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PHYTOCHEMICAL SCREENING, *IN-VITRO* MEMBRANE STABILIZING AND THROMBO-LYTIC ACTIVITIES OF *LOPHOPETALUM JAVANICUM*

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ABSTRACT: In the present study, different extracts of *Lophopetalum javanicum* (Zoll) leaves were evaluated for erythrocyte membrane stabilizing and thrombolytic activities. Phytochemical analysis of the plant was also performed to detect presence of different kinds of phytoconstituents. In order to predict anti-inflammatory activity the plant extracts were subjected to check erythrocyte membrane stabilization using heat and hypotonic solution induced hemolysis method. The crude methanolic extract, the aqueous and chloroform, pet-ether soluble fractions of *L. javanicum* leaves demonstrated strong membrane stabilizing activity compared to acetyl salicylic acid. In thrombolytic study, the crude methanolic extract, pet-ether, chloroform and carbon tetrachloride soluble fractions demonstrated strong thrombolytic activity in human blood specimen. Phytochemical screening revealed the presence of flavonoids, steroids, tannins and triterpenoids as major constituents in the extracts of the plant which may be responsible for the observed biological effects.

INTRODUCTION: *Lophopetalum javanicum* (Family: Celastraceae) is a plant species found mainly in lowland rainforest, sometimes in hill up to 1,400 m. It is native in Indonesia, Malaysia, Papua New Guinea, the Philippines, Thailand, and Vietnam [1]. Synonyms of the plant are *Hippocratea maingayi* (non Laws.) Vidal, *Lophopetalum celebicus* Koord, *Lophopetalum fimbriatum* (non Wight) F. Vill, *Lophopetalum fuscescens* Kurz, *Lophopetalum intermedium* Ridl, *Lophopetalum oblongifolium* King, *Lophopetalum oblongum* King, *Lophopetalum paucinervium* Merr, *Lophopetalum toxicum* Loher, *Solenospermum javanicum* Zoll, *Solenospermum oblongifolius* Loes, *Solenospermum paucinervium* Loes, *Solenospermum toxicum* Loes. Its common names are abuab (Filipino), perupok (Malay), madang-gambici (Indonesian), mandalaksa, tatokwa, kachang rimba, perupok dual, buyun, sampol etc. It is an emergent tree up to 56 m tall and 125 cm dbh. The leaves are opposite, simple, penni-veined and the flowers are ca. 6 mm in diameter, white-yellow, placed in panicles. The Fruits are ca. 72 mm long, green-brown, dehiscent capsules filled with wind dispersed winged seeds (wing completely enclosing seed). The bark is used as a constituent of dart poison. To the best of our knowledge no scientific work on this plant has been performed yet.

As a part of our continuing investigation of medicinal plants of Bangladesh, the crude methanol extract, aqueous and organic soluble fractions of *Lophopetalum javanicum* were studied for phytochemical screening, erythrocyte membrane stabilizing and thrombolytic activities for the first time and we, here in, report the results of our preliminary investigations.

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MATERIALS AND METHODS:

Plant material: The leaves of *Lophopetalum javanicum* were collected from Botanical garden, Mirpur, Dhaka, Bangladesh. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no.36570). The sun dried and powdered leaves (500 gm) of *L. javanicum* was macerated in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45 ºC) and reduced pressure. The concentrated methanolic extract (ME) was fractionated by modified Kupchan partitioning method [2] and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental purposes.

Preliminary phytochemical screening: One gram of the methanol extract of *Lophopetalum javanicum* were dissolved in 100 ml of its own mother solvent and subjected to preliminary phytochemical screening to detect the presence various classes of phytoconstituents. Phytoconstituents like saponins, tannins, steroids, flavonoids and triterpenoids were identified by characteristic color changes using standard procedures [3, 4].

Membrane stabilizing activity: The membrane stabilizing activity of the extractives was determined by using hypotonic solution-induced and heat-induced hemolysis of human erythrocyte membranes by the method developed by Shinde *et al* [5] and modified by Sikder *et al* [6]. In hypotonic solution-induced method, the test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) is mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extracts (1.0 mg/mL) or acetyl salicylic acid (0.1 g/mL). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation –

% inhibition of haemolysis = 100 x (OD_1-OD_2/OD_1)

Where, OD_1=optical density of hypotonic - buffered saline solution alone (control) and OD_2= optical density of test sample in hypotonic solution.

In heat-induced haemolysis, isotonic buffer containing aliquots (5 ml) of the different extracts were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54 o C for 20 min in a water bath, while the other pair was maintained at (0-5) o C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

% Inhibition of hemolysis = 100 x [1-(OD_2-OD_1/OD_3-OD1)]

Where, OD_1= optical density of unheated test sample, OD_2= optical density of heated test sample and OD_3=optical density of heated control sample.

Thrombolytic activity: The thrombolytic activity of all extracts was evaluated by the method developed by Daginawala *et al* [7] and modified by Kawsar *et al* [8] using streptokinase (SK) as the standard. In short, the plant extract (100 mg) suspended in 10 ml of distilled water was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22-micron syringe filter. For clot lysis, venous blood (500 µl) drawn from healthy volunteers was distributed in different sterile pre weighed microcentrifuge tube and incubated at 37°C for 45 minutes.

After clot formation, the serum was completely removed without disturbing the clot and the clot weight was determined. 100 µl aqueous solutions of different partitionates and crude extract was added separately to each microcentrifuge tube with the pre-weighed clot. Then, 100 µl (30,000 I.U) of commercial streptokinase (SK) and 100 mg of distilled water were separately added to the control tube as positive and negative controls, respectively.

