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Kazi Nuruddin Al Masud
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Mahbuba Mohoshina Runa
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Rafiqul Hasan Khan
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Monika Nasrin Munni
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Nura Ahmed
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Amina Ferdous Chowdhury
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Shakera Islam Keya
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Correspondence
Kazi Nuruddin Al Masud
Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Study of cytotoxic and thrombolytic activity of *Baccaurea ramiflora* in different extracts

Kazi Nuruddin Al Masud, Mahbuba Mohoshina Runa, Rafiqul Hasan Khan, Monika Nasrin Munni, Nura Ahmed, Amina Ferdous Chowdhury and Shakera Islam Keya

Abstract
This study was conducted to evaluate the thrombolytic activity and cytotoxic activity of *Baccaurea ramiflora*. The plant belongs to the Euphorbiaceae family and locally used for the treatment of tumors, boils and buboes. The aqueous, methanolic, ethanolic, ethyl acetate, chloroform and their cyclohexane soluble partitioning materials extracts of *Baccaurea ramiflora* was used to evaluate the thrombolytic property and the cytotoxic activity. Streptokinase was used as standard for the thrombolytic activity evaluation and the highest% of clot lysis was found in the the aqueous soluble fraction, which was 88.21. Vincristine sulphate was used as standard for cytotoxic activity and the methanolic extracts provides comparatively best IC50 value which was 25.19 µg/ml.

Keywords: *Baccaurea ramiflora*, cytotoxic activity, thrombolytic activity, different extracts

Introduction
From the beginning of the modern era traditional medicinal plants play a significant part in maintaining healthcare system in most countries. These medicinal plants are the great sources for therapeutically active compounds. In addition, therapeutic active compounds have been used for treating various type of diseases [1]. Medicinal plants provide different types of pharmacological and biological properties [2]. Medicinal plants like *Achillea wilhelmsii* provides antimicrobial and antioxidant activity [3]. Different parts of plant *Nitraria schoberi* L. shows antioxidant properties [4]. Plant extracts of *Piper* genus provide some important properties like anti-dermatophyte, anti-fusarium and cytotoxic activity [5]. Some other plants showed antifungal, antiparasitic activity [6, 7].
Baccaurea ramiflora C.B. Clarke is a plant belongs to Euphorbiaceae family. Euphorbiaceae are a large family comprises 150 genera and 3000 species [8]. Some species have been used in traditional medicine, mainly against fever, cough, colds, snakebite, pains, and infectious and inflammatory diseases. Euphorbiaceae species chemical compounds like flavonoids, steroids, phenolic glucosides, simple phenolics, quinones, xanthones and some compounds showed antimicrobial, anti-inflamatory, antioxidant, and antitumor properties [9]. Common name of the *Baccaurea ramiflora* is "Thread-like Boeica". This plant is located tropic areas like Bangladesh, India etc. Medicinal plants are great source of cytotoxic activity which means capability of killing cancer cells [11]. However, leaf, stem and bark almost every part of the plant can shows pharmacological properties which will be very helpful to deal with various disease conditions [12]. Moreover, thrombolytic activity also very important property where compounds able to destroy blood clots [10]. So, to make some contribution to medical science the current study was take on to discover cytotoxic and thrombolytic activities of *Baccaurea ramiflora* plant.

Materials and Methods
Collection of the seeds: *Baccaurea ramiflora* plant was selected during this research work for investigating different pharmacological activities. The fresh leaves of the plant were collected from Kumilla during June July 2017. The plant sample was then sent to National Herbarium of Bangladesh (NHB), Mirpur for identification where they identified the plant and provided a verification number 46964.

Extraction of seed material: The plants were dried under sun for a few days and finally oven dried to remove all the moisture content.

Then the seeds were crushed to coarse consistency. The coarse grains were extracted in a decreasing polarity order. The coarse plant material (900g) was taken and soaked with 1500 ml of methanol for 3 consecutive days at 25 ºC. The extract was filtered and the filtrate was kept for further extraction. In the same manner the filtrate was soaked in different solvents by polarity decreasing order.
Aqueous > Methanol > Ethanol > Ethyl Acetate > Chloroform > Cyclohexane
For every case, the extract was preserved and solvent evaporation was done by using rotary evaporator. Finally, all the extracts of *Baccaurea ramiflora* was kept under laminar airflow for protecting it from any type of contamination.

