

Evaluation of Cytotoxicity and Antibacterial Activities of Methanolic Extract of *Antidesma bunius* (Linn.) (Family Euphorbiaceae) Leaf

**Sonia Zaman^{1*}, Md. Shariful Islam¹, Sabiha Ferdowsy Koly¹, Tahmid Faisal¹
and Kamruzzaman Rakib²**

¹Department of Pharmacy, Southeast University, Dhaka, Bangladesh.
²Department of Pharmacy, North South University, Dhaka, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author SZ designed the study, wrote the protocol, managed the analyses of the study and made necessary corrections after peer review process. Author MSI carried out the laboratory tests and prepared the draft of the manuscript and prepared the plant extracts and managed the literature searches. Author SFK performed the graphical evaluations and checked the manuscript. Authors TF and KR reviewed the scientific contents of the manuscript. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2018/40004

Editor(s):

(1) Julius Olugbenga Soyinka, Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

Reviewers:

(1) Ilham Zahir, Sultan Moulay Slimane University, Morocco.

(2) Emelia Oppong Bekoe, University of Ghana, College of Health Sciences, Ghana.

Complete Peer review History: <http://www.sciencedomain.org/review-history/23639>

Original Research Article

Received 4th January 2018

Accepted 10th March 2018

Published 14th March 2018

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 ethyl acetate 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.

Keywords: 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 : Dichloromethane extract

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 medicinal 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 medicinal 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 contains a widespread 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 antibacterial 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 Plant Material

Antidesma bunius 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.

2.2 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 was 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.

2.3 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.

2.4 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*, *Sarcina lutea*, *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.

3.2 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 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 *Sarcina lutea* 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, 12mm 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.

3.3 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 LC₅₀ 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 LC₅₀ of 0.589 μ g/ml.