All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid 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 as shown below:

% of clot lysis = (weight of released clot /clot weight) ×100

RESULTS AND DISCUSSION:

Preliminary phytochemical screening: Phytochemical studies showed the presences of flavonoids, tannins, triterpenoids and steroids methanol extract, flavonoids and tannins in n-hexane, tannins in CSF and flavonoids, triterpenoids and steroids in AQSF of the leaves of *L. javanicum* (**Table 1**). However, saponin was absent in methanol extract and all fractionates of the plant.

TABLE 1: ANALYSIS OF PHYTOCHEMICALS IN DIFFERENT EXTRACTS AND FRACTIONS OF *L. JAVANICUM.*

Phytochemicals	ME	n-hexane	CSF	AQSF
Flavonoids	+	+	-	+
Saponins	-	-	-	-
Tannins	+	+	+	-
Triterpenoids	+	-	-	+
steroids	+	-	-	+

'+' = presence. '-' =absence; ME= Methanolic extract; n-hexane = n-hexane soluble fraction; CSF = Chloroform soluble fraction; AQSF =Aqueous soluble fraction of the methanolic extract of *L. javanicum.*

Membrane stabilizing activity: The various extracts of *L. javanicum* leaves (1.0 mg/mL) showed significant activity against lysis of human erythrocyte membrane induced by hypotonic solution or heat (**Table 2**). In hypotonic solution induced condition, PESF showed highest protection of erythrocyte membrane (69.08% % inhibition of haemolysis) which was similar to haemolysis inhibited by the standard acetyl salicylic acid (0.10 mg/mL). Methanolic extract and other solvents soluble fractions significantly inhibited haemolysis of RBC. In heat induced condition, methanolic extract and different solvents soluble fractions of *L. javanicum* inhibited 16.05 % and 4.23 -28.12 % hemolysis of RBC, respectively as compared to 42.12% inhibited by acetyl salicylic acid.

Since human red blood cell (HRBC) membranes are similar to lysosomal membrane components [9], the inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory effect of the plant extract. Membrane stabilization results in prevention of leakage of serum proteins and fluids into the tissues during a period of increased permeability caused by inflammatory mediators [10]. Phytochemical screening showed that the plant extract contains flavonoids which have been reported to possess potent anti-inflammatory property [11, 12]. The anti-inflammatory activity is probably due to the inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid [13].

TABLE 2: EFFECTS OF DIFFERENT EXTRACTS AND FRACTIONS OF *L. JAVANICUM* LEAVES ON HAEMOLYSIS OF ERYTHROCYTE MEMBRANE.

Samples	Concentration (mg/mL)	% inhibition of haemolysis	
		Hypotonic solution induced	Heat induced
ME	1.0	52.72±0.87	16.05±0.76
PESF	1.0	69.08±0.38	13.55±0.82
CTCSF	1.0	55.64±0.79	14.43±0.71
AQSF	1.0	57.68±0.93	28.12±0.38
CSF	1.0	66.33±0.88	4.23±0.37
Acetyl salicylic acid	0.1	71.9±0.78	42.12±0.38

ME = Methanolic extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = Aqueous soluble fraction of methanolic extract of *L. javanicum.*

Thrombolytic activity: As a part of discovery of cardio protective drugs from natural resources, the extracts of *L. javanicum* were assessed for thrombolytic activity using a simple and rapid *in-vitro* clot lysis model (**Figure 1**). Addition of 100 µl (30,000 I.U.) SK (positive control) to the clots and subsequent incubation for 90 minutes at 37°C exhibited 66.57% lysis of clot, whereas distilled water (negative control) showed a negligible percentage of lysis of clot (3.87%). Methanolic extract and all fractions exhibited significant thrombolytic activity. The highest thrombolytic activity (55.61%) was demonstrated by the methanolic extract of *L. javanicum*. The thrombolytic activity is probably due to the plant`s diverse composition like flavonoids, tannins and terpenoids [14].

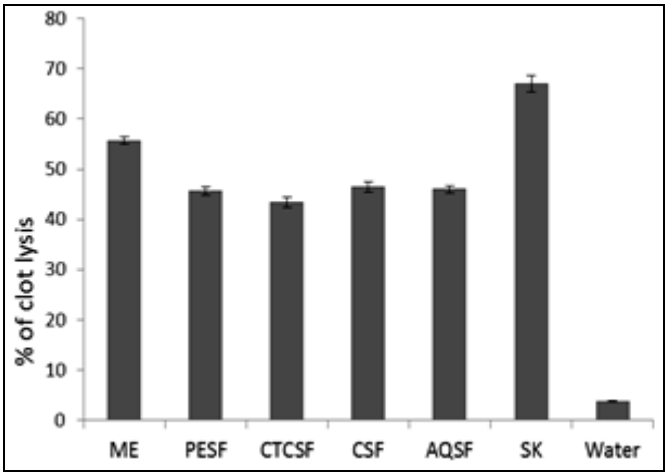

FIGURE 1. THROMBOLYTIC ACTIVITY OF CRUDE EXTRACT AND DIFFERENT FRACTIONS OF *L. JAVANICUM* (ME = Methanolic extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = Aqueous soluble fraction of the methanolic extract, SK = Streptokinase).

CONCLUSION: Results of the present study for the first time indicate that the *L. javanicum* leaves possess significant membrane stabilizing and thrombolytic activities and suggests that the plant may be a safe, economical and easily available source of natural agents used in inflammation and cardiovascular disorders involving blood clot. Further studies will be conducted for isolation and purification of the active principles of the plant responsible for the observed biological effects.

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