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Investigation of the Different Ethnopharmacological Activity of Fractional Root Extracts of *Mussaenda roxburghii* in *in vitro* Model

Farzana Akther Sumi[1], Prawej Ansari[2,3*], Biswajit Sikder[3], Anaytulla[2], Nadia Akter Zhumur[3], Mustafe Khalid Mohamed[2], Sabbir Anwar[3], Mitali Debnath[3] and Rokibul Hasan[4]

[1]University of Science and Technology Chittagong, Foy's Lake, Khulshi, Chittagong-4202, Bangladesh.
[2]Department of Pharmacy, International Islamic University, Chittagong, 154/A, College Road, Chittagong-4203, Bangladesh.
[3]Department of Pharmaceutical Sciences, School of Health and Life Sciences, North South University, Dhaka-1229, Bangladesh.
[4]Department of Pharmacy, Northern University of Bangladesh, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Authors FAS designed the current project, performed the experiments. Author PA wrote the manuscript, carried out the experimental process; responsible for data interpretation and statistical analysis. Authors BJS and AU helped in experiments and preparing the manuscript. Authors NAZ, MKM, SA, MD and RH participated in experiments and data collection. Author PA also edited the manuscript. All authors read and approved the final version of the manuscript.

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ABSTRACT

Purpose: With this study, we wanted to reveal that the root extract of *M. roxburghii* has thrombolytic activity or not and targeted to estimate its toxicity, potentiality to inhibit inflammation by the inhibition of protein denaturation.
Methods: *In vitro* thrombolytic assay was used in the purpose of clot lysis capacity measurement of fractional crude extracts of *M. roxburghii*. We took streptokinase as a positive control. Brine

**Corresponding author: E-mail: chemist89ansari@gmail.com;*

shrimp lethality bioassay was performed to measure the toxic potentiality of the plant extracts. The *in vitro* anti-inflammatory assay was designed using egg protein model to check the percent protection rate of protein denaturation.

Results: In the thrombolytic assay, ethyl acetate extract of *M. roxburghii* showed significant ($p < 0.0001$) clot lysis activity with 48.85±7.38%. The positive control Streptokinase results 76.90±9.01% and negative control water results very little, 7.55±3.33%. The apoptotic activity study showed good result with EC_{50} of 32.46 µg/ml with significant limit of <0.002. In *in vitro* anti-inflammatory study the ethyl acetate extract of *M. roxburghii* showed highest percent of inhibition with 48.43±2.23% ($p<0.001$).

Conclusions: From our study outcome, the ethyl acetate and dichlormethane extracts of root of *M. roxburghii* have good thrombolytic, anticancer and anti-inflammatory activity. Hence, further study is needed to identify the phytochemicals incorporated with thrombolytic activity, the new chemicals may aid in the improvement of the treatment of atherosclerosis or embolism like diseases. We should also take result of pain inhibition and anti-cancer study, as well.

Keywords: Anti-inflammatory; antitumor; atherosclerosis; M. roxburghii; Brine shrimp; thrombolysis.

1. INTRODUCTION

From past few years, medicinal plants are getting more attention from investigator or researcher, to find out potential sources of new thrombolytic agents [1,2], as well as they also paying interest on cytotoxic plant extract [3]. Working with different medicinal plants extract it is approved, that some of them can lyses thrombus as streptokinase do [4,5]. Some of the plant extract also increase lethality of the cell due to their known toxic potentiality. Brine shrimp lethality bioassay is performed for evaluating the level of toxicity according to the method of Persoone, 1980 and Goldstein et al. 1974.