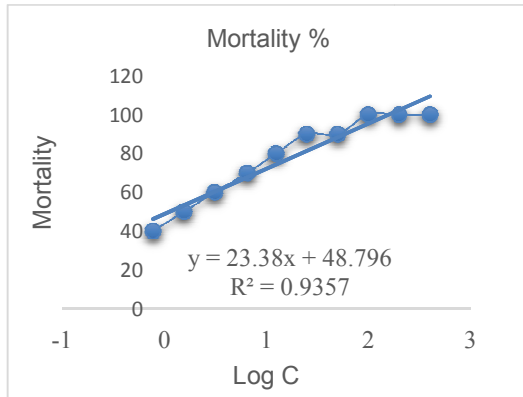


Fig. 1. Vincristine Sulphate

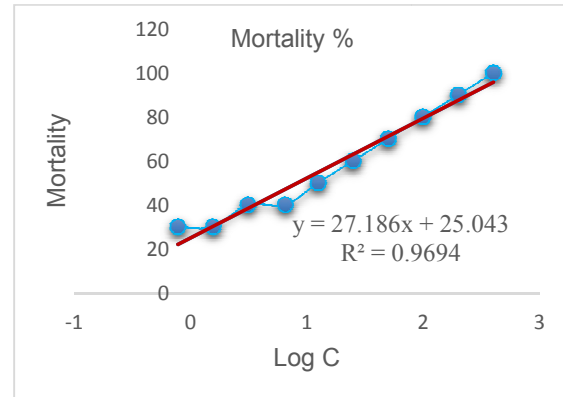


Fig. 2. Methanolic extract of *A. bunius*

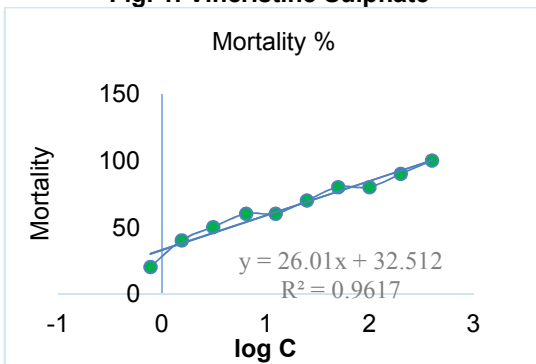


Fig. 3. Dichloromethane fraction

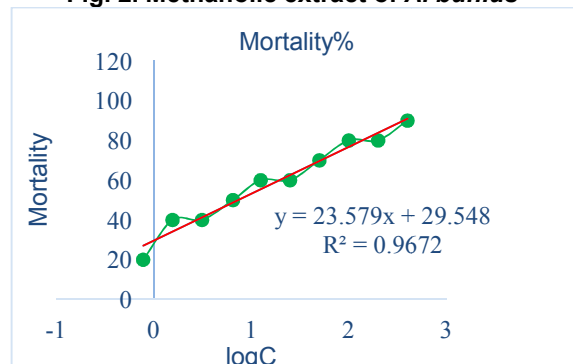


Fig. 4. N-hexane fraction

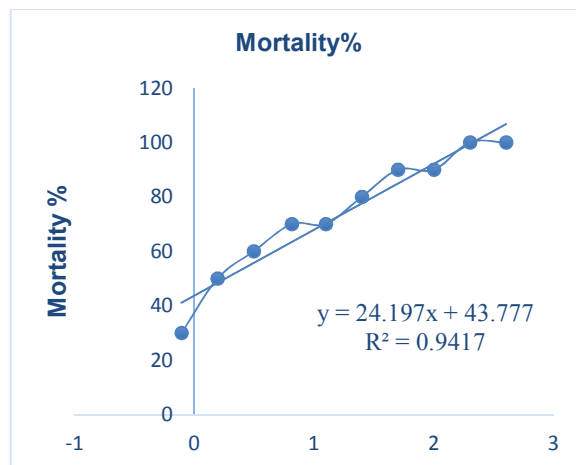


Fig. 5. Ethyl acetate fraction

Fig. 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. bunius* from linear correlation between logarithms of concentration versus percentage of mortality

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

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>Sarcina lutea</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 α</i>	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.

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.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Pharmacy, Southeast University, Bangladesh for their support during the research and also thankful to Jahangirnagar University, Bangladesh for supplying the test microorganisms.

CONSENT

It is not applicable.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rahman MS, Rahman MZ, Wahab MA, Chowdhury R, Rashid MA. Antimicrobial activity of some indigenous plants of Bangladesh. Dhaka University Journal of Pharmaceutical Sciences. 2008;7(1):23-6.
2. Kawsar SM, Huq E, Nahar N. Cytotoxicity assessment of the aerial parts of *Macrotyloma uniflorum* Linn. International Journal of Pharmacology. 2008;4(4):297-300.
3. Bulbul IJ, Nahar L, Haque M. Antibacterial, cytotoxic and antioxidant activity of chloroform, n-hexane and ethyl acetate extract of plant *Cocciniacordifolia*. AgricBiol JN Am. 2011;2(4):713-9.
4. De Pasquale A. Pharmacognosy: The oldest modern science. Journal of ethnopharmacology. 1984;11(1):1-6.
5. Kopp G, Lean JL. A new, lower value of total solar irradiance: Evidence and climate