Drugs and chemicals: Most of the chemicals used in this research were purchased from well-known vendors and some of them were received as gifts from different leading pharmaceutical companies in the country. First of all, lyophilized Altepase (Streptokinase) was obtained from Sanofi Bangladesh Ltd whereas Beacon Pharmaceuticals Limited, Bangladesh provided Vincristine sulphate. Methanol was purchased from a reputed chemicals supplier known as Active Fine Chemicals Limited, Bangladesh. Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific, UK.

Cytotoxic activity: The cytotoxic potentialities of all the extracts of *Baccaurea ramiflora* were performed on brine shrimp nauplii by following a method described by Meyer which is also a valid method to determine other pharmacological activities, e.g. antimicrobial property, antiviral property, pesticides and tumor-resistant property, etc. [13. 14]. At the very beginning, 38gm of salt was mixed thoroughly in one liter of water in a container to prepare artificial sea water which is simply 3.8% NaCl solution [15]. Collected egg of brine shrimps (*Artemia salina*) were hatched in the artificial sea water which were afterward used in the Brine Shrimp Lethality Bioassay test. The test sample were prepared by dissolving 4mg of crude extract in DMSO and serial dilution technique was followed to prepared varied concentration of sample. The test solutions were then introduced into the labelled vials containing 10 live brine shrimp and incubate d for 24 hours. When the incubation for 24 hours were done, all the vials were examined using magnifying glass to count the survived brine shrimp nauplii and the data were used to calculate the cytotoxicity potential of the plant *Baccaurea ramiflora.*

Thrombolytic activity:
Standard drug and test solution preparation: Lyophilized Altepase, 30,000 IU Streptokinase (Streptase®, by Sanofi-Aventis) vials were used as standard drug to compare the thrombolytic property of *Baccaurea ramiflora.* On the other hand, test sample were prepared by dissolving crude extracts in methanol with vigorous shaking and kept overnight to decant to release the supernatants which were further reformed into 2mg/ml. 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml concentration by following serial dilution method. Those methanolic supernatants were formerly filtered through 0.22microne syringe filter to get the clear test solution which were used to observe the thrombolytic activity of the plant.

Thrombolytic analysis: Prasad et.al illustrated a method which was followed as a standard method to observe the thrombolytic activity of the plant [16]. Blood sample from healthy persons were collected who do not have any history of oral contraceptives, anticoagulants, and drug history in recent past. 5 ml of blood were then dispersed into different micro centrifuge tubes (0.5 ml/tube) which were then incubated at room temperature for 45 minutes. As a result, blood cells clotted at the bottom while serum floated at the top of the tubes which were then removed from the tube without irrupting the clots at the bottom. Weights of the clots were measured and test samples were introduced into the tubes congaing clots with proper labeling. Simultaneously, streptokinase as a positive control and distilled water as negative control were also added in separate tubes. All the tubes were then incubated for 90 minutes at 37º C temperature. When the incubation was complete, fluid in each tube was taken away and weight variation of clots between after and before of lysis was calculated and represented as the percentage of lysis.

wt. of tube and clot – wt. of tube tube = wt. of clot
wt. of tube and clot – weight after lysis = wt. of lysis

$\frac{wt.of\ lysis}{wt.of\ clot}$ x 100 =% of lysis

Statistical Analysis: Every analysis was performed in triplicate under strict aseptic conditions to ensure consistency of all findings. For each extract triplicate data was taken and the final data was taken by the triplicate data's mean ± SD (Standard Deviation), which was analyzed by Microsoft excel.

Results and Discussion

Cytotoxic activity

Brine shrimp lethality bioassay: Lethal Concentration (LC50) is a standard measure of the toxicity of the substance that kills half of the test nauplii at a specific time which refers to the presence of cytotoxicity activity.