When a blood clot (thrombus) developed inside the circulatory system, it causes vascular blockage and leads to serious consequences of atherothrombotic diseases, such as acute myocardial or cerebral infarction, and ultimate result is death. At present different thrombolytic agents, like as alteplase, streptokinase, urokinase and tissue plasminogen activator (TPA), are being used to dissolve clots [6]. All these anti-thrombotic agents consist of some shortcomings, including the limited fibrin specificity and bleeding tendency. Due to these limitations, continuous attempts are in progress to develop better-quality recombinant alternatives of these drugs [7]. From very past time, it is human tradition to find cure of different diseases in herbal preparations, because they are often alleged as safe [8]. Several studies have established that, diets with experimentally proved thrombolytic effect can ease hazard of thrombosis. Proper identification and analysis of plant chemicals, from both *in vitro* and *in vivo* study, can replace present anti-thrombotic drugs, so far [9,10].

Inflammation is a multifaceted biological reaction of vascular tissues to injurious spurs. It is also a defensive feedback of living organism to remove those stimuli and initiate the therapeutic progression [11]. Inflammation starts; the cells endure activation and release inflammatory intermediaries. These mediators like histamine, serotonin, prostaglandins and other systems such as complement, clotting, fibrinolytic and kinin system [12]. These mediators propagate inflammation and together, they cause increased vasodilatation and permeability of blood vessels. Thus, blood flow increased, which exudate the plasma proteins and fluids, and migration of leukocytes, mainly neutrophils, outside the blood vessels towards the injured tissues. Inflammation is either acute or chronic. Noticeable vascular changes occur in acute inflammation, including increased vasodilatation and capillary permeability, which is predisposed by various inflammatory mediators. Chronic inflammation is the propagation of inflammatory impetuses, which gradually move into the cells present at the site of inflammation. Simultaneous destruction and healing of the tissues elongate the inflammatory process.

We have attempted this present study intended to establish physiochemical standards of the plant *Mussaenda roxburghii* locally known as, *Chauri-Chaonri Gach Sildaura* (Chakma) *Supaila* (Marma) and *Nakaling*, *Paokanling* (Khumi). It is used in the treatment of boils, fever and rheumatism [13]; abdominal pain, bleeding, breast pain, cirorhosis, epilepsy, food poisoning, gout, headache, hyper acidity, lipoma, edema, para-paralysis, paralysis, pediatric disease, pyorrhea, rheumatism, skin disease, snakebite, swelling of armpit gland and tumour [14]; rheumatism [15]; boils and headache [16]; boils,

headache and jaundice [17] and boils. Other uses include, leaves of this plant is used as vegetable. The leaf part of *Mussaenda roxburgii* showed mild antibacterial activity with cytotoxic property and it have enriched anti-oxidative content like phenolic, flavonoid etc. [18,19].

2. MATERIALS AND METHODS

2.1 Preparation of Extract

M. roxburghii leaves were collected from hilly region of Chittagong district of Bangladesh in May 2013 and were identified by the taxonomist Ast. Prof. Md. Sheikh Bakhtiar Uddin, University of Chittagong, and Bangladesh National Herbarium, Chittagong branch. One voucher specimen was deposited in Herbarium and the accession number is MFK137.CTG.UH. After isolating the root parts of *M. roxburghii* form collected sample, it was dried in open air, under a shed for approximately 15 days, and then the sample grounded to coarse powder with the help of suitable grinder. The mother extraction was made using 4 liter of methanol in a sealed container for another 7 days. Then the mixture was filtered through Whatman filter paper. After 7 to 8 days evaporation of methanol occurred and concentrated methanolic extract was acquired. The obtained extract was further fractioned for proper investigation.

2.2 Collection of Blood Sample

Whole blood sample (n=20) of 4 ml were collected from the healthy volunteers without a history of oral contraceptive or anticoagulant therapy. For each treatment ten tubes were taken and experiment was repeated thrice. The blood was withdrawn from median cubital vein. The ethical committee of our institution University of Science and Technology Chittagong approved the whole process, the consent number was Pharm-R&D-46/07'13-04.

2.3 Volunteer's Agreement Form

An agreement form was supplied to each volunteer that contains the full explanation of our purpose and the procedure of sampling. Each of them was suggested to read it carefully and if they found disagree with any point they can quit immediately.

