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BRINE SHRIMP LETHALITY BIOASSAY, THROMBOLYTIC AND ANTIBACTERIAL ACTIVITIES OF METHANOL EXTRACT OF *BOEHMERIA PLATYPHYLLA* D DON LEAVES

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ABSTRACT

Extract from the leaves of *Boehmeria platyphylla* D Don were screened for their brine shrimp lethality bioassay**,** thrombolytic and antimicrobial activities. The cytotoxicity was surveyed with the brine shrimp lethality bioassay and thrombolytic impact with human blood. The brine shrimp lethality bioassay was utilized to assess cytotoxicity (LC_{50} = 75.26 µg/ml) contrasted with Vincristine sulfate (LC_{50} = 0.74 µg/ml). It was also assessed as thrombolytic activity when contrasted with streptokinase. It has significant thrombolytic movement (73.17±2.08%) contrasted with standard streptokinase (81.32±1.46%). The extract indicated zone of inhibition against Gram positive bacteria (*Bacillus subtilis* and *Bacillus cereus*) and Gram negative bacteria (*Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli*, *Pseudomonas aeruginosa*) at 1000 µg/disc. Gram negative bacteria *Staphylococcus aureus* demonstrated no action against *B. platyphylla* leaves extract at both doses. Relative percentage inhibition of the extract against each bacterium also calculated. These results indicated that *B. platyphylla* have favorable thrombolytic, cytotoxic and antibacterial effects and capacities of *B. platyphylla* extract to be processed for pharmaceutical use.

Key word: *Boehmeria platyphylla*, Thrombolytic, cytotoxic, antibacterial

INTRODUCTION

Thrombosis is the arrangement of blood coagulation inside a vein, impeding the stream of blood through the circulatory framework. At the point when a vein is harmed, the body utilizes platelets (thrombocytes) and fibrin to shape a blood coagulation to avoid blood misfortune. Notwithstanding when a vein is not harmed, blood clumps may shape in the body under specific conditions [1, 2]. In the patient admitted to doctor's facility, thrombosis is a noteworthy reason for intricacies and once in a while demise. In UK, for occasion, the Parliamentary Health Select Committee heard in 2005 that the yearly rate of death because of thrombosis was 25,000, with no less than half of these being doctor's facility obtained [3]. Streptokinase (SK) has a place with a gathering of pharmaceuticals known as fibrinolytics, and edifices of streptokinase with human plasminogen can hydrolytically actuate other unbound plasminogen by initiating through bond cleavage to deliver plasmin. SK is utilized as a viable and cheap thrombolysis solution now and again of myocardial dead tissue (heart assault) [4] and pulmonary embolism [5]. Regular items have served as a hotspot for tumor chemotherapy. The brine shrimp lethality bioassay

has routinely been utilized as a part of the essential screening of the rough concentrates to survey the poisonous quality towards saline solution shrimp. The bioassay has a decent connection with cytotoxic movement in some human strong tumors and in addition pesticidal action [6]. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant [7][8, 9]. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality [10]. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs [11, 12].

Boehmeria platyphylla D Don is a monoecious or dioecious, 1-1.5 (-3) m tall, shrub with 4-angled, glabrescent twigs. Leaves mostly opposite, with 2.5-20 cm long petiole; lamina 3-costate, broadly ovate to orbiculate, 6-22 cm long, 5-15 cm broad, sparsely appressed hairy, scabrous, dentate, somewhat cuneate, truncate or subcordate at the base, acuminate; stipules triangular-lanceolate, 8-12 mm long. Cymose clusters of flowers arranged on axillary, drooping, up to 30 cm long spikes. Flowers white, tetramerous; bracts lanceolate, 3-4 mm long. Sepals c. 1 mm long, pubescent, acute. Stamens exserted. Style long exserted. Achenes pale brown, c.l mm long, beaked, glossy. *B. platyphylla* is a species of plant in the Urticaceae family. The Urticaceae are subject to many bacterial, viral, fungal, and nematode parasite diseases [13][14] .
The purpose of the present study focuses on the scientific investigation of brine shrimp lethality bioassay, thrombolytic and antibacterial activity of *Boehmeria platyphylla* (*B. platyphylla*) leaves.