Table 1: LC50 values of the test samples of whole plants of *Baccaurea ramiflora*

Sample Name	Regression line	R2	LC50 (µg/ml)
VS	y = 30.799x + 60.653	0.973	0.47
WE	y = 0.0867x + 29.341	0.3537	268.28
ME	y = 0.1417x + 47.848	0.5116	25.19
EE	y = 0.1275x + 40.266	0.4511	98.35
AE	y = 0.1014x + 40.874	0.7292	94
CHE	y = 0.161x + 36.911	0.6875	83.29
CYE	y = 0.1267x + 29.344	0.5234	263.03

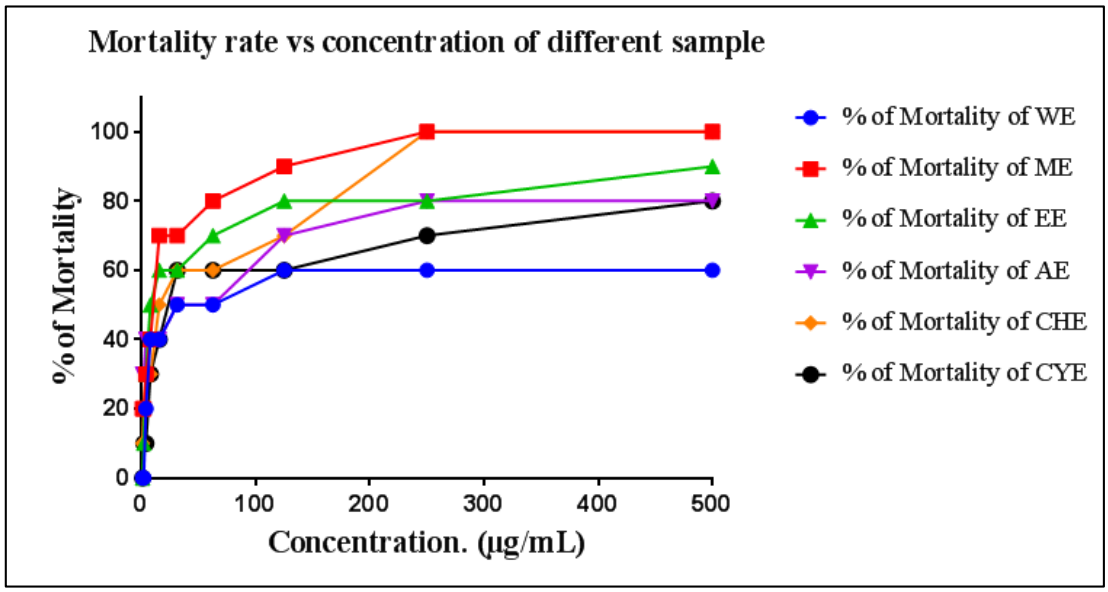

Fig 1: Graphical representation of% of mortality and concentrations of different sample.

In this study, vincristine sulphate used as a standard showed that the LC50 value was 0.45 µg / ml. compared to the standard among extractive ME, it showed the highest lethality with a value of 15.19 µg / ml. The LC50 values of WE, EE, AE, CHE and CYE were respectively 238.28 µg / ml, 76.87 µg / ml, 90 µg / ml, 81.29 µg / ml and 163.03 µg / ml (Table 4). After this analysis, it was clarified that the plant *Baccaurea ramiflora* has a cytotoxic activity. The graphic representation showed the relationship between the percentages of morality and the different concentration samples where the percentage of mortality increases with concentration. (Figure 2). Each sample regression line provided in Table 1. Therefore, this plant can be used as a cytotoxic agent with adequate purification and isolation.

Thrombolytic activity

To identify blood thinning medications from plant source different extractives of *Baccaurea ramiflora* were studied for thrombolytic activity. All the results were displayed in the table 2.