2.4 Test for Thrombolytic Activity

A 100 mg of the crude extracts was suspended in 10 ml of distilled water and for proper suspension; it was shaken on a vortex mixer thoroughly. The suspension kept at least for 10-12 hour and draw off the soluble supernatant through a 0.22-µm syringe filter. A 100 µl of the earlier aqueous preparation was added to the microcentrifuge tubes containing the clots to check thrombolytic activity [20].

Experiments for clot lysis were carried as reported previously [21]. Briefly, 4 ml of venous blood strained from the healthy volunteers was dispersed in nine different previously weighed sterile microcentrifuge tubes (0.5 ml/tube), three for each different groups, and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight. To each microcentrifuge tube containing pre-weighed clot, 100 µl of crude extracts was added. Same procedure was followed for positive control, 100 µl of Streptokinase and a negative control, 100 µl of distilled water. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, released fluid was removed and tubes were again weighed to observe the difference in weight after clot lysis. Difference obtained in weight taken before and after clot, lysis was expressed as percentage of clot lysis.

2.5 Test for Cytotoxic Property

Three fractional extracts of *M. roxburghii* was subjected to cytotoxic study. A 1 mg of crude sample was taken from each and a stock solution of 1000 µg/ml was prepared with dimethyl sulfoxide (DMSO). A series of solutions of different concentrations were prepared from the stock solution by serial dilution method and the concentrations were as – 1000 µg/ml, 500 µg/ml, 200 µg/ml, 100 µg/ml, 75 µg/ml and 50 µg/ml. Then the samples were subjected to brine shrimp lethality bioassay [22,23] for cytotoxic studies. In each test tube, containing different concentrations of test sample, 10 brine shrimp nauplii (*Artemia salina*) were added. One control group was used in this study, to validate the method as well as the result due to the activity of the test agent. DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii to use as negative control group. We used vincristine sulfate as comparable standard in this protocol. After 24 hours, the test tubes were observed, keeping under magnifying glass and the numbers of survived nauplii in each test tube were

counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for the extract.

2.6 Anti-inflammatory Effect Measurement

The mixture of 5 ml of reaction consisted of 0.2 ml of egg albumin (from hen), 2.8 ml of phosphate buffer (pH 6.4) and 2 ml of varying concentrations of extract so that final concentrations become 100, 200, 400 µg/ml. Similar volume of distilled water served as control. Then the mixtures were incubated at (37°C±2) in incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800). Acetyl salicylic acid at the final concentration of 100, 200, 400 µg/ml was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\%\ of\ inhibition = \frac{Abs_{control} - Abs_{test}}{Abs_{control}}\ X\ 100$$

2.7 Statistical Analysis

The paired t-test was done to analyze the data of percent of clot lysis by plant extract using the software GraphPad prism 6. Data are expressed as mean ± standard deviation. P < 0.05 was hypothesized as lower limit of significance. The calculation of EC_{50} for toxicity data establishment was performed using the same software.

3. RESULTS AND DISCUSSION

3.1 Percent Yield

The percent of compound yield from initial extract was calculated by the following equation:

$$\%\ of\ yeild = \frac{Final\ weight\ of\ extract}{Initial\ weight\ of\ extract}\ X\ 100$$

Table 1. Percent yield from the root extract *M. roxburghii*

Extract	Percent (%)
Methanol	5.54
Ethyl acetate	0.77
Dichloromethane	3.28

3.2 Phytochemical Screening

A fresh methanolic root extract of *M. roxburghii* was tested qualitatively for different chemical constituents. Qualitative phytochemical tests were carried out with the extract following the method described previously [24].

Table 2. Preliminary phytochemical screening of *M. roxburghii*

Phyto compound	Presence/Absence
Carbohydrate	+
Cholesterol	--
Steroid	+
Alkaloid	+
Tannin	+
Cardiac glycoside	--
Resin	--
Saponin	+
Reducing sugar	+
flavonoid	+
Terpenoid	+

Presence (+), absence (--)

3.3 Thrombolytic Assay Result

Streptokinase (100 µl) was taken as positive control, 90 minutes later, it showed 76.9±9.01% clot lysis with P <0.0001. Water was as negative control, which resulted 7.55±3.33% clot lysis. The highest percent of clot lysis was shown by ethyl acetate extract of *M. roxburghii*, 48.85±7.38% (P < 0.0001). The result of thrombolytic assay is represented in Table 3 and graphically shown in Fig. 1.

