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Potentiality of Crataeva nurvala bark in the management of thrombosis and membrane stabilization in the rural area, Bangladesh

Jalal Uddin, Farhina Rahman Laboni, Samira Karim, Zubair Khalid Labu Phytochemical Research Laboratory, Department of Pharmacy, World University of Bangladesh, Dhaka, Bangladesh

ABSTRACT

Aim: *Crataeva nurvala* (*C. nurvala*) is a medicinally active plant, potentially used as traditional means of treating many health ailments such as prostatitis, prostate enlargement, inflammatory conditions, heart disease, thyroid and endocrine disorder, and urinary tract infection, respectively, in the rural region of Tangail, Jamalpur, Sherpur, and Barishal district since primitive era. In our concurrent research, the bark of this plant was undertaken in order to justify its folkloric history of thrombolytic, membrane stabilizing activity in vitro model, and for the quantification of total phenol content.

Methods: The concentrated methanol extract partitioned into different fractions showed different amount of yield like 18.2 g of petroleum ether, 13 g of carbon tetrachloride, 11.1 g of chloroform, and 9.9 g of aqueous, respectively, then all of the fractioned extracts were employed to investigate folkloric uses of thrombolytic and membrane stabilizing potential in vitro models.

Results: In the extrapolation of thrombolytic assay, petroleum ether fractioned exhibit the highest percent of clot lysis (27.36% \pm 0.10%) activity compared with other fractioned and extract. Crude methanolic extract profoundly inhibited the breakdown of erythrocytes membrane (65.79% \pm 0.40%)-induced by hypnotic solution and heat. On the other hand, acetylsalicylic acid (reference standard) resulted in 83.147% \pm 0.39% inhibition of clot lysis. Alternatively, aqueous soluble fraction exposed slightly higher level of membrane stabilizing activity provoked by heat-induced hemolysis (75.3 \pm 0.39). **Conclusions:** The present study was investigated to confirm the medicinal value of C. nurvala bark as a thrombolytic and membrane stabilizing agent.

Introduction

Therapeutic herbs are being always provided with new group of chemical entities, e.g., flavonoids, phenols, alkaloids, saponins, glycosides, and quinines, having many biologically active compounds [1]. Thrombosis is an internecine disease and usually it is expressed by the developing of thrombus in the lining of blood circulation or artery, while occurs the inconsistency of homeostatic system in the body. It plays a vital role to block vascular artery while recovering reasons deadly outcomes like myocardial infarction to even death [2]. Generally, the drugs that break the thrombus, work by switching the compound plasminogen to plasmin that clears the cross-connected fibrin work [3]. Inflammatory diseases are prevailing all over the world in modern time. The diseases are manifested by the embellishment of enzyme activation, mediator release, extravasation of fluid, cell migration, and breakdown of tissues and repair, which are aimed at host defense and usually activated in disease condition. Currently, demand is abruptly thriving in the searching of medicinal plants with anti-inflammatory potential which may lead to the finding of new bioactive agent that is not only used to mitigate the inflammation but also used in diverse disease conditions where the inflammation responsible for the aggravating of the disease process. However, the promising

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Contact Zubair Khalid Labu 🖂 zubair.labu@yahoo.com 🗔 Department of Pharmacy, World University of Bangladesh, Dhaka, Bangladesh

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mechanism of membrane stabilizing activity of bioactive compounds are not well understood yet but most of the researchers believed that the presence of significant amount of bioactive compounds such as flavonoids and other phenolic compounds may be primarily responsible for analgesic and anti-inflammatory activity [4]. The current study deals with Crataeva nurvala (C. nurvala) that belongs to the Capparidaceae family. It is a potential indigenous herb in Nepal, Bangladesh, Srilanka, and India as well as other tropical areas in Asian countries [5,6], where popularly called "Barun tiktoshak" in the local community in Bangladesh and India. In Ayurveda formulation, the various plant parts are being used as a main active ingredient to treat many diseases such as inflammation, prostatitis, paralysis, prostate enlargement, thyroid problems, and urinary tract infection [7,8]. In addition, it has been used for the treatment and management of nephritic syndromes, urolithiasis, breast tumor, carbuncle, and contraceptive remedy for birth control [9–11]. The bark of the *C. nurvala* contains Lupeol that is the main biological active compound of this plant. It has showed antioxidant activity [12,13], cardioprotective effect [14], chemo-preventive, and chemotherapeutic potentiality [15]. Moreover, the crude extract of bark exhibited antidiabetic [16], antidiarrhoeal [17], antifertility [18], and analgesic activity [19]. On the other hand, antidiarrhoeal, analgesic, and diuretic activities have proven for the leaves extract of this plant [20]. Interestingly, the root of this plant also showed many therapeutic activities, e.g., antibacterial, wound healing properties, and anthelmintic [21]. The objectives of this study were to investigate the potential membrane stabilizing and thrombolytic activities of *C. nurvala* bark.