Table 2:% of clot lysis of different extract and standard

Sample	% of clot lysis
Blank	5.076
Aqueous	88.21
Methanol	63.44
Ethanol	51.24
Acetone	49.21
Chloroform	33.25
Cyclo-Hexane	25.21
Pet Ether	23.15
Streptokinase	78.98

The expansion of 100 µl of Streptokinase, a positive control (30,000 i.u. units), coagulation and subsequent incubation for one and a half hours at 37 ° C indicated a clot lysis of 66.77%. On the other hand, purified water used as negative control which showed insignificant rate of clot lysis 5.07%. It was found that the average distinction in the clot lysis rate between positive and negative control is exceptionally critical. In this study, aqueous extractive showed very high and surprising thrombolytic activity 84.91%. Moreover, other extractive like methanol, ethanol, acetone, chloroform, cyclo-hexane and pet ether showed clot lysis respectively 62.29%, 53.28%, 46.27%, 38.71%, 29.07% and 22.43%. From this experiment, it can be said that some of the *Baccaurea ramiflora* extracts showed gentle to direct lysis of the clot. Therefore, this plant can be used as some thrombolytic agents with its best pharmaceutical possibilities.

Conclusion

The lethality assay of the brine shrimp indicated that there was significant cytotoxic activity in the different extractives of the *Baccaurea ramiflora* plant. Among them methanol extract showed the most potent cytotoxic property. Moreover, this plant showed significant level of thrombolytic activity so it can be a good source of anti-thrombolytic agents. All the experiments performed in this study are based on crude extracts and are considered preliminary and a more sophisticated investigation is needed to reach a concrete conclusion on the results of this study.

Reference

1. Kirtikar KR, Basu BD. Indian Medicinal Plants. International Book Distributors, Allahabad. 1996; 3:2247.
2. Akter R, Uddin SJ, Grice ID, Tiralongo E. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. Journal of Natural Medicines. 2014; 68:246-252.
3. Alfatemi SMH, Rad JS, Rad MS, Mohsenzadeh S, da Silva JAT. Chemical composition, antioxidant activity and *in vitro* antibacterial activity of *Achillea wilhelmsii* C. Koch essential oil on methicillin-susceptible and methicillin-resistant Staphylococcus aureus spp. 3 Biotech. 2015; 5:39-44.
4. Sharifi Rad J, Hoseini-Alfatemi SM, Sharifi Rad M, Iriti M. Free radical scavenging and antioxidant activities of different parts of *Nitraria schoberi* L. Journal of Biologically Active Products from Nature. 2014; 4:44-51.
5. Tangarife-Castaño V, Correa-Royero JB, Roa-Linares VC, Pino-Benitez N, Betancur-Galvis LA, Durándcd DC *et al*. Anti-dermatophyte, anti-Fusarium and cytotoxic activity of essential oils and plant extracts of Piper genus. Journal of Essential Oil Research. 2014; 26:221-227.
6. Bhagwat MK, Datar AG. Antifungal activity of herbal extracts against plant pathogenic fungi. Archives of

Phytopathology and Plant Protection. 2014; 47:959-965.
7. Horn D, Duraisingh MT. Antiparasitic chemotherapy: From genomes to mechanisms. Annual Review of Pharmacology and Toxicology. 2014; 54:71-94.
8. "The Plant List - A Working List for All Plant Species." Home - The Plant List, www.theplantlist.org/browse/A/Gesneriaceae/Boeica/
9. Verdan, Maria Helena, and Maria Elida Alves Stefanello. "ChemInform Abstract: Secondary Metabolites and Biological Properties of Gesneriaceae Species." ChemInform, 2013; 44:12 doi:10.1002/chin.201312269.
10. Bekker J, Ploem S, Jong KP. Early Hepatic Artery Thrombosis after Liver Transplantation: A Systematic Review of the Incidence, Outcome and Risk Factors. American Journal of Transplantation. 2009; 9(4):746-757.
11. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. International Journal of Applied Science and Engineering. 2005; 3:125-134.
12. Omale, James, Okafor, Polycarp Nnacheta. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. African Journal of Biotechnology. 2008; 7(17):3129-3133.
13. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D, McLaughlin J. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Planta Medica, 1982; 45(5):31-34. https://doi.org/10.1055/s-2007- 971236
14. McLaughlin JL, Rogers LL, Anderson JE. The Use of Biological Assays to evaluate Botanicals. Drug Information Journal. 1998; 32(2):513-524. https://doi.org/10.1177/009286159803200223
15. Ali R, Marjan H, Jannatul FR, Hasanuzzaman M, Islam M. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna Journal of Phytomedicin. 2014; 4(6):430-6.
16. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. Thromb J. 2006; 4(14):1-4.