MATERIAL AND METHOD

Plant material: Fresh leaves of *B. platyphylla* were collected from Bandarban, Chittagong, Bangladesh in the month of March 2015. It was authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Preparation of Extract: The leaves were dried for a period of 10 days under shade and ground. The ground leaves (450 gm) were soaked in sufficient amount of ethanol for one week at room temperature with occasional shaking and stirring then the whole mixture was filtered and the filtrate thus obtained was concentrated using a water bath to get a viscous mass. The viscous mass was kept at room temperature under a ceiling fan to get a dried extract (yield value, 5.3%). The extract prepared was for pharmacological screening.

Chemicals and equipment: To the commercially available lyophilized streptokinase (SK) vial (Square Pharmaceuticals Ltd. Dimethyl sulfoxide) of 1500000 I.U., 5mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 μL (30,000 I.U.) was used for *in vitro* thrombolysis. Methanol purchased from Merck (Germany). Dimethyl sulfoxide (DMSO) and Vincristine sulfate (2mg/vial; Techno Drugs Limited Bangladesh). Kanamycin (30µg/disc, Oxoid, England) was used as a standard antibiotic disc.

Brine Shrimp Lethality Bioassay: Brine shrimp lethality bioassay was carried out with the method as described by Meyer *et al.* [15, 16] to investigate the cytotoxicity of methanol extract of *B. platyphylla* leaves. The dried extract preparations were re-dissolved in DMSO to obtain a solution of 10 mg/ml which was subjected to serial dilution to get the concentrations between 12.5 µg/ml- 400 µg/ml. Standard drug Vincristine Sulphate (VS) was used as positive control at concentrations of 5 µg/ml - 0.312 µg/ml. A 5.0 ml of artificial sea water was added into all the test tubes. Simple zoological organism (*Artemia salina*) was used as a convenient monitor for cytotoxic screening. The eggs of the brine shrimps were collected from local aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (Prepared by using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 24 h under the light. The hatched shrimps were allowed to grow by 48 h to get shrimp larvae called nauplii. After 48 h, active nauplii were attracted to one side in a glass petri dish by using a micropipette. The nauplii were then separated from the eggs by aliquoting them in another glass petri dish containing artificial sea water and used for the assay. Suspension containing 10 nauplii was added into each test tube and was incubated at room temperature (25±1°C) for 12 h under the light. The tubes were then examined after 24 h and the number of surviving larvae in each tube was counted with the aid of a 3X magnifying glass. Experiments were conducted along with VS in a set of three tubes per dose. The concentration that would kill 50% of the nauplii (LC_{50}) was determined from a linear regression equation using the software "Microsoft excels 2007".

***In vitro* Thrombolytic activity**

Blood specimen: Whole blood (1.5 ml) was drawn from healthy human volunteers (n = 12) without a history of oral contraceptive or anticoagulant therapy. A new consent, approved by Mohammed Abu Sayeed, Assistant professor & Head of Department of Pharmacy, International Islamic University Chittagong, Bangladesh, for collection of blood samples from Human volunteers. Blood collection were conducted by Md. Shariful Islam (Lab technician, Department of Pharmacy, IIUC) and preservation were conducted by Abdul Karim (Lab technician, Department of Pharmacy, IIUC), who stored the clot containing Eppendorf tube in the refrigerator in Microbiology lab, Department of Pharmacy, IIUC. A 500 µl of blood was transferred to each of the three previously weighed Eppendorf tube tubes to form clots.