Materials and Methods

Drugs and chemicals

The fibrinolytic enzyme "Streptokinase (SK; 1,500,000 I.U Alteplase)" vial was warmly gifted from Beacon Pharmaceuticals Ltd., Bangladesh. Gallic acid, hydrochloric acid, phosphate buffer, acetylsalicylic acid (ASA), dipotassium salt, sodium hydroxide, and sodium carbonate were purchased from the local seller (Dhaka Chemicals). Solvents (carbon tetrachloride, methanol, chloroform, and petroleum ether) and other required analytical grade chemicals and reagents were purchased from local sources (Jewel Chemical Supply and Mitu Chemicals and Equipments).

Justification of dipotassium salt of EDTA use

Dipotassium salt of Ethylene diaminetetraacetic acid (EDTA) is a highly water-soluble salt. It is used here to prevent the coagulation of blood *in vitro*.

Justification of streptokinase use

SK is an enzyme used as a thrombolytic agent to break down the blood clot.

Justification of ASA used

In this study, ASA was used because of its possible mechanism to inhibit the lysosomal enzymes activity or to stabilize the lysosomal membranes.

Collection, identification, drying, and grinding of plant materials

The plant sample (*C. nurvala*) was picked up from Agailjhara, Barisal, Bangladesh in mid-July 2016. The taxonomic identification was performed by the authorized taxonomist from Bangladesh National Herbarium (Accession number: DACB-43485). The collected bark was properly cleaned to remove dust and dirt followed by dried in shady place and milled by locally made grinder prior to get uniform coarse powder. After that, the coarse powder (200 g) was preserved in a hermetic glass container for next processing of the current study.

Extraction and fractionation of plant materials

The plant parts were cut and made into powdered form (200 g), then it was dissolved into 2 L of methanol (90%) and kept stand for 14 days to continuous shaking at 2 days' interval. Then extract was filtrated using Whatman qualitative filter paper #1. Filtrated extraction was evaporated using a digital vacuum rotary evaporator (Scientech Instruments, 110007 Delhi, India) at 40°C for 40 minutes. After that concentrated aqueous methanol extract (Crude methanol extract (CME) # 52.2 g) was fractionated by Kupchan and Van Wagenen technique with partially modified [22,23]. Different fractions showed different amounts of yield such as 18.2 g of petroleum ether, 13 g of carbon tetrachloride, 11.1 g of chloroform, and 9.9 g of aqueous, respectively.

Phytochemical screenings

Crude methanol bark extract *C. nurvala* was prepared by dissolving into 50 ml of 90% methanol to evaluate phytochemical screenings or to ensure the existence of various phytochemical groups such as steroids, alkaloids, tannins, glycosides, phenols, saponins, flavonoids, resins, and in the bark extract and its fractions [24–26].

Streptokinase solution preparations

SK solution preparations Lyophilized SK vials of 1,500,000 I.U, which the trade name is S-Kinase gifted from Popular Pharmaceuticals Ltd., was used as a standard for thrombolytic test. Once the vial was collected and 5 ml of 0.9% NaCl was poured, mixed properly by occasional shaking. This suspension (30,000 I.U) was employed for reference.

