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Simplified the Screening and *In Vitro* Appraisal of Antioxidant, Cytotoxic, Thrombolytic, Antimicrobial and Membrane Stabilizing Activities of *Lablab Purpures* at a Time

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Abstract

To simplify the different biological investigation of the methanolic extract and solvent-solvent partitioning of *Lablab purpures* (*L. purpures*) bark. In-vitro anti-oxidant study was determined using total DPPH radical scavenging assay. In vitro antimicrobial study was measured by observing zone of inhibition. The cytotoxic activity was studied using brine shrimp lethality bioassay and thrombolytic activity by clot disruption method. The antioxidant potential was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteau reagents using butylated hydroxytolune (BHT) and ascorbic acid as standards. The Aqueous soluble fraction revealed the highest free radical scavenging activity (IC_{50} = 48.76 μg/mL). The antimicrobial screening of the bark of *L. purpures* exhibited mild to moderate activity in test microorganisms. The CSF showed the maximum relative percentage inhibition against *Salmonella parathyphi* (34.2%) for bacteria and *C. albicans* (28.8%) for fungi whereas, lowest relative percentage inhibition against *Sarcina lutea* (22.0%) for bacteria and *Aspergillus niger* (24.4%) for fungi. In the brine shrimp lethality bioassay, The LC_{50} values of Carbon tetrachloride and N-Hexane soluble fraction were found 92.18 μg/mL, and 68.95 μg/mL respectively while the LC_{50} values of standard Vincristine sulphate was 1.37 μg/mL. The methanolic extract and its organic soluble fractions of *Lablab purpureus* at concentration 2.0 mg/mL, significantly protected the lysis of erythrocyte membrane induced by hypotonic solution and heat as compared to the standard, acetyl salicylic acid (0.10 mg/mL). The MSF and AQSF produced 61.48 % and 53.75% inhibition of hemolysis of RBC caused by hypotonic solution respectively, whereas acetyl salicylic acid (0.10 mg/mL) showed 76.42%. Ethanol extract of *L. purpures* and all of its different partitions exhibited moderate thrombolytic activity of 37.25%-2.40%. Very good preliminary screening and simplified experiments were able to show the different biological activity of methanolic extract and its soluble fractions of *L. purpures* at a time.

Key words: *L. Purpures*, Antioxidant, Antimicrobial, Cytotoxic, Thrombolytic

1. Introduction

Plant kingdom is the highest versatile sources of phytochemicals and 80% people of the developing countries are used their traditional plant for their primary health care but only very small fractions of those medical plants have been proved scientifically. Now a day, among the world the use of medicinal plants to solve the healthcare problem is increasing. The active principles differ from plants to plants due to their biodiversity and produce a definite physiological action on the human body that develops interest on their medicinal properties and importance in the pharmaceutical industries. Today about 300 species of medicinal and aromatic plants as well as their secondary metabolites are used worldwide in the pharmaceuticals, foods, cosmetics and perfume industries[1].The investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs[2].

In Bangladesh thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has

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been in vogue since ancient time. *Lablab purpureus* sweet or Hyacinth bean is an important vegetable crop in Bangladesh In Bangladesh Hyacinth bean is grown for fodder and cover crop, the leaves and seeds contain 20-28% protein, with well balance amino acid composition[3]. The fresh pods and green seeds are eaten boiled or used in curries, mature seeds are used as pulses, often in soup. In addition to starch, beans contain proteins and amino acids as principal Chemical Constituents. Leaves and fruits contain sterols (including cholsetorol and its derivatives), fatty acids, palmitic, palmitoleic, linoleic and linolenic acids. A pyridine alkaloid, trigonelline and sterols have been isolated from tissue cultures of seeds, stems and leaves. Pod exudates contain as many as 42 different fatty acids and their Me-esters (C11-C25). Seeds contain antimicrobial proteins, globulins, albumins and lectins; leghaemoglobin, amionpropylamino alcohols and a number of polyamines including diaminopropane, putrescine, spermine, thermospermine, aminopropylhomosperemidine and spermidine, lipids, fatty acids, enzyme, β-N-acetylhexosaminidase and carbohydrates. Cytokinins have been isolated from immature seeds. Pectic polysaccharides have been isolated from the seed husks[4-7]. A biologically active substance obtained from immature seeds and identified as 3-O-β-D-glucopyranosyl gibberellin A1 was much less active than gibberellin A1. L-pipecolic acid has been isolated from legumes[8]. Literature reviews indicated that no combined studies in antioxidant, antimicrobial and thrombolytic activities of leaves of *Lablab purpureus* have so far been undertaken. Taking this in view and as a part of our ongoing research[9] on Bangladeshi medicinal plants, the present study was aimed to evaluate the comparison of antioxidant, antimicrobial, as well as thrombolytic activities of the methanolic extract and its various organic soluble fractions of the leaves of *Lablab purpureus* in different experimental model.