Statement on informed consent of the donors: The volunteer donors were supplied a consent form which informed the title of the research project, name and detail contact of investigators as well as purpose of the research. Description of the research mentioning step-by-step brief of the proposed research, inclusion and exclusion criteria of the donors, whether donors will receive any therapy or not, volume of blood to be taken, possible discomfort of the puncture sites, time required for the blood sampling. Benefits of the volunteer described. It was indicated to the consent form that the volunteers might refuse to donate blood at any time. Donor whether could withdraw his sample data was disclosed. The sample was restricted for that individual study not for future research projects was presented in the consent form. Potential harm, injuries, discomforts or inconvenience associated with donors in this study was added as informed consent statement. If there was known harm to the donors, the potential harm, current knowledge regarding the probability of the occurrence of the harm, clinical importance of the harm; and any relevant knowledge regarding the probability of reversibility. Treatment alternative and possibility of the research was described. Confidentiality statement was included in the consent form in the way that "confidentiality will be respected and no information that discloses the identity of the participant will be released or published without consent unless required by law of states. Finally identification of investigators was provided in case of further query. The consent form was concluded with major questions on above disclosures in Yes/NO form followed by the signature (with date) of the donor.

***In Vitro* Thrombolytic Study procedure:** Experiments for clot lysis were carried as reported earlier [17-19]. Briefly, 1.5 ml venous blood drawn from the healthy volunteers was distributed in three different pre weighed sterile Eppendorf tube (0.5 ml/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each Eppendorf tube containing pre-weighed clot, 100 µl of methanol extract of *B. platyphylla* leaves were added separately. As a positive control, 100 µl of SK and as a negative non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated with the blood samples of the 12 volunteers.

***In vitro* Antibacterial activity**

Microorganisms: Seven bacterial species, gram-positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and gram-negative *Salmonella typhi, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa*. These microbes were obtained from the department of Pharmacy, International Islamic University Chittagong.

Media preparation and maintenance of bacteria: All of the bacterial strains were grown and maintained on Nutrient agar (Merck, India) media at 37 °C and pH (7.4±0.2). The bacteria were subculture overnight.

Preparation of concentration: In the study of the antibacterial activity, all the extracts were diluted in their solvent. So methanol extract diluted in methanol and other also. The concentrations corresponding to the extracts given in Table 2 are expressed in terms of µg/ disk.

Preparation of discs: The discs of about 5 mm in diameter were cut by punching machine from Whatman No.1 filter paper. The discs were taken in a petri dish and sterilized by autoclaving, dried in oven at 180^0C.

Antibacterial screening by disk diffusion technique: The antibacterial effects were tested by the disc diffusion method [20-22] with some minor modification. The filter paper discs (5 mm in diameter) were individually impregnated with 21 µl of 700 µg/disk and 30 µl of 1000 µg/disk of leaves extract of *B. platyphylla* and then placed onto the

agar plates which had previously been inoculated with the test microorganisms (within 15 min). The Petri dishes were kept at 4 °C for 3 h before incubation at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicate. Blank disc impregnated with distilled water was used as negative control and disc of Kanamycin (30 µg / disc) as positive control.

Determination of relative percentage inhibition: The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula [23].

Relative percentage inhibition of the test extract:

$$\frac{100 \times (x - y)}{(z - y)}$$

Where,

x = total area of inhibition of the test extract

y = total area of inhibition of the solvent (methanol)

z = total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition.

Statistical analysis: The results were expressed as mean±SD from triplicate experiment for zone of inhibition from triplicate experiments for Antibacterial activity. All other results are expressed as mean ± standard error of the mean (SEM). Data were analyzed using one way factorial ANOVA tests using SPSS Data Editor for Windows, Version 22.0 (SPSS Inc., USA) followed by Dennett's tests on each group except negative control for antibacterial activity. The results obtained were compared with the control groups for antithrombotic activity by using Tukey test and $P < 0.01$, $P < 0.001$ and $P < 0.0001$ was considered to be statistically significant in Dennett's and Tukey tests. GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) was used for graphical presentation.

RESULTS

***In Vitro* Thrombolytic activity:** In thrombolytic activity assay, addition of 100µl streptokinase as positive control (30,000 I.U.) to the clots and subsequent incubation for 90 minutes at 37°C, showed 81.32±1.46 % lysis of clot. On the other hand, distilled water treated as negative control exhibited a negligible percentage of lysis of clot (6.81±0.97%). The mean difference in clot lysis percentage between positive and negative control was found statistically very significant (P<0.0001). In this study, the crude methanol extract of *B. platyphylla* exhibited thrombolytic activity (73.17±2.08%) (Figure 1).