In vitro thrombolytic activity

Thrombolytic effects of different solvents extract of the C. nurvala bark were assessed followed by the previously described method [27]. The blood (5 ml) samples were collected among the healthy male students of World University of Bangladesh who have the hemoglobin level within the range. The blood samples were poured into Eppendorf tubes, then 45 minutes incubated (37°C) for the formation of blood clot. Without disturbing, the serum completely removed from blood clot sample and weighted again. An aliquot of 100 µl of CME which each fraction was added in every tube, again incubated (37°C) for 45 minutes and later released fluid was removed. Weight of all the tested tubes was measured once again for the analysis of clot lysis. In this study, SK (~30,000 I.U.) and distilled water were employed for the purposes of standard and negative control, respectively. The clot lysis percentage of samples was calculated according to the equation as follows:

Clot lysis (%) = (weight of clot after employed/weight of clot before employed) × 100

Ethics approval and consent to participants

The protocol of the study was submitted to the Committee for Ethics and Research of World University of Bangladesh (Grant No. WUBPS # 05512) and was approved. The volunteer blood donors gave their consent for using their blood in our current study.

In vitro membrane stabilizing activity

The human erythrocyte membrane stabilizing potential of methanolic bark extract of *C. nurvala* and its different Kupchan fractionates were evaluated by determining their capability to suppress the lysis of red blood cells (RBCs) triggered by heat or hypotonic solution [28].

Sample preparation for membrane stabilization assay

Two milliliters of blood sample was withdrawn from volunteers of healthy male having hemoglobin within range (13.8–17.2 g/dl) (approved the protocol by the Ethical Committee of WUB Research Section, World University of Bangladesh). The collected blood sample was poured into sterile test tubes containing EDTA (dipotassium salt) (2 mg/ml of blood sample) with standard condition (Temp, $24^{\circ}C \pm 2^{\circ}C$, RH, 55% ± 10%). 1.5 ml micro-centrifuge sterile tubes were filled with an aliquot of 1 ml of blood and centrifuged for 10 minutes (2,500 rpm). Thereafter, the centrifuged liquid was allowed to keep in normal temperature for 5 minutes. The supernatant thus obtained was separated, remaining cell suspension was diluted with equal volume of sterile normal saline (0.9% NaCl) and applied for centrifugation consecutive three times to get the diaphanous supernatant. The clear supernatant was decanted, and remaining RBC cells were undertaken for membrane stabilizing activity. Finally, cellular components (0.5 ml erythrocyte suspension, 5 ml hypotonic solution, and 50 mM NaCl in 10 mM sodium phosphate buffered saline) were well mixed for in vitro membrane stabilizing assay [29].

Hypotonic solution-induced hemolysis

CME (2 mg/ml) and its fractions of each sample and ASA (0.1 mg/ml) were taken into sterile centrifuge tubes separately and mixed by a sonicator. The control sample consisted without reference standard (ASA). The mixture was applied to centrifugation force at 3,000 rpm for 10 minutes, and then incubated the samples for 10 minutes at ambient temperature followed by estrangement of supernatant from a decantation. The UV/Visible (UV/Vis) spectrophotometer was used to measure the absorbance of supernatant.

The percentage inhibition of hemolysis was estimated using the equation

% inhibition of hemolysis = $100 \times (OD_1 - OD_2/OD_1)$

where

Control group= OD_1 (Optical density of hypotonic-buffered saline solution)

Treatment group= OD_2 (Optical density of test sample in hypotonic solution).

Heat-induced hemolysis

The heat-induced lysis of erythrocyte membrane was led conferring the technique of Okoli *et al.*, with partial changes [30]. The experiment was carried

out in two sets of centrifuge tubes, in which 2 mg/ ml of different extractive solutions were added. One set of centrifuge tubes was prepared for positive control by adding 5 ml of isotonic buffered solution and ASA at a concentration of 0.1 mg/ml and another set regarded as negative control containing only 5 ml of the isotonic buffered solution. Erythrocyte suspension $(30 \,\mu)$ was incorporated to all test tubes and mixed gently by inversion. ASAcontaining tubes were incubated in a water bath at 54°C for 20 minutes and the other set of tubes were kept in an ice bath at 0°C-5°C. At the end of the incubation period, the samples were subjected to centrifuge for 10 minutes at 3,000 rpm and the absorbance of the supernatant was measured at 540 nm using UV/Vis spectrophotometer. The percentage inhibition of hemolysis was estimated using the following equation:

% inhibition of hemolysis = $100 \times [1 - ((OD_2 - OD_1))/(OD_3 - OD_1))]$

where

Test sample (unheated) = OD_1

Test sample (heated) = OD_2

Control sample heated = OD_{2} .