2. Experimental Section

2.1. Plant Materials and Extraction

The fresh leaves of *Lablab purpureus* were collected from Jessor, Bangladesh in July, 2012 and identified by DR. M.A. Razzaque Shah PhD, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh and the voucher specimen maintained in our laboratory for future reference. The both plant materials were shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve#40 and stored in an air-tight container. The dried powder material of leaves (1.0 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness; *in vacuo* at 40°C to render the MeOH extract 250 g. This extract was suspended in H_2O and then successively partitioned with hexane (C_6H_{12}), carbontetrachloride (CCl_4), chloroform ($CHCl_3$), to afford the C_6H_{12} (20 g), CCl_4 (50 g), $CHCl_3$ (70 g), fractions and the H_2O residue (110 g).

2.2. Chemicals

Streptokinase was collected from Beacon pharmaceutical Ltd., Bangladesh. Aspirin was bought from Incepta Pharmaceuticals Ltd., Bangladesh. Artemia salina leach (brine shrimp eggs), sea salt (NaCl), n-hexane, carbon tetra chloride, chloroform, methanol, ascorbic acid, tert-butyl-1-hydroxytoluene, Na_2CO_3 solution (7.5%), nutrient agar medium, and ethanol were obtained from Merck, Germany and were in analytical grade. RBCs were collected from the human male (45 kg) brown complexion and free from diseases. The collected RBC was kept in a test tube with an anticoagulant EDTA under standard conditions of temperature (28±2)°C and relative humidity (65±10)%.

2.3. Test Microorganisms

Strains of both fungi and bacteria (Gram positive and Gram negative) were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). *Bacillus cereus* ATCC 14579, *Bacillus megaterium* ATCC 13578, *Bacillus subtilis* ATCC 6059, *Staphylococcus aureus* ATCC 6538, *Sarcina lutea* ATCC 9341, *Escherichia coli* ATCC 25922, *Pseudomonus aeruginosa* ATCC 27853, *Salmonella paratyphi* ATCC 9150, *Salmonella typhi* ATCC 13311, S*higella boydii* ATCC 9234, *Shigella dysenteriae* ATCC 9361, *Vibrio mimicus* ATCC 33653, *Vibrio parahemolyticus* ATCC 17802, *Candida albicans* ATCC 90028, *Aspergillus niger* ATCC 1004 and *Sachoromyces cerevacae* ATCC 60782 were used as test microorganism. All these bacterial and fungal species are recommended by ATCC for their susceptibility assay. The strains are maintained and tested on Nutrient Agar media (NA) for bacteria and Sabourand dextrose agar media (SDA) for fungi.

2.4. Free Radical Scavenging Activity Measured by 1,1-Diphenyl-2-picryl-hydrazyl (DPPH)

The free radical scavenging activity of MeOH extract and its organic soluble fractions, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.*[10]. Plant extract (0.1 mL) was added to 3mL of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was measured after 30 min and the percentage inhibition activity was calculated from $[(A_0-A_1)/A_0]\times100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (μg/mL) versus % inhibition.