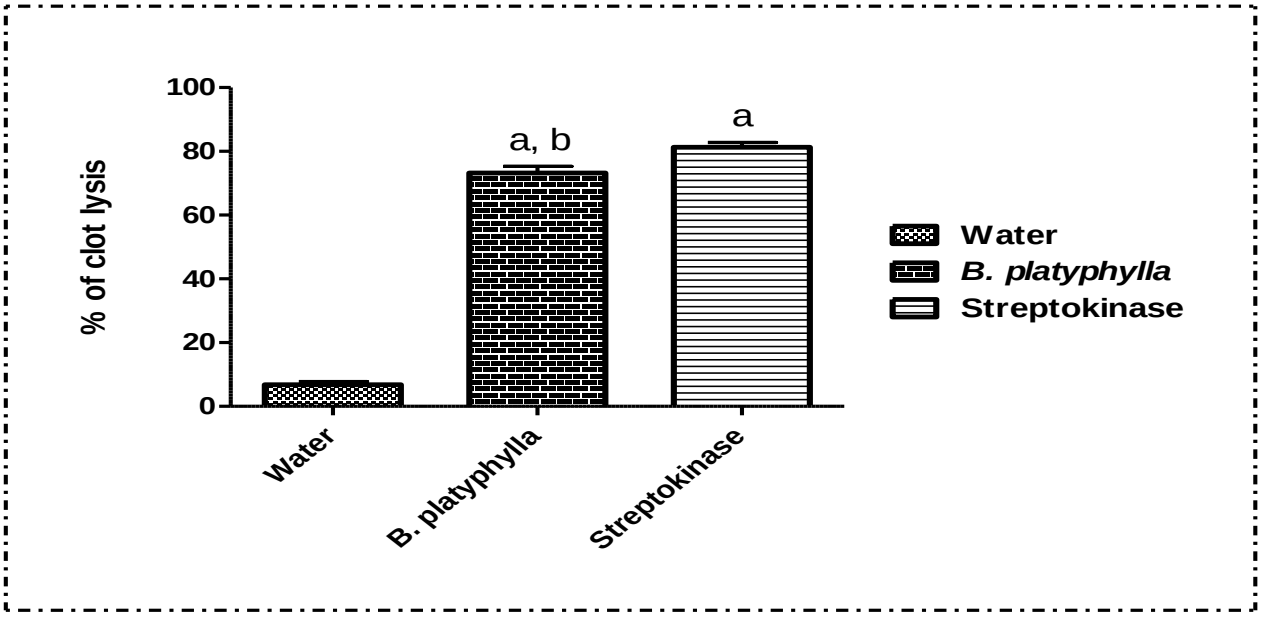

Figure 1: Thrombolytic activity of methanol extract of *B. platyphylla* leaves. Values are mean ± SEM (n = 12); [a]P <0.0001, Tukey test as compared to negative control (Water), [b]$P < 0.001$, compared to positive control (Streptokinase). Statistical representation of the effective clot lysis percentage by drugs preparations, positive thrombolytic control (streptokinase), and negative control (sterile distilled water) processed by Tukey test by using SPSS for windows, version 22.0.

***In vitro* Brine Shrimp Lethality Bioassay:** In brine shrimp lethality bioassay, the methanolic extract of *B. platyphylla* leaves showed optimistic result in comparison with the positive control vincristine sulphate. By plotting the log of concentration (log C) versus percent (%) of mortality for all test samples showed an approximate linear correlation. From the graph, the median lethal concentration (LC_{50}) Cytotoxic effect of the extract is summarized in the Figure 2. The LC_{50} for ethanol extract of *B. platyphylla* leaf were found to be 75.26µg/ml respectively, and that of Vincristine Sulphate was 0.74µg/ml. DMSO was used as negative control to validate the test method.

