

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

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ABSTRACT

Aims: The aim of this study was to find out the cytotoxicity and antibacterial activity of the methanolic extract of leaf of *Antidesma bunius* (Family Euphorbiaceae).

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 was used as a positive control. The antibacterial activity was evaluated using the disk diffusion technique. Kanamycin was used as standard.

Results: Crude methanolic extract and its different fractions demonstrated variable cytotoxic activity. Especially, ethyl acetate soluble fraction displayed considerable toxicity toward brine shrimps. The LC₅₀ value of ethyl acetate soluble fraction was 0.589µg/ml, dichloromethane soluble fraction was 0.737µg/ml, n-hexane soluble fraction was 0.894µg/ml and crude methanolic extract was 0.913µg/ml. The LC₅₀ value of standard vincristine sulphate was 0.545µg/ml. The n-hexane soluble fraction exhibited the highest inhibition against microbial growth especially against *E.coli* DH5α by having a zone of inhibition of 19mm.

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.

ABBREVIATIONS

DMSO: Dimethyl sulphoxide;

LC₅₀: Lethal concentration 50;

ME: Methanolic extract;

EA: Ethyl acetate extract;

NH: n-hexane extract

DCM: Dichloromethaneextract

1. 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 nursed with thousands of unhealthy products. The level of sensibility in front of diseases is very high. To cure these diseases, the use of medicinal plants can represent the best solution. Medicinal plants 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 bunius* (Family-Euphorbiaceae) is traditionally used as sudorific and in the treatment of snakebite, in Asia [6]. Decoction is used to promote perspiration in febrile condition; juice of the plant is useful in the treatment of insomnia. Fresh juice of the fruits is used in the manufacture of wine as an antioxidant. Roots and leaves are antihelminthic and also used in indigestion cough and stomachache. The seeds are used against round worms and threadworms, coughs, flatulence, intestinal colic and also used as pesticide [6]. Also this plant contain a widespread of pharmacological activity including cytotoxicity, anti-diabetic, antioxidant, antiradical and pesticide agent etc. [7-14]. In this study, we focused on the evaluation of cytotoxic and anti-bacterial activity of the methanolic extract of leaf of *A. bunius* and its ethyl acetate, n-hexane and dichloromethane partitionate.

2. MATERIALS AND METHODS

2.1.1 Plant material: *Antidesma buniis* fresh leaves were collected from Chittagong in the month of August, 2017 and identified by an expert taxonomist. A voucher specimen was submitted to the national herbarium, Mirpur, Dhaka, Bangladesh. Accession number: DACB 43490.

2.1.2 Plant materials extraction and fractionation: About 400g of dried and powdered plant material was soaked in 1.7 liter of methanol in an amber glass container for about 14 days at room temperature with occasional shaking. After 14 days, the solution were filtered using cotton filter and Whitman's filter paper number 1. The filtrate was concentrated to solid mass by using a rotary evaporator [15-16]. The concentrated methanolic extract leaves were fractionated by modified Kupchan method [17] and the resultant fractions were dichloromethane, ethyl acetate, n-hexane soluble fractions.

2.2 Cytotoxicity evaluation: The brine shrimp lethality bioassay is widely used for determination of cytotoxic activity of the plant extracts [18-20]. 4mg of each of the extracts were dissolved in DMSO and solutions of various concentrations such as 400 µg/ml to 0.781µg/ml were obtained by serial dilution technique. Vincristine Sulfate was used as the positive control and DMSO was used as the negative control respectively [21-23]. Next ten matured shrimps were taken to each of the experimental vials and the control vial. The number of the nauplii that died after 24 hours was counted and the LC₅₀ was calculated from the regression equation, obtained from the logarithm of sample concentration versus percentage mortality of the shrimp nauplii.

2.3 Antibacterial test: The antibacterial activity of the extracts was determined by disc diffusion technique [24-25] against thirteen bacteria. The bacterial strains used for the experiment were collected from the University of Jahangirnagar, Savar, Dhaka, Bangladesh. Each of the four extracts was again dissolved in respective solvents to be applied on sterile filter paper at 400µg/disc and cautiously dried to evaporate the remaining solvent. Standard antibiotic, kanamycin (30 mg/disc) was used as a positive control. All extracts were tested against six Gram-positive and seven Gram negative bacteria (*Bacillus subtilis*, *Staphylococcus epidermis*, *Bacillus megaterium*, *Sarcinalutea*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella gs-B (N)*, *E.coli DH5 α* , *Proteus mirabilis*, *Vibrio cholera*). The antibacterial activities of the extracts were ascertained by measuring the respective zone of inhibition in millimeters.

3. RESULTS AND DISCUSSION

3.1 Cytotoxicity test: Crude methanolic extract, n-hexane fraction, ethyl acetate fraction, dichloromethane fraction of the plant were tested. Brine shrimp lethality bioassay was tested using the following the procedure of Meyer [18]. This method was applied for the determination of toxic property of the extractives. The LC₅₀ values for standard vincristine sulphate, methanolic extract, ethyl acetate fraction, n-hexane fraction, dichloromethane fraction were found to be 0.545µg/ml [Fig 1].

0.913 µg/ml [Fig 2], 0.589µg/ml [Fig 5], 0.894µg/ml [Fig 4], and 0.737µg/ml [Fig 3] respectively which indicates that the plant has potent cytotoxic effect.