2.5. *In vitro* Thrombolysis Activity

Phosphate buffered saline (PBS) (5 mL) was added to the commercially available lyophilized streptokinase vial (1,500,000 I.U.) and mixed properly. This suspension was used as a stock from which appropriate dilutions were made to observe the thrombolytic activity. Experiments for clot lysis were carried as reported earlier[11]. In brief, 2 mL venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile microcentrifuge tube (0.5 mL/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) 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 microcentrifuge tube containing pre-weighed clot, 100 μL of extract/fractions (10 mg/mL) of was added. As a positive control, 100 μL of streptokinase 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.

2.6. Brine Shrimp Lethality Bioassay

The toxic potentiality of the plant crude extract was evaluated using Brine Shrimp lethality bioassay method[12] whereas 6 graded doses (viz,. 10, 20, 40, 80, 160, and 320 μg/mL) were used. Brine shrimps (*Artemia salina* Leach) nauplii Ocean 90, USA were used as test organisms. For hatching, eggs were kept in brine with a constant oxygen supply for 48 hours. The mature nauplii were then used in the experiment. DMSO was used as a solvent and also as a negative control. The median lethal concentration LC_{50} of the test sample after 24 hours was obtained by a plot of percentage of the dead shrimps against the logarithm of the sample concentration. Tamoxifin, a well-known anticancer drug was used as a reference standard in this case.

2.7. Antimicrobial Activity

Crude methanolic extract was dissolved in 10% DMSO to get a concentration of 400 μg/mL and sterilized by filtration by 0.45 μm Millipore filters. Standard antibacterial agents Ciprofloxacin (30 μg/disc) were prepared. Antimicrobial tests were then carried out by modified agar diffusion method[13,14] using 100 μL of suspension containing 10^8 CFU/mL of bacteria, 10^6 CFU/mL of yeast and 10^4 spore/mL, spread on nutrient agar (NA) and subourand dextrose agar (SDA), respectively[14]. Bacteria were cultured overnight at 37°C and fungi at 28°C for 72 hours used as inoculums. Nutrient agar (20 mL) was dispensed into sterile universal bottles. These were then inoculated, mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6 mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50 μL of the extract at a concentration of 400 μg/mL and were allowed for diffuse (45 min). The plates were then incubated at 37°C for 24 hours for bacteria. The above procedure was followed for fungal assays and the media used was sabourand dextrose, incubated at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicate.

2.8. Determination of Relative Percentage Inhibition

The relative percentage inhibition with respect to positive control was calculated by using the following formula[15]. Relative percentage inhibition of the test extract = $[\{100\times(a-b)\}/(c-b)]$. Whereas, a: total area of inhibition of the test extract; b: total area of inhibition

of the solvent; c: total area of inhibition of the standard drug. The total area of the inhibition was calculated by using area = r^2; where, r = radius of the zone of inhibition.

2.9. Preparation of Erythrocyte Suspension

Fresh whole blood (3 mL) collected from healthy volunteers into heparinised tubes was centrifuged at 3,000 rpm for 10 min. A volume of normal saline equivalent to that of the supernatant was used to dissolve the red blood pellets. The volume of the dissolved red blood pellets obtained was measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of NaH_2PO_4, 1.15 g of Na_2HPO_4 and 9 g of NaCl in 1 L of distilled water. The reconstituted red blood cells (resuspended supernatant) were used as such.

The effects of the *Lablab purpureus* extract/fractions on haemolysis of HRBC induced by heat and distilled water was evaluated using the method of Shinde *et al.*[16] with some modifications.

2.10. Heat Induced Haemolysis

Samples of the extract/fractions used were dissolved in isotonic phosphate buffer solution. A set of 5 centrifuge tubes containing respectively, 5 mL graded doses of the extracts (2 mg/mL) were arranged in quadruplicate sets (4 sets per dose). Two sets of control tubes contained 5 mL of the vehicle and 5 mL of 200 μg/mL of indomethacin respectively. HRBC suspension (0.1 mL) was added to each of the tubes and mixed gently. A pair of the tubes was incubated at 54°C for 20 min in a regulated water bath. The other pair was maintained at 10°C in a freezer for 20 min. Afterwards, the tubes were centrifuged at 1,300 g for 3 min and the haemoglobin content of the supernatant was estimated using Spectronic 21D (Milton Roy) Spectrophotometer at 540 nm. The percent inhibition of haemolysis by the extract was calculated thus: % Inhibition of Haemolysis = $[(1-\{(OD_2-OD_1)/(OD_3-OD_1)\})\times 100]$; Where OD_1= absorbance of test sample unheated; OD_2= absorbance of test sample heated; OD_3= absorbance of control sample heated.