- significance. Geophysical Research Letters. 2011;16:38(1).
6. Lim TK. *Antidesma bunius*. In Edible Medicinal and Non-Medicinal Plants. Springer Netherlands. 2012;220-224.
 7. Elya B, Malik A, Mahanani PI, Loranza B. Antidiabetic activity test by inhibition of α -Glucosidase and phytochemical screening from the most active fraction of Buni (*Antidesma bunius* L.) stem barks and leaves.
 8. Belina-Aldemita MD, Sabularse VC, Dizon EI, Hurtada WA, Torio MA. Antioxidant properties of bignay [*Antidesma bunius* (L.) Spreng.] Wine at different stages of processing. The Philippine Agricultural Scientist. 2013;27:96(3).
 9. Butkhup L, Samappito S. Changes in physico-chemical properties, polyphenol compounds and antiradical activity during development and ripening of maoluang (*Antidesma bunius* L. Spreng) fruits. Journal of Fruit and Ornamental Plant Research. 2011;19(1):85-99.
 10. Jose Rene L. Micor, Custer C. Deocariz and Elmer-Rico E. Mojica. Biological Activity of Bignay [*Antidesma bunius* (L.) Spreng] Crude Extract in *Artemiasalina*. Journal of Medical Sciences. 2005;5(3): 195-198.
 11. Lawag IL, Aguinaldo AM, Naheed S, Mosihuzzaman M. α -Glucosidase inhibitory activity of selected Philippine plants. Journal of ethnopharmacology. 2012;144(1):217-9.
 12. Elya B, Malik A, Mahanani PI, Loranza B. Antidiabetic activity test by inhibition of α -Glucosidase and phytochemical screening from the most active fraction of Buni(*Antidesma bunius* L.) stem barks and leaves. International Journal of Pharma Tech Research. 2012;4(4):1667-1671.
 13. El-Tantawy WH, Soliman ND, El-Naggar D, Shafei A. Investigation of antidiabetic action of *Antidesma bunius* extract in type 1 diabetes. Archives of physiology and biochemistry. 2015;121(3):116-22.
 14. Belmi RM, Giron J, Tansengco ML. Antidesmabunius (bignay) fruit extract as an organic pesticide against *Epilachna spp.* Journal of Asian Scientific Research. 2014;4(7):320.
 15. Jeery GH, Basse J, Mendham J, Denney RC. Vogel's Textbook of Quantitative Chemical Analysis. 5th ed. England: Longman Group UK Ltd. 2000;161.
 16. Haque M, Jahan T, Rashid MA. Antibacterial and cytotoxic activities of *Alocasia fornicata* (Roxb.). International Journal of Nutrition, Pharmacology, Neurological Diseases. 2014;4(5):29.
 17. VanWagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC, Swithenbank C. Ulosantoin, a potent insecticide from the sponge *Ulosaruetzleri*. The Journal of Organic Chemistry. 1993;58(2):335-7.
 18. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Plantamedica. 1982;45(05):31-4.
 19. Zhao G, Hui Y, Rupprecht JK, McLaughlin JL, Wood KV. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asiminatriloba*. Journal of Natural Products. 1992;55(3):347-56.
 20. Persoone G. Proceeding of the international symposium on brine shrimp; Artemiasalina; University press; Written; Belgium. 1988;1-3.
 21. Rahman MS, Begum B, Chowdhury R, Rahman KM, Rashid MA. Preliminary cytotoxicity screening of some medicinal plants of Bangladesh. Dhaka University Journal of Pharmaceutical Sciences. 2008;7(1):47-52.
 22. Khan A, Rahman M, Islam MS. Antibacterial, antifungal and cytotoxic activities of amblyone isolated from *Amorphophallus campanulatus*. Indian Journal of Pharmacology. 2008;40(1):41.
 23. Sarkar S, Farooque MA. Antimicrobial and cytotoxic activities of 2-aminobenzoic acid and 2-aminophenol and their coordination complexes with Magnesium (Mg-II). Pakistan Journal of Biological Sciences. 2004;7(1):75-11.
 24. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. Journal of ethnopharmacology. 1988;23(2-3):127-49.
 25. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966;45(4): 493.

26. Cichewicz RH, Thorpe PA. The antimicrobial properties of chile peppers (*Capsicum species*) and their uses in Mayan medicine. *Journal of Ethnopharmacology*. 1996;52(2):61-70.
27. Srinivasan D, Nathan S, Suresh T, Perumalsamy PL. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology*. 2001;74(3):217-20.

© 2018 Zaman et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history/23639>