Morphological and angiographic studies confirmed that atherosclerotic lesion is the major reason behind the complication of atherosclerosis that is the result of formation of coagulation and it is one of the leading cause of death at present time [25]. Thrombogenicity of the atheroma is resolved for the most part by the dependability of a sinewy top and substance of tissue element that initiates the thickening waterfall when presented to standard stream of blood [26]. While all these components works together, the regular blood flow through the blood vessel is impaired, due to clotting [27]. Under pathological circumstance where platelets, vessel divider and plasma proteins (essential haemostasis) are initiated, coagulation happens. Thrombus formed due to certain coagulation factor activation, in that situation, it is evident that treatment with antiplatelet agents improves

survival rate [28]. In addition, recent epidemiologic studies evident that experimentally proved thrombolytic/fibrinolytic specialists from characteristic sources are equipped for lessening danger of thrombosis more than others and they are safe, as well [29-32].

Plants are playing most important role that is ensuring safety and efficacy, because of the reported immunomodulatory effects, they are getting more attention [33,34]. In our present study, the ethyl acetic acid derivation concentrate of *M. roxburghii* demonstrated the most noteworthy thrombolytic action and had the critical action. The other two concentrate additionally indicated great impact (Table 3 & Fig. 1). Evidence bacterial contaminants of plants have plasminogen receptors and they can tie plasminogen. The receptor binds plasminogen and convert to plasmin later on results in fibrinolysis [35], although plant species also have ability to expel their thrombolytic or fibrinolytic impacts by their substance of specific proteases proteins. Singular synthetic segment action relationship, which can investigate the other new hint for the watched thrombolytic impacts of this plant part, will be the following stride of the examination follow-up of this study.

3.4 Cytotoxic Assay

The dichloromethane extracts showed highest lethality with LC_{50} of 32.46 µg/ml, it indicates the biologically active compounds are present little bit higher than other fraction of this crude drug. The methanolic extract and ethyl acetate extract of *M. roxburghii* shows LC_{50} of 52.38 µg/ml and 50.22 µg/ml, respectively. The percent of mortality was calculated from the number of dead nauplii, after application of crude extract. The LC_{50} for the concentrate was computed from Fig. 2. The watched quality was so deadly and it was even more than standard (48.04 µg/ml).

Table 3. Result of thrombolytic assay

Group	Percent of clot lysis	Standard deviation	p-value
Water	7.553	3.334	
Streptokinase	76.9	9.005	< 0.0001
DCMR	47.1	7.589	< 0.0001
MEMR	44.37	7.805	< 0.0001
EAMR	48.85	7.38	< 0.0001

Values presented here as mean ± SD, the result obtained from paired t-test and each group was compared with negative control, the extract results statistically significant when compared with water.