2.11. Hypotonicity Induced Haemolysis

Samples of the extract used in this test were dissolved in distilled water (hypotonic solution). The hypotonic solution (5 mL) containing graded doses of the extracts (2 mg/mL) were put into duplicate pairs (per dose) of the centrifuge tubes. Isotonic solution (5 mL) containing graded doses of the extracts (100-800 μg/mL) were also put into duplicate pairs (per dose) of the centrifuge tubes. Control tubes contained 5 mL of the vehicle (distilled water) and 5 mL of 200 μg/mL of indomethacin respectively. Erythrocyte suspension (0.1 mL) was added to each of the tubes and mixed gently. The mixtures were incubated for 1 hour at room temperature (37°C), and afterwards, centrifuged for 3 min at 1,300 g. Absorbance (OD) of the haemoglobin content of the supernatant was estimated at 540 nm using Spectronic 21D (Milton Roy) spectrophotometer. The percentage heamolysis was calculated by assuming the heamolysis produced in the presence of distilled water as 100%. The percent inhibition of haemolysis by the extract was calculated thus: % Inhibition of Haemolysis = $[(1-\{(OD_2-OD_1)/(OD_3-OD_1)\})\times 100]$; Where OD_1= absorbance of test sample in isotonic solution; OD_2= absorbance of test sample in hypotonic solution; OD_3= absorbance of control sample in hypotonic solution.

2.13. Statistical Analysis

All the *in vitro* experimental results were given as mean±SEM of three parallel measurements and data were evaluated by using student's t test. *P* values$<$0.001 were regarded as significant.

3. Results and Discussion

3.1. Antioxidant Activity

Anti-oxidant activity of the methanolic extract of *L. purpureus* (MSF) and its different organic soluble fractions HXSF, CTCSF, CSF and AQSF were tested for DPPH scavenging activity. IC_{50} value in different extract/fractions was found 48.76-191.97 (Fig. 1(a)). Among all extract/fraction, the highest IC_{50} was found in AQSF as 48.76 whereas the standard ASC and BHT was showed as 8.71 and 26.26 respectively. Significant amount % of inhibition was also present in all extract/fractions at 5-320 μg/mL (Fig. 1(b)).

The stable DPPH radical model is widely used and was found relatively quick method for the evaluation of free radical scavenging activity. DPPH is a stable free radical that accepts an electron or hydrogen radical to

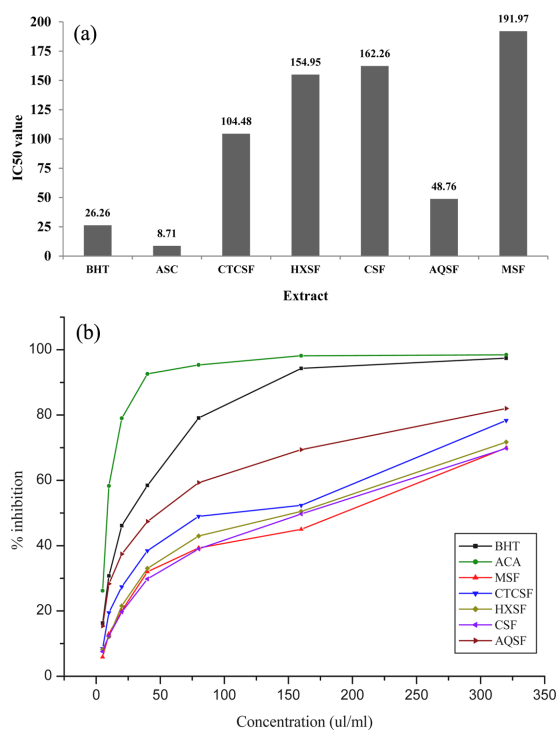

Fig. 1. Free radical (DPPH) scavenging activity of *Lablab purpureus* leaves. IC_{50} value (a), % inhibition of DPPH (b) in various concentrations of standard and *Lablab purpureus* methanolic extracts with its various organic soluble fractions. MESF: Methanolic extract; CTCSF: Carbon tetrachloride soluble fraction; HXSF: N-Hexane soluble fraction; CSF: Chloroform soluble fraction; AQSF: Aqueous soluble fraction; BHT: tert-butyl-1-hydroxy-toluene; ASC: Ascorbic acid.