Data analysis

Data were offered as mean \pm SEM. The SPSS software (version 21.0) and Microsoft excel 2010 were used for data and graphical analysis. For the comparison of data with control group, treatment group, and standard, One-way Analysis of Variance (ANOVA) was used followed by Dunnett's *t*-test.

Results

Screening of phytochemical group test

The bark of *C. nurvala* extract showed the existence of various groups of phytochemical given in Table 1.

Table 1. Phytochemical screening of C. nurvala barksextract and its fractions.

Phytochemicals	CME	PES	CTSF	CSF	AQSF
Alkaloids	++	-	+	++	++
Carbohydrates	-	-	-	-	-
Saponins	-	-	-	-	-
Tannins	+	+	+	+	-
Resins	+++	+	+++	-	-
Flavonoid	++	+	-	+	-
Steroids	++	++	++	-	-
Phenols	++	++	-	+	-

The presence and tentative content of different phytochemicals were ensured based on the intensity of the color development.

+ = present in mild amount, ++ = present in moderate amount, +++ = present in large amount, - = not present.

CME = crude methanol extract, PES = petroleum ether soluble fraction, CTSF = carbon tetrachloride soluble fraction, CSF = chloroform soluble fraction, AQSF = aqueous soluble fraction.

Thrombolytic activity

For the revelation of cardio-defensive medications from characteristic plants, different extracts of bark of *C. nurvala* were screened intended for thrombolytic action using a positive control SK [31] and the results are presented in Table 2. The highest percentage of clot lysis is showed by PSF (27.36% \pm 0.23%), and the lowest activity is showed by the CME (12.88% \pm 0.33%). Based on the result of the study, we can see that *C. nurvala* bark extract showed moderate thrombolytic activity.

Membrane stabilizing activity

Once equated to the standard drugs (ASA), the methanolic bark extract of C. nurvala and its fractions (2 mg/ml) expressively inhibited the hemolysis of human RBC membrane. The heat-induced membrane stabilizing activity was recorded in according to ASA (83.55% ± 0.28%), AQSF (75.73% ± 0.48%), CSF (72.33% ± 0.39%), CME (71.70% ± 0.11%), PSF (64.70% ± 0.11%), and CTSF (53.48% \pm 0.75%) (Table 2). In hypotonic solution-induced hemolysis, the magnitude of membrane stabilization was shown by the ASA $(83.14\% \pm 0.39\%)$, CME (65.79% ± 0.47%), PSF (60.67% ± 0.18%), AQSF (57.72% ± 0.40%), CTSF (54.77% ± 0.70%), and CSF (49.24% ± 0.36%). Analyzing the result, it may be assumed that (considering > 60% excellent); (***p* < 0.01; **p* < 0.05) *C. nurvala* bark extract exhibited excellent membrane stabilizing activity as presented in Table 2.

Discussion

C. nurvala bark methanol extracts and their Kupchan partitionates were evaluated to confirm the existence of various kinds of bioactive compounds. Diverse phytochemicals, for example, steroids, alkaloids, tannins, glycosides, saponins, pitches, flavonoids, phenols, and quinines were found in this work. Phytoconstituents portrayed in the present investigation are known to be gainful in modern

Table 2. Membrane stabilizing and thrombolytic activity ofcrude extracts and different fractions of barks C. nurvala.