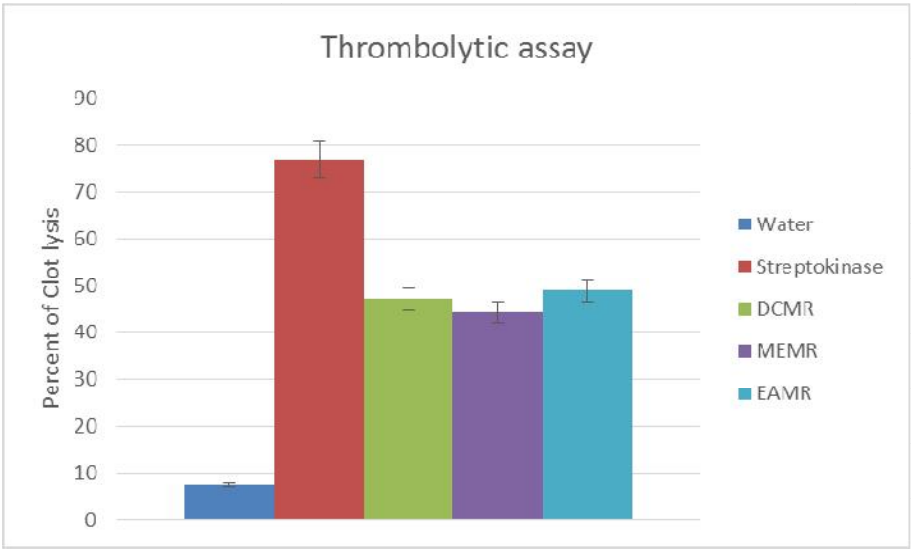

Fig. 1. The graphical and comparative representation of thrombolytic assay of different extracts of *M. roxburghii* root

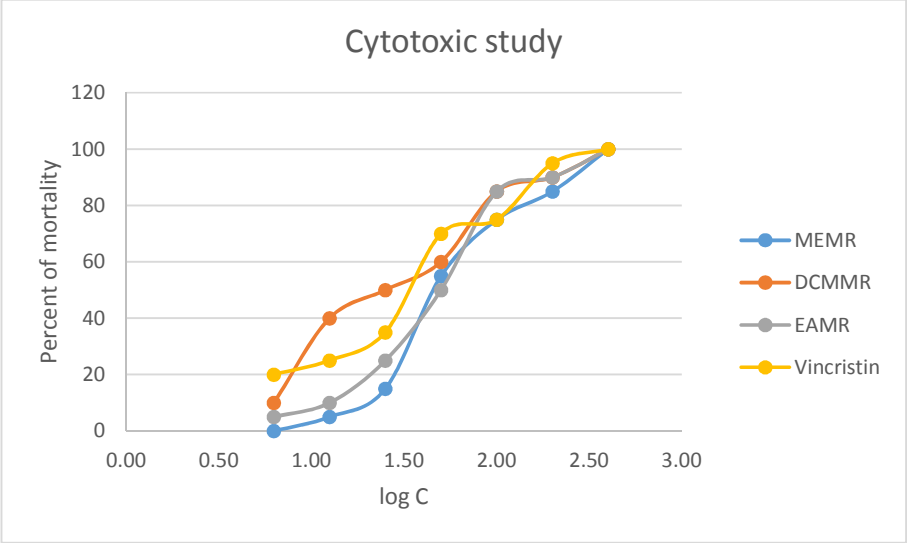

Fig. 2. Schematic representation of percent of mortality done by distinctive division of *M. roxburghii* on Brine shrimp lethality bioassay, the watched highest toxic concentration was 32.46 µg/ml, which was, even more than compared standard *vincristine* (48.04 µg/ml)

3.5 In vitro Anti-inflammatory Activity Evaluation

In our present study, the anti-inflammatory activity of ethyl acetate extract of *M. roxburghii* showed mean inhibition of protein denaturation of 48.43±2.32% at the dose of 400 µg/ml, whereas, the compared standard aspirin at same dose inhibited 79.32±2.62% (Table 4, Fig. 3). The ethyl acetate extracts of *M. roxburghii* demonstrated great mitigating action with a direct measurement reaction. The ability of the extract of *M. roxburghii* to inhibit heat-induced denaturation of protein was discovered to be measurably noteworthy ($p < 0.0003$). Further examination is asked for in conclusion to recognize the dynamic constituents too the careful instrument of activity in charge of the reported anti-inflammatory property of different fractional extract of *M. roxburghii*.