become a stable diamagnetic molecule[17]. Based on the data obtained from this study, DPPH radical scavenging activity of AQSF fractions of *L. purpureus* leaves was significant. It was revealed that aqueous fraction of *L. purpureus* leaves did show the proton donating ability and could serve as free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in human body.

3.2. Thrombolysis Activity

Fig. 2 shows the effect of the extract on clot lysis activity. The percentage (%) clot lysis was statistically significant ($p<0.001$) when compared with vehicle control. The plant extract/fractions showed moderate clot lysis activity (32.18%, 37.25%, 32.35%, and 29.17% for MSF, CTCSF, HXSF and CSF respectively) whereas standard streptokinase showed 65% clot lysis activity.

Platelets play an important role in the process of atherothrombosis by adhering to the damaged regions (caused by reactive oxygen species) of the endothelial surface. The activated platelets form platelets to platelets bonds, binds also to leucocytes bringing them into a complex process of plaque formation and growth[18]. Plasmin, natural fibrinolytic agent, lyses clot by breaking down the fibrinogen and fibrin contained in a clot. Streptokinase forms a 1:1 stochiometric complex with plasminogen that can convert additional plasminogen to plasmin[19]. Moreover, phlorotannin, isolated from

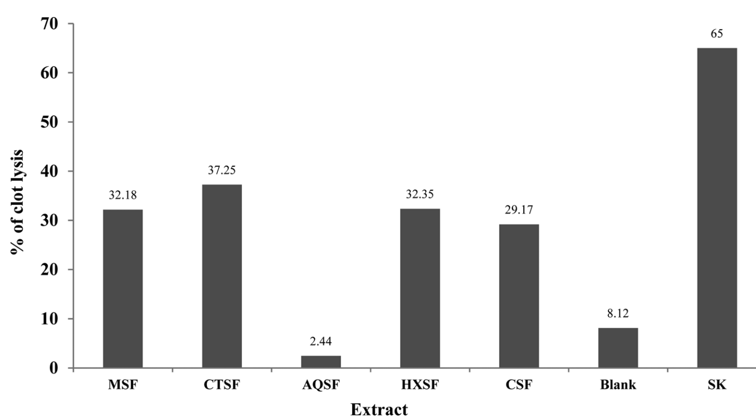

Fig. 2. Thrombolysis activity of *Lablab purpureus* leaves. % clot lysis activity of standard and *Lablab purpureus* methanolic extracts with its various organic soluble fractions. MSF: Methanolic extract; CTCSF: Carbon tetrachloride soluble fraction; HXSF: N-Hexane soluble fraction; CSF: Chloroform soluble fraction; AQSF: Aqueous soluble fraction; SK: Streptokinase.

marine brown algae, have a unique property in promotion of dissolution of intravascular blood clot via antiplasmin inhibition[20]. Since phytochemical analysis showed that the crude extract contains tannin, alkaloid and saponin could be participated for it's clot lysis activity.

3.3. Toxicity Studies

Cytotoxicity activity MSF, HXSF, CTCSF, CLSF, AQSF and standard VS were tested using brine shrimp lethality bioassay. The lethal concentration LC_{50} of the test samples were obtained after 24 hours in a plot of percentage of the shrimps died against the log concentration (toxicant concentration) of the sample. In toxicity studies, the extract was found to be moderately effective ($p<0.001$) and showed an LC_{50} value of 38.54, 22.18, 41.38 μg/mL for MLPL, HXLPL, and CTLPL respectively whereas 0.45 μg/mL was observed for the standard vincristine sulphate (VS) (Fig. 3).