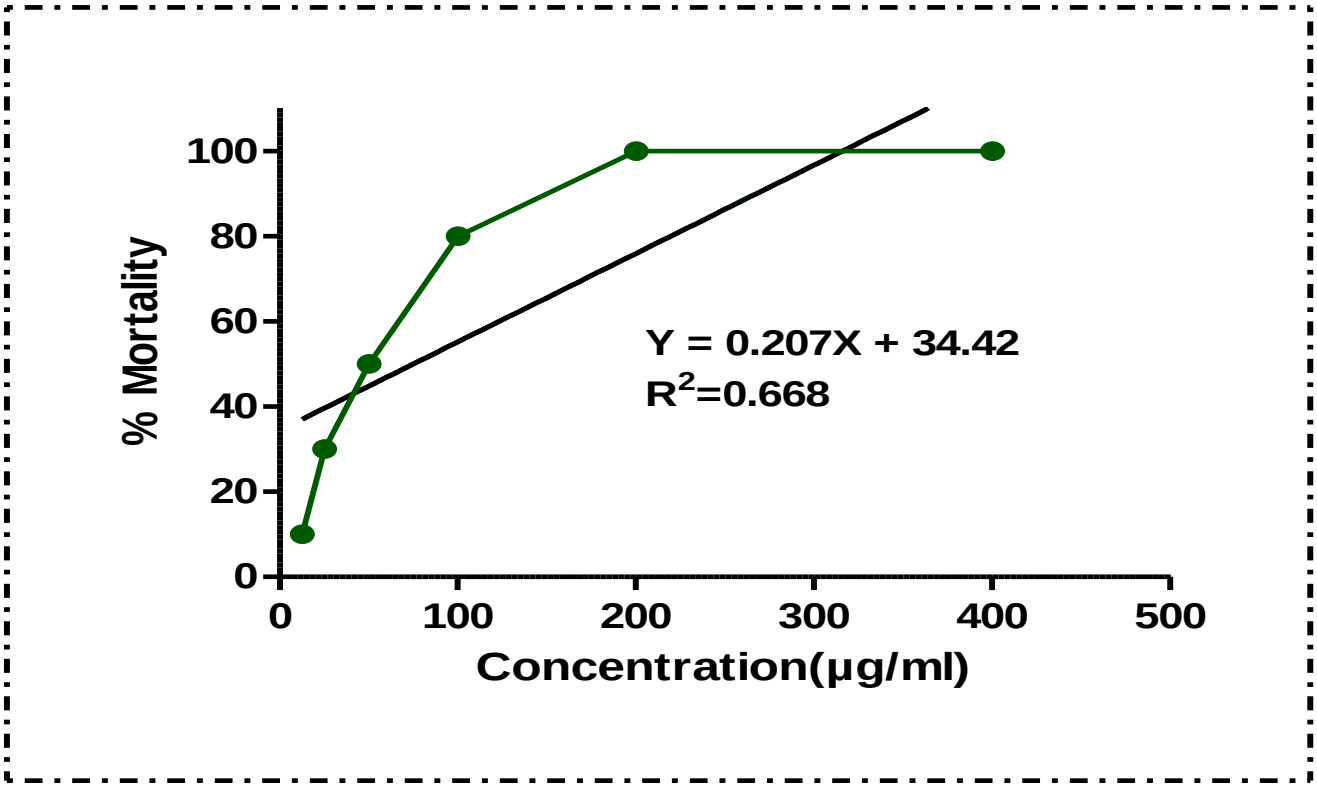

Figure 2: Effects of various concentrations of methanol extract of *B. platyphylla* leaves on the viability of brine shrimp nauplii after 24 hrs incubation. Percent mortality of brine shrimps of methanol extract of *B. platyphylla*. Data are shown as mean ± SEM of ten shrimps for each concentration.