Assessment of anti-inflammatory property of *M. roxburghii* was done by *ex vivo* protein denaturation method using egg albumin of hen. The denaturation of egg protein (albumin) was induced by heat treatment. Inflammation, that are

Table 4. Tabulation of percent of inhibition of protein denaturation in *in vitro* method

Group	Dose	Absorbance	% of inhibition
Control	---------	0.549±0.004	-------
Aspirin	100 µg/ml	0.416±0.018	24.22±2.62[a]
	200 µg/ml	0.214±0.012	42.91±1.68[a]
	400 µg/ml	0.114±0.011	79.32±1.77
MEMR	100 µg/ml	0.496±0.006	9.7±1.66[b]
	200 µg/ml	0.451±0.034	17.96±6.02[b]
	400 µg/ml	0.380±0.016	30.82±3.16
EAMR	100 µg/ml	0.454±0.009	17.41±2.05[c]
	200 µg/ml	0.424±0.023	22.75±4.24[c]
	400 µg/ml	0.283±0.014	48.43±2.32
DCMMR	100 µg/ml	0.495±0.009	9.84±1.03[d]
	200 µg/ml	0.434±0.021	20.94±3.42[d]
	400 µg/ml	0.344±0.010	37.31±2.20

The results represented here are mean ± SD, the obtained significant limit (p-value) from one-way ANOVA is as following [a]<0.0001, [b]<0.002, [c]<0.0003, [d]<0.0005.

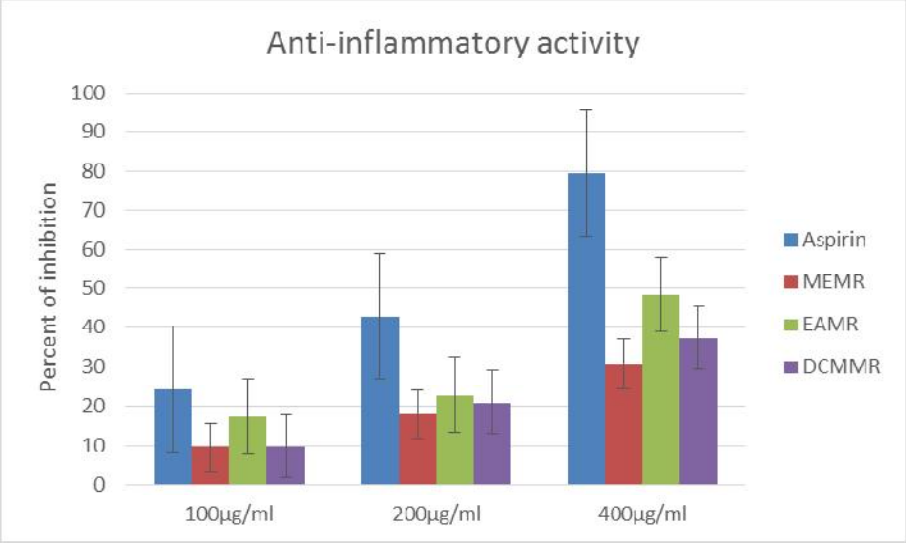

Fig. 3. Graphical representation of anti-inflammatory activity of different fraction of root extract of *M. roxburghii*

associated with Type-III hypersensitive antigens or anaphylactic reaction is evaluated by anti-denaturation assay. Type-III hypersensitive reaction is responsible for several diseases such as serum sickness; glomerular nephritis etc [36]. *Ex vivo* Heat-denatured proteins represent the similar effectiveness of native proteins that influence delayed hypersensitivity [37]. It was already proved that prevention of protein denaturation is also done by NSAIDs like indomethacin and aspirin beside their regular mechanism of inhibition of Prostaglandins [38]. Thus, protein denaturation method is the convenient protocol to check the anti-inflammatory activity, heat induced inflammation. From our present study, the extract exhibited considerable anti-inflammatory activity. *M. roxburghii* is fit for controlling the creation of auto antigen response and in this manner it restrains the denaturation of proteins and its effect, the comparable standard drug was Aspirin. The preliminary phytochemical study says the plant is enriched with secondary metabolite, as phenolic compounds and tannins; they may be in charge of this movement.