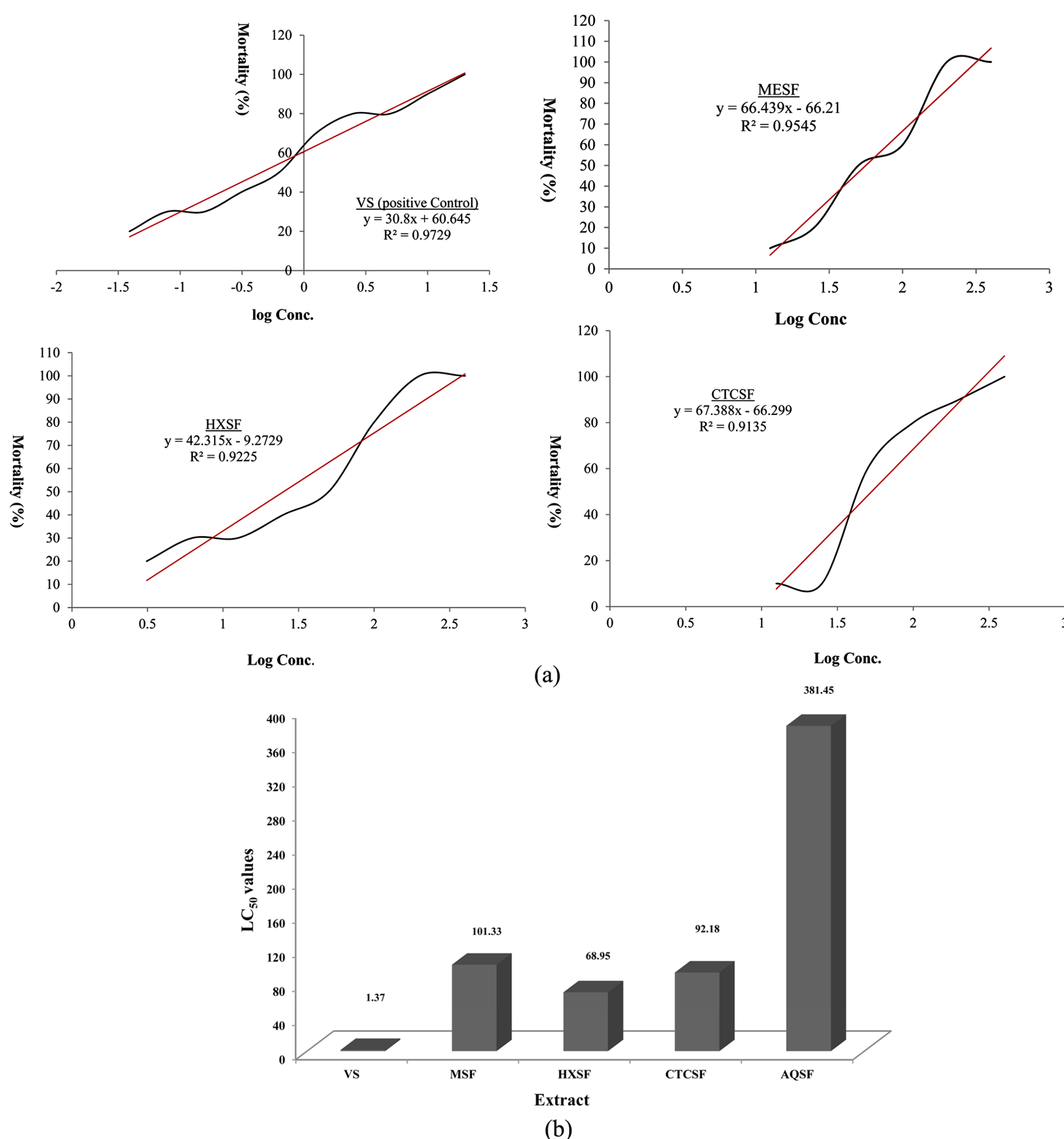

Fig. 3. Toxicity study of *Lablab purpureus* leaves (3a), LC_{50} of standard and *Lablab purpureus* methanolic extracts with its various organic soluble fractions (3b), MSF: Methanolic extract; CTCSF: Carbon tetrachloride soluble fraction; HXSF: N-Hexane soluble fraction; CSF: Chloroform soluble fraction; AQSF: Aqueous soluble fraction; VS: Vincristine Sulphate.

