table (Table 1). The n-hexane soluble fraction (NH) exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 0 mm to 19 mm. The maximum zone of inhibition produced by NH, which was found to be 19 mm against *E.coli DH5* α followed by 17 mm, 14 mm, 13 mm, 12 mm, 11 mm, and 10 mm against *Staphylococcus epidermis*, *Proteus mirabilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli* and *Sarcina lutea* respectively. The dichloromethane soluble fraction (DCM) exhibited some inhibition against microbial growth having zone of inhibition ranged from 0 mm to 17 mm. The maximum zone of inhibition produced by DCM was found to be 17 mm against *Salmonella typhi* and *Proteus mirabilis* followed by 16 mm, 15 mm, 14 mm, 12 mm and 10 mm against *Bacillus*

megaterium, *Bacillus subtilis*, *Staphylococcus epidermis*, *E.coli DH5* α , *Escherichia coli*. The methanolic extract soluble fraction (ME) exhibited some inhibition against microbial growth having zone of inhibition ranged from 0 mm to 17 mm. The maximum zone of inhibition produced by ME was found to be 17 mm against *Proteus mirabilis* followed by 11 mm, 10 mm against *Escherichia coli* and *Bacillus megaterium* respectively.

Table 1. Antibacterial activity of the leaves of *A. bunius*

Test microorganisms	Diameter of zone of inhibition (mm)				
	MESF	EASF	N-HSF	DCMSF	Kanamycin
Gram positive bacteria					
<i>Bacillus subtilis</i>	0	0	11	15	50
<i>Bacillus megaterium</i>	10	8	13	16	58
<i>Enterococcus faecalis</i>	0	0	19	0	42
<i>Staphylococcus aureus</i>	0	0	12	0	45
<i>Sarcinalutea</i>	0	0	10	0	47
<i>Staphylococcus epidermis</i>	0	0	17	14	41
Gram negative bacteria					
<i>Escherichia coli</i>	11	16	11	10	48
<i>E.coli DH5</i> α	0	0	19	12	44
<i>Pseudomonas aeruginosa</i>	0	0	0	0	45
<i>Salmonella typhi</i>	0	0	11	17	43
<i>Salmonella gs-B (N)</i>	0	0	0	0	39
<i>Proteus mirabilis</i>	17	12	14	17	56
<i>Vibrio cholerae</i>	0	0	0	0	32

5.1.1.1 Discussion: Plants are main source of potentially useful compounds for the development of new chemotherapeutic agents. The confirmation of antimicrobial activity against both Gram-positive and Gram-negative bacteria may be representative of the presence of a broad spectrum of antibiotic compounds [26-27]. In present study, we also demonstrated that the methanolic extract of *A. bunius* and its different fractions have highly potent antimicrobial activity. The n-hexane soluble fraction (NH) exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 0 mm to 19 mm. The maximum zone of inhibition produced by N-H was found to be 19 mm against *E.coliDH5* α . In the cytotoxic activities test by using the method brine shrimp (*Artemia salina*) lethality bioassay [18] where vincristine sulfate (with LC₅₀ of 0.051 μ g/ml) is used as standard, the crude methanolic extract and its different fractions showed considerably cytotoxic activity but dichloromethane fraction exhibited the highest activity with lowest LC₅₀ of 0.673 μ g/ml.

CONCLUSION: From this study, it was concluded that the *A. bunius* possesses antibacterial and cytotoxic activities. Therefore, we consider that the plant may be utilized for the advancement of traditional medicine. We would like to tell that further investigation of *A. bunius plant* is necessary for the development as a safer antibiotic and also as an anticancer agent.

ETHICAL APPROVAL

The protocol of the experiment was approved by the animal ethics committee of the Department of Pharmacy, Southeast University, Dhaka, Bangladesh. The animals care and health were maintained according to the guidelines of National Institutes of Health.

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ABBREVIATIONS

- DMSO: Dimethyl sulphoxide;
- LC₅₀: Lethal concentration 50;

208 ME: Methanolic extract;

209 EA: Ethanolic extract;

210 NH: N-hexane

211 DCM: Dichloromethane