4. CONCLUSION

In the conclusion, different fractional extracts of the *M. roxburghii* is suitable for designing as thrombolytic agent either in combinatorial use or alone. The extract also found as highly toxic, thus, can effective apoptotic agent, so it can be future lead as anti-tumor drug. Further work is expected to disconnect the metabolites that lysed the thrombi or showing that high extent apoptotic property. The result of *in vitro* anti-inflammatory study should also be taken under consideration, during search for new anti-inflammatory agent. The whole study showed that society pharmaceutical can be as powerful as advanced engineered prescription to lessen danger of cardiogenic issue and other danger element like deep vein thrombosis, and can be safe remedy of chronic painful condition like rheumatoid arthritis, as well.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Elumalai A, Eswariah MC, Chowdary VCH, Kumar R, Anusha M, Naresh K. Screening of thrombolytic activity of *Bougainvillea glabra* leaves extract by *In-Vitro.* Asian J Res Pharm Sci. 2012;2(4):134-136.
2. Emran TB, Rahman MA, Uddin MMN, Rahman MM, Dash R, Layzu C, Uddin MZ.

Effects of organic extracts and their different fractions of five Bangladeshi plants on in vitro thrombolysis. BMC Compl Alt Med. 2015;15:128-36.

3. Hossain MK, Hassan MM, Parvin MN, Hasan MM, Islam MS, Haque MA. Antimicrobial, cytotoxic and thrombolytic activity of *Cassia senna* leaves (Family: Fabaceae). J App Pharm Sci. 2012;2(6): 186-190.
4. Gennaro AR. Remington; The science and practice of pharmacy; Thrombolytic agents; 20th ed. Lippincott Williams & Wilkins; New York. 2000;1256-1257.
5. Sweta P, Rajpal SK, Jayant YD, Hemant JP, Girdhar MD, Hatim FD. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Throm J. 2006;4(14):1-4.
6. Collen D. Coronary thrombolysis: Streptokinase or recombinant tissue-type plasminogen activator. Ann Intern Med. 1990;112:529–538.
7. Marder VJ. Recombinant streptokinase – Opportunity for an improved agent. Blood Coagul Fibrin. 1993;4:1039–1040.
8. Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. Circulation. 1995;91:1182–1188.
9. Basta G, Lupi C, Lazzerini G, Chiarelli P, L'Abbate A, Rovai D. Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis: An in vitro study on blood of normal subjects and patients with coronary artery disease. Thromb Haemost. 2004;91: 1078-1083.
10. Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. Nutrition. 2005; 21:580–587.
11. Potterat O, Hamburger M. Drug discovery and development with plant-derived compounds. Prog Drug Res. 2008;65(45): 47–118.
12. Verstraete M. Third-generation thrombo-lytic drugs. Am J Med. 2000; 109(1):52–8.
13. Uddin SB. A comparative ethnobotanical study among the tribal communities of chittagong hill tracts, Bangladesh. PhD thesis; University of Aberdeen. 2001;122-128.
14. Uddin SN. Traditional uses of ethnomedicinal plants of the Chittagong hill tracts. Bangladesh National Herbarium, Mirpur 1, Dhaka 1216, Bangladesh. 2006; 992.
15. Khisha T, Karim R, Chowdhury SR, Banoo R. Ethnomedical studies of chakma communities of Chittagong hill tracts, Bangladesh. Bangladesh Pharmaceutical Journal. 2012;15(1):59-67.
16. Alam MK. Medical ethnobotany of the Marma tribe of Bangladesh. Economic Botany. 1992;46(3):330-335.
17. Yusuf M. Crotalaria pallida Ait. In: Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmed M, Ahmed ATA, Rahman AKA, Haque EU. (eds). Encyclopedia of flora and fauna of Bangladesh, Vol. 8. Angiosperms: Dicotyledons (Fabaceae-Lythraceae). Asiatic Society of Bangladesh, Dhaka. 2009;40-41.
18. Islam F, Kuddus MR, Latif F, Hossain MK. Preliminary antimicrobial activity and cytotoxicity of leaf extracts of *Mussaenda roxburghii* Hook. F. Bol Latinoam Caribe Plant Med Aromat. 2013;12(6):612–617.
19. Latif F, Islam F, Kuddus MR, Hossain MK. Antioxidant, thrombolytic and membrane stabilizing activities of *Mussaenda roxburghii* Hook. F. iP-Planet. 2013;1(1): 13-19.
20. Ezeugwu CO, Okonta JM, Nwodo NJ. Antidiabetic properties of ethanolic fruit extract of *Solanum aethiopicum* L. Res J Pharmaceut Allied Sci. 2004;2(2):251–254.
21. Read MA. Flavonoids: Naturally occurring anti-inflammatory agents. Am Pathol. 1995;147:235-237.
22. Rahman MA, Sultana R, Emran TB, Islam MS, Chakma JS, Rashid HU, et al. Effects of organic extracts of six Bangladeshi plants on *in vitro* thrombolysis and cytotoxicity. BMC Compl Alt Med. 2013; 13(25):1472–6882.
23. 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.
24. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edition; London: Chapman and Hall. 1998;302. ISBN: 0-412-57270-2
25. Goldstein AL, Kalkan SM. Principles of drug action; 2nd ed; Willey Biochemical Health Publications. 1974;376-381.
26. Meyer BB, Ferringi NR, Futman FJ, Jacobsen LB, Nichols DE, Mclaughlin JL. Brine shrimp a convenient general