The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts to assess the toxicity towards brine shrimp, which could also provide an indication of possible toxicity of the test materials. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay[12]. The variation in BSLA results (Fig. 3) may be due to the difference in the amount and kind of toxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts. Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or μg/mL) to brine shrimp is indicative of the presence of potent toxic and probably insecticidal compounds which warrants further investigation. BSLA results may be used to guide the researchers on which crude plant extracts/fractions to prioritize for further fractionation and isolation of thesebioactive compounds.

3.4. Anti-microbial Activity

Values of the observed diameter zone of inhibition (mm) excluding cap diameter. Incubation conditions for bacteria -24 hours at 37°C and for fungi -48 hours at 25°C. Assay was performed in triplicate relative percentage of inhibition are indicated at parenthesis.

Table 1 expresses the antibacterial and antifungal

Table 1. *In vitro* antimicrobial activity of Lablab purpureus leaves on various bacterial and fungal strains

	Test Microorganism	MESSC	HXSF	CTCSF	CSF	AQSF	CIPRO
Gram Positive (+)	*Bacillus cereus*	9 (22.5%)	11 (27.5%)		12 (30.0%)	-	40
	Bacillus megaterium	10 (20.0%)	-	9 (18.0%)	9 (18.0%)	-	50
	Bacillus subtilis	-	-	-	9 (23.0%)	-	39
	Staphylococcus aureus	9 (21.4%)	9 (21.4%)	-	13 (30.9%)	11 (26.1%)	42
	Sarcina lutea	11 (22.0%)	-	12 (24.0%)	11 (22.0%)	-	50
Gram Negative (-)	*Escherichia coli*	12 (25.5%)	12 (25.5%)	13 (27.4%)	14 (29.7%)	-	47
	Pseudomonas aeruginosa	-	8 (17.7%)	-	13 (28.8%)	-	45
	Salmonella paratyphi	11 (31.4%)	11 (31.4%)	8 (22.8%)	12 (34.2%)	-	35
	Salmonella typhi	-	-	-	11 (29.7%)	-	37
	Shigella boydii	-	9 (20.0%)	8 (17.7%)	11 (24.4%)	-	45
	Shigella dysenteriae	9 (19.1%)	8 (17.0%)	-	12 (25.5%)	-	47
	Vibrio mimicus	-	-	-	12 (25.5%)	-	47
	Vibrio parahemolyticus	8 (17.7%)	-	-	14 (31.1%)	-	45
Fungi	*Candida albicans*	10 (22.2%)	10 (22.2%)	9 (20.0%)	13 (28.8%)	-	45
	Aspergillus niger	11 (24.4%)	9 (20.0%)	13 (28.8%)	11 (24.4%)	-	45
	Sacharomyces cerevacae	-	-	-	13 (27.0%)	-	48

Zone of inhibition in mm (% of relative activity).

activity (zone of inhibitions) of the methanolic extract of the leaves of *Lablab purpureus* and its organic soluble fractions. Chloroform extract (CSF) showed very good activity against all microorganisms whereas MSF, HXSF and CTSF showed good to moderate activity of selected microorganism. The range of zone of inhibition for MSF was 8-12 mm. However, CSF showed the highest zone of inhibition against *Vibrio parahemolyticus* and *Escherichia coli* (zone of inhibition 14 mm).

Results of relative percentage inhibition are reported in table 1. The CSF showed the maximum relative percentage inhibition against *Salmonella parathyphi* (34.2%) for bacteria and *C. albicans* (28.8%) for fungi whereas, lowest relative percentage inhibition against *Sarcina lutea* (22.0%) for bacteria and *Aspergillus niger* (24.4%) for fungi.

Infectious diseases are resulted from the infection, presence and growth of pathogenic biological agents in an individual host organism. Antimicrobials prevent or slow down the transmission of infectious diseases. Phytoconstituents such as saponins, phenolic compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections. The results of our study are in agreement with the study of Priya and Karthika[21].