***In vitro* Antibacterial activity**

Determination of zone of inhibition: Antibacterial activity results of *B. platyphylla* leaves extract are given in Table 1. The extract at two different concentrations 1000 µg/disc and 700 µg/disc showed significant ($P < 0.01$ and $P < 0.001$) as compared with standard Kanamycin 30µg/disc showed zone of inhibitions against Gram positive *Staphylococcus aureus (Nil)*, *Bacillus subtilis* (9.0±0.50, 7.0±0.50), *Bacillus cereus* (9.3±0.58, 7.3±0.58), *Salmonella typhi* (10±0.50, 8.5±0.50), *Salmonella paratyphi* (8.8±0.29, 7.3±0.58), *Escherichia coli* (11.2±1.26, 9.3±0.29), *Pseudomonas aeruginosa* (12.5±0.50, 9.5±0.50) respectively. The extract showed the highest zone of inhibition against the Gram negative *P. aeruginosa* (12.5±0.50) at concentration 1000 µg/disc. However, *S. aureus* showed the no antibacterial activity to the extract *B. platyphylla* leaves. Relative percentage inhibition of the test extract presented in Table 2

Table 1: Results of antibacterial activity testing of *B. platyphylla* leaves.

Name of the bacteria	Negative control (Methanol)	Methanol extract of *B. platyphylla* leaves		Kanamycin
	30 µl/disc	1000µg/disc	700µg/disc	30µg/disc
Gram Positive				
Staphylococcus aureus	-	-	-	22.2±0.76
Bacillus subtilis	-	9.0±0.50[a]	7.0±0.50[a]	18.2±0.29
Bacillus cereus	-	9.3±0.58[b]	7.3±0.58[b]	25±0.50
Gram Negative				
Salmonella typhi	-	10±0.50[b]	8.5±0.50[b]	25.3±0.58
Salmonella paratyphi	-	8.8±0.29[b]	7.3±0.58[a]	20.3±0.29
Escherichia coli	-	11.2±1.26[a]	9.3±0.29[a]	23.5±0.50
Pseudomonas aeruginosa	-	12.5±0.50[b]	9.5±0.50[b]	25.5±0.50

Values are mean inhibition zone (mm) ± S.D of three replicates. The different superscripted (a, b) values have significantly different ([a]P < 0.01 and [b]P < 0.001) as compared with standard (Kanamycin) in same row in Dunnett's test by SPSS. - - - = no zone of inhibition.

Table 2: Relative percentage inhibition of Methanol extract of *B. platyphylla* leaves with their doses compare to standard antibiotics.

Name of the bacteria	Relative percentage inhibition (%)	
	Methanol extract of *B. platyphylla* leaves	
	1000µg/disc	700µg/disc
Gram Positive		
Staphylococcus aureus	0	0
Bacillus subtilis	24.5	14.9
Bacillus cereus	13.8	8.5
Gram Negative		
Salmonella typhi	15.6	11.3
Salmonella paratyphi	18.7	12.9
Escherichia coli	22.7	15.7
Pseudomomas aeruginosa	24.0	13.9

Values calculated from their mean values.

DISCUSSION

A few thrombolytic medications acquired from different sources are utilized for the treatment of thrombosis. thrombolytic specialists are utilized to upset effectively shaped blood clusters in clinical settings where ischemia may be lethal (intense myocardial dead tissue, pneumonic embolism, ischemic stroke, and blood vessel thrombosis). Thrombolytic medications break up blood clumps by enacting plasminogen, which shapes a cut item called plasmin. Plasmin is a ptoteolytic compound that is fit for breaking cross-connections between fibrin atoms, which give the auxiliary honesty of blood clusters.