bioassay for active plant constituents. Planta Medica. 1982;5:31-34.
27. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011; 473:317-325.
28. Fuentes E, Guzmán L, Alarcón M, Moore R, Palomo I. Thrombolytic/fibrinolytic mechanism of natural products. Fibrinolysis and Thrombolysis; chapter 5. 2014;107-121.
29. Ananyeva NM, Kouiavskaia DV, Shima M, Saenko EL. Intrinsic pathway of blood coagulation contributes to thrombogenicity of atherosclerotic plaque. Blood. 2002;99: 4475-4485.
30. Zinkstok SM, Vermeulen M, Stam J, de Haan RJ, Roos YB; Antiplatelet therapy in combination with rt-PA thrombolysis in ischemic stroke (ARTIS): Rationale and design of a randomized controlled trial. Cerebrovasc Dis. 2010;29:79-81.
31. Rahman MA, Sultana R, Bin Emran T, Islam MS, Chakma JS, Rashid HU, Hasan CM. Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity. BMC Compl Alt Med. 2013;13:25.
32. Yamada K, Naemura A, Sawashita N, Noguchi Y, Yamamoto J. An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents. Thromb Res. 2004;114: 213-220.
33. Suzuki Y, Kondo K, Matsumoto Y, Zhao BQ, Otsuguro K, Maeda T, Tsukamoto Y, Urano T, Umemura K. Dietary supplementation of fermented soybean, natto, suppresses intimal thickening and modulates the lysis of mural thrombi after endothelial injury in rat femoral artery. Life Sci. 2003;73:1289-1298.
34. Rajput MS, Mathur V, Agrawal P, Chandrawanshi HK, Pilaniya U. Fibrinolytic activity of kaempferol isolated from the fruits of *Lagenaria siceraria* (Molina) Standley. Nat Prod Res. 2011;25:1870-1875.
35. Licciardi PV, Underwood JR. Plant-derived medicines: A novel class of immunological adjuvants. Int Immunopharmacol. 2011; 11(3):390–8.
36. Ahmad F, Khan RA, Rasheed S. Study of analgesic and anti-inflammatory activity from plant extracts of *Lactuca scariola* and *Artemisia absinthium.* J Islamia Acad Sci. 1992;5:111–114.
37. Gell PGH, Benacerraf B. Studies on hypersensitivity-II delayed hypersensitivity to denatured proteins in guinea pig. Immunology. 1959;2:64.
38. Insel PA. Analgesic-Antipyretics and anti-inflammatory agents: drugs employed in the treatment of rheumatoid arthritis and gout. In Goodman and Gilman's The Pharmacological Basis of Therapeutics; Edited by Gilman AG, Rall T, Nies A, Taylor P; Pergamon, NY. 1990;638–681.

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