3.5. Membrane Stabilization Effects

The methanolic extract and its organic soluble fractions of *Lablab purpureus* at concentration 2.0 mg/mL, significantly protected the lysis of erythrocyte membrane induced by hypotonic solution and heat as compared to the standard, acetyl salicylic acid (0.10 mg/mL). The MSF and AQSF produced 61.48% and 53.75% inhibition of hemolysis of RBC caused by hypotonic solution respectively, whereas acetyl salicylic acid (0.10 mg/mL) showed 76.42% (Fig. 4(a)). The membrane stabilizing effects of *Lablab purpureus* on heat induced hemolysis increased in the order of CTSF>AQSF>CSF>HXSFL>MSF (Fig. 4(b)).

The results showed that MLPL extracts was potent on

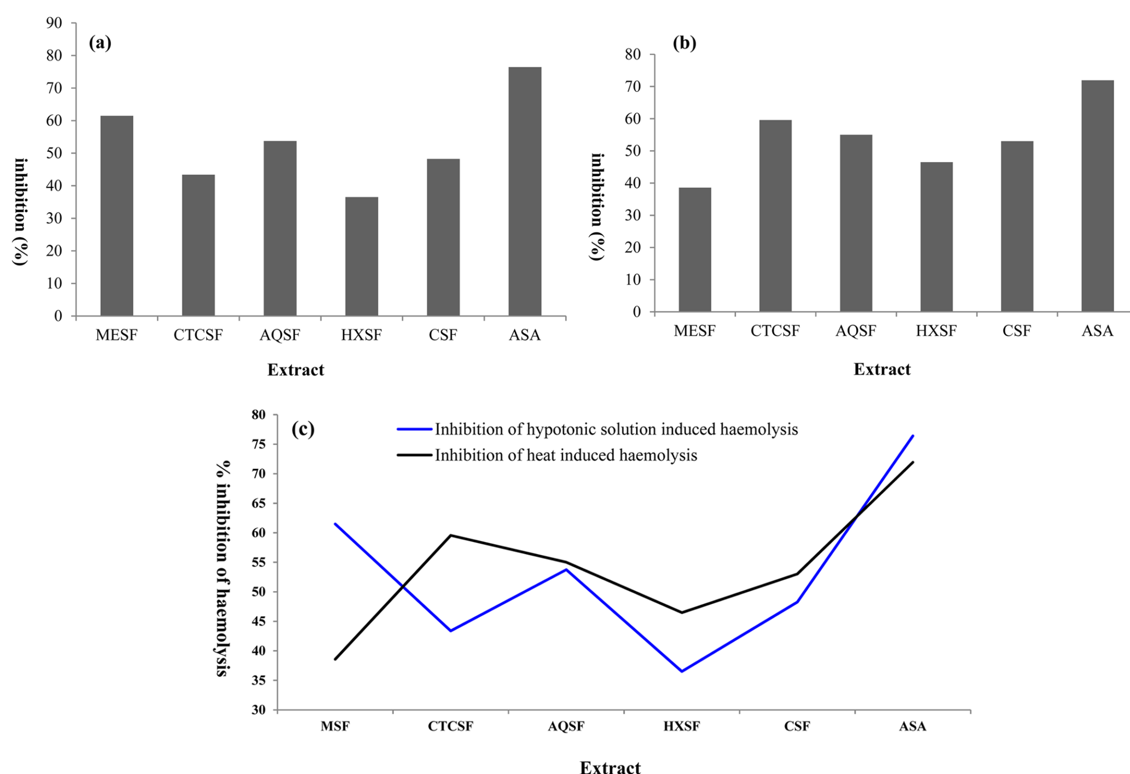

Fig. 4. Membrane stabilizing effects of *Lablab purpureus* on hypnotic solution induced hemolysis (a); heat induced hemolysis (b); Comparison of activity on hypotonic solution and heat-induced hemolysis of erythrocyte membrane (c). ASA: Acetyl Salicylic Acid

human erythrocyte in hypotonic induced lyses whereas CTLPL was potent on heat induced lyses. The activity was comparable to standard anti-inflammatory drug (aspirin). It has been reported