Due to these activities, thrombolytic medications are likewise called "plasminogen activators" and "fibrinolytic drugs."There are three noteworthy classes of fibrinolytic medications: tissue plaminogen activator (tPA), streptokinase (SK) and urokinase (UK). While drugs in these three classes all can powerful break up blood clusters, they vary in their point by point components in ways that change their selectivity for fibrin clumps. Some are adjusted further with the utilization of recombinant innovation so as to make these thrombolytic medications more site particular and compelling. Cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work [24]. Brine shrimp lethality test is carried out in order to reveal new anticancer compounds. It was watched that the antimicrobial impact of plant extract fluctuates starting with one plant then onto the next in distinctive inquires about completed in diverse areas of the world. This may be because of numerous components, for example, the impact of atmosphere, soil structure, age and vegetation cycle stage, on the quality, amount and arrangement of extricated item, diverse bacterial strains [25, 26]. Moreover, different studies found that the type of solvent has an important role in the process of extracting [27-30]. Outcomes of this study showed that the gram-negative bacteria indicated more activity to the plant extract than gram-positive bacteria, for example, *Pseudomonas aeruginosa* displayed more action than *Bacillus subtilis* when they were tried with *B. platyphylla* leaves. Since lipopolysaccharide (LPS) layer of gram-negative bacteria in external film have a high hydrophobicity which goes about as an in number porousness obstruction against hydrophobic atoms [31]. Hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram- negative bacteria because cell wall of the gram- positive bacteria contained only peptidoglycan [32, 33].

CONCLUSION

Crude methanol extract of *B. platyphylla* leaves indicated thrombolytic, cytotoxic and antibacterial impact, it can be expected that distinctive dynamic auxiliary metabolites were available in this concentrate and maybe some of these mixes may work in a synergistic way. On the other hand, further studies are important to illustrate the component lying with these impacts. On the other hand, this is the first write about this example and it may serve as a stride with respect to the natural and pharmacological exercises of this specimen.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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REFERENCES

1. Furie B, Furie BC. Mechanisms of thrombus formation. *New England Journal of Medicine* 2008; **359**: 938-49.
2. Handin R. Chapter 53: bleeding and thrombosis. *Kasper DL, Braunwald E, Fauci AS, et al Harrison's Principles of Internal Medicine* 2005.
3. Hunt BJ. Awareness and politics of venous thromboembolism in the United Kingdom. *Arteriosclerosis, thrombosis, and vascular biology* 2008; **28**: 398-9.
4. Sikri N, Bardia A. A history of streptokinase use in acute myocardial infarction. *Texas Heart Institute Journal* 2007; **34**: 318.
5. Meneveau N, Schiele F, Vuillemenot A et al. Streptokinase vs alteplase in massive pulmonary embolism. *Eur Heart J* 1997; **18**: 1141-8.
6. Mclaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. *Drug information journal* 1998; **32**: 513-24.
7. World Health Organization (WHO) (2001). Traditional medicine. Fact sheet number 134. Revised May, 2003. Available on http/www.who.int/media centre fact sheet/fs/134.
8. Aibinu I, Ohaegbulam V, Adenipekun E et al. Extended-spectrum β-lactamase enzymes in clinical isolates of Enterobacter species from Lagos, Nigeria. *Journal of Clinical Microbiology* 2003; **41**: 2197-200.
9. Aibinu I, Aednipekun E, Odugbemi T. Emergence of Quinolone Resistance amongst Escherichia coli strains isolated from Clinical infections in some Lagos State Hospitals, in Nigeria. *Nigerian Journal of Health and Biomedical Sciences* 2004; **3**: 73-8.