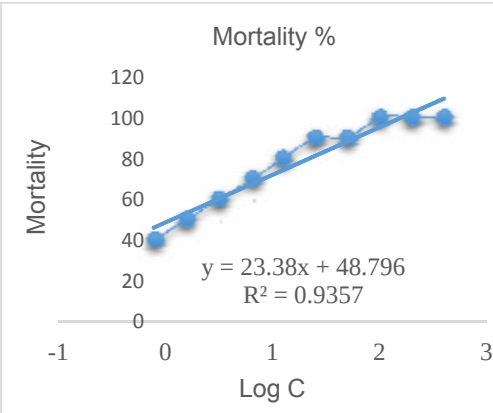


Figure 1. Vincristine Sulphate

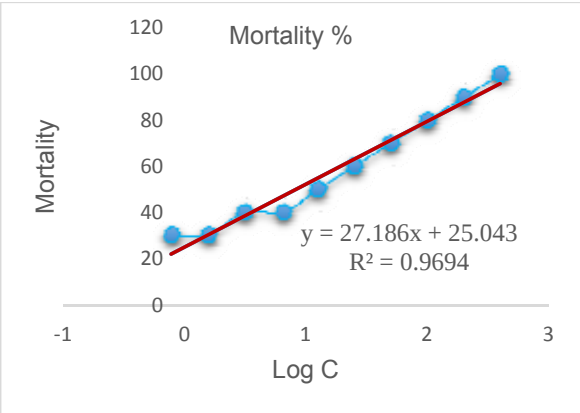


Figure 2. Methanolic extract of *A. bunius*

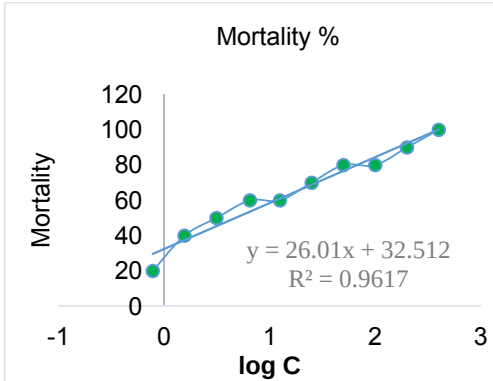


Figure 3. Dichloromethane fraction

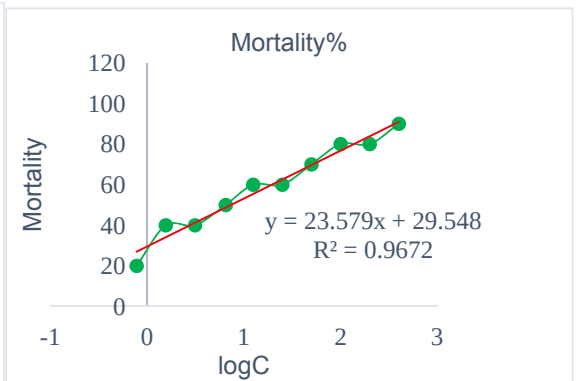


Figure 4. N-hexane fraction

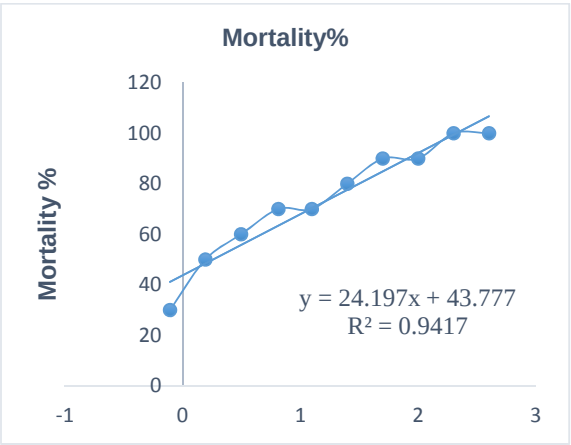


Figure 5. Ethyl acetate fraction

Figure 1, 2, 3,4 and 5: Determination of LC₅₀ values for standard Vincristine Sulphate, methanolic extract, dichloromethane fraction, n-hexane fraction, ethyl acetate fraction of leaves *A. buni* from linear correlation between logarithms of concentration versus percentage of mortality.

3.2 Antibacterial test: The methanolic extract of the leaves of *A. buni* (ME) and its different partitionates- ethyl acetate (EA), n-hexane (NH), dichloromethane (DCM) soluble fractions were subjected to antibacterial screening with a concentration of 400 µg/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. 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 *Sarcinalutea* respectively. The dichloromethane soluble fraction (DCM) exhibited some inhibition against microbial growth. 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 and 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. buni*

Test microorganisms	Diameter of zone of inhibition (mm)				
	ME	EA	NH*	DCM	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

Here, NH exhibited the highest inhibition against microbial growth.

3.1.1 Discussion: Plants are the main source of potentially useful compounds for the development of new chemotherapeutic agents. In our study, we demonstrated for the first time about the antibacterial activity of *A. bunius*. The Results of our study on *A. bunius* agree with the results of earlier studies conducted on the same plant which showed the cytotoxic activity of *A. bunius* [10]. 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 and the maximum zone of inhibition produced by n-hexane was found to be 19 mm against *E.coli DH5* α . In the cytotoxic activity test by using the method brine shrimp (*Artemia salina*) lethality bioassay [18] where vincristine sulfate (with LC_{50} of 0.545 μ g/ml) is used as standard, the crude methanolic extract and its different fractions showed considerably cytotoxic activity but ethyl acetate fraction exhibited the highest activity with lowest LC_{50} of 0.589 μ g/ml.

4. 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.

Consent: NA

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