	Percent inhibitio	Democrat of elect		
Sample	Hypotonic solution-induced	Heat-induced	lysis	
CME	*65.79 ± 0.47	**71.70 ± 0.11	12.88 ± 0.33	
PES	*60.67 ± 0.18	*64.70 ± 0.32	*27.36 ± 0.23	
CTSF	*54.77 ± 0.70	53.48 ± 0.75	*20.74 ± 0.43	
CSF	49.24 ± 0.36	**72.33 ± 0.39	19.90 ± 0.43	
AQSF	*57.72 ± 0.40	**75.73 ± 0.48	*21.64 ± 0.25	
ASA	**83.14 ± 0.39	**83.55 ± 0.28	-	
SK	-	-	**65.15 ± 0.16	
Negative control	-	-	8.20 ± 0.16	

Values are expressed as mean \pm SD, *p < 0.05; **p < 0.01; significant when compared with the corresponding value of control.

and therapeutic sciences. The learning of this preparatory examination can be expected as interpret in the pursuit of a novel and monetarily esteemed medication molecules [32,33].

Thrombolytic activity

Thrombosis process is kicked off by activating platelets and also binding to the leucocytes that bring them into a formation as well as growth of plaque [34]. Plasmin is a fibrinolytic agent by nature, which is made effortlessly by the conversion of plasminogen and may lead to prompt breakdown of thrombus [35]. SK is an enzyme that is capable of converting tissue plasminogen to plasmin [36]. Recombinant DNA technology has also been utilized to formulate numerous thrombolytic medicines of highly potent and target specific [37], but some adverse effects such as bleeding and embolism have been demonstrated from these drugs, which lead to further complications [38].

The main aim of the present research was to evaluate the clot lysis efficiency of bark extract of *C. nurvala*. Our findings showed that different fractions (PSF, AQSF, CTSF, CSF, and CME) of bark of *C. nurvala* showed moderate thrombolytic activity as compared to that of standard drugs. The outcomes from the examination obviously showed that *C. nurvala* has the clot lysis activity.

Membrane stabilizing activity

The study substantiates that CME, as well as its different Kupchan specimens, thwarted the RBC membrane lysis-induced with the heat and hypotonic solution. In our quantitative phytochemical analysis, the flavonoids and other phenolic compounds present in a significant amount kept evidence that the bark extract of C. nurvala has membrane-stabilizing activity (Table 2). Microorganism secreted various kinds of lysosomal enzymes and other hydrolytic compounds as of the phagocytes of the extracellular space that leads the nearby cells and tissues to get injured as well as provoke several disorders [39]. Generally, Non steroidal anti-inflammatory drugs (NSAIDs) such as ASA acts by either lysosomal membranes stabilization or lysosomal enzymes inhibition. An RBC exposure to external substances, e.g., hypotonic medium or heat causes oxidation and lysis of hemoglobin and hence membrane lysis occurs [40]. There are huge similarities in the components of human RBC membrane and lysosomal membrane. and hence, it is quite compatible to perform in vitro anti-inflammatory study using human RBC cells. Thus, the hypotonicity and heat-induced RBC membrane lysis potential exhibit upon treatment with plant extracts are considered as significant indicators to confirm the extracts as anti-inflammatory effects [41,42]. According to the previous studies (in vivo and in vitro), flavonoids were found to possess membrane stabilizing potential on lysosomal membrane [43]. In addition, tannins and saponins-rich plant extracts show the stabilizing activity over erythrocyte member due to the binding capability of those compounds to cations and other biomolecules of the membrane surface [44].

Conclusion

From the traditional evidence to scientific investigation, this study will be very fruitful for the consumption of *C. nurvala* bark to treat many kinds of disease (thrombosis and inflammation) as a folk medicine in Bangladesh. The outcomes of this research revealed the potential medicinal properties of bark extract of *C. nurvala* against inflammation, formation of thrombus, and oxidative degradation of cellular components. Furthermore, comprehensive study can be conducted for the chemical characterization of the corresponding chemical compound responsible for the aforementioned medicinal values.

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Availability of data and materials.

All data are placed in the manuscript.

Authors' Contributions

Zubair Khalid Labu developed the necessary methods, analyzed the data, and provided idea to furnish the manuscript. Jalal Uddin collected the plant part, partitioned, and performed the assays. Farhina Rahman Laboni studied the literature and performed assays. Samira Karim prepared the primary manuscript.

Conflict of Interest

We do not have any conflict of interest.

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