10. Williams R. Antimicrobial resistance a global threat. *Essential Drug Monitor* 2000; **28**: 1.
11. Chowdhury KAA, Kabir MSH, Chowdhury TA, Hasan M, Kader SMA, Alam MS, Hossain J, Hossain MS and, Hasanat A. Antibacterial activity on some gram positive and gram negative bacteria and antihelmintic activity on Tubifex tubifex worm of methanol extract of Macaranga denticulata (MUELL. ARG.) bark. *International Journal of Pharmacy* 2015; **5**: 985-90.
12. Moreillon P, Que Y, Glauser M. Staphylococcus aureus. *Mandel GL, Bennett JE, Dolin R Principles and practice of infectious disease* 2005; **6**: 2321-51.
13. The American Phytopathological Society. Common Names of Plant Diseases: Diseases of Foliage Plants (House Plants): Urticaceae".. 26 March 1993. Archived from the original on 30 November 2011.
14. Chase A. Influence of host plant and isolate source on Myrothecium leaf spot of foliage plants. *Plant disease* 1983; **67**: 668-71.
15. Meyer B, Ferrigni N, Putnam J et al. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica* 1982: 31-4.
16. Rahman MA, Sultana R, Bin Emran T et al. Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity. *BMC Complement Altern Med* 2013; **13**: 1472-6882.
17. Prasad S, Kashyap RS, Deopujari JY et al. Effect of Fagonia Arabica (Dhamasa) on *in vitro* thrombolysis. *BMC Complementary and Alternative Medicine* 2007; **7**: 36.
18. Tarek MI, Hasanat A, Kabir MSH, Chowdhury TA, Rahman MM, Hossain ME. *In vitro* thrombolytic and cytotoxic activity of methanolic extract of Syzygium operculatum leaves. 2015; **4**:87-89.
19. Kabir MSH, Murad MAH, Hasanat A, Hamid MA, Islam MI, Chowdhury TA, Hasan M, Hossain MM, Masum MAA, Uddin MR. Evaluation of total flavonols, total proanthocyanidins content and thrombolytic activity of methanol extracts of three bangladeshi plants. *International Journal of Pharmacy* 2015; **5**: 747-51.
20. Bauer A, Kirby W, Sherris JC et al. Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology* 1966; **45**: 493.
21. Kabir MSH, Al Noman MA, Rahman MM, Ara J, Hossain MM, Hasanat A, Zaheed F. Antibacterial Activity of Organic and Aqueous Extracts of Hopea odorata Roxb. Leaves and their Total Flavonoid Content. *British Microbiology Research Journal* 2015; **9**: 1-7.
22. Hasanat A, Kabir MSH, Hossain MM, Hasan M, Masum MAA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria ASMG. Antibacterial activity of methanol extract of Macaranga denticulata leaves and in silico PASS prediction for its six secondary metabolites. *World Journal of Pharmaceutical Sciences* 2015; **3**: 1258-66.
23. Kumar KA, Rai KL, Umesha K. Evaluation of antibacterial activity of 3, 5-dicyano-4, 6-diaryl-4-ethoxycarbonyl-piperid-2-ones. *Journal of Pharmaceutical and Biomedical Analysis* 2002; **27**: 837-40.
24. Cardellina, J.H., Fuller, R.W., Gamble, W.R., Westergaard, C., Boswell, J., Munro, M.H.G., Currens, M. and Boyd M.P. 1999. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV inhibitory natural products extracts. In: Bohlin, L., Bruhn, J.G. (Eds), Bioassay Methods in Natural Product Research and Development. Kluwer Academic Publishers, Dordrecht, pp. 25-36.
25. Masotti V, Juteau F, Bessière JM, Viano J. Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. *Journal of agricultural and food chemistry* 2003; **51**: 7115-21.
26. Angioni A, Barra A, Coroneo V et al. Chemical composition, seasonal variability, and antifungal activity of Lavandula stoechas L. ssp. stoechas essential oils from stem/leaves and flowers. *Journal of agricultural and food chemistry* 2006; **54**: 4364-70.
27. Al-Zubaydi SR, Al-Hmdany MA, Raesan S. Antibactrial effect of some medicinal plant extracts against some pathogenic bacteria strains. *Journal of Duhok University* 2009; **12**: 244-9.
28. Bakht J, Muhammad T, Ali H et al. Effect of different solvent extracted sample of Allium sativum (Linn) on bacteria and fungi. *African Journal of Biotechnology* 2013; **10**: 5910-5.
29. Bokhari FM. Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia. *Mycopath* 2009; **7**: 51-7.
30. Bedi N, Bedi P, Bodiwala HS et al. Scientific evaluation of an innovative herbal medicine for relief in respiratory disorders. *Canadian journal of pure & applied sciences* 2010; **4**: 1249-55.
31. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in applied microbiology* 1998; **26**: 118-22.
32. Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. *Microbiological reviews* 1985; **49**: 1.
33. Lambert R, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology* 2001; **91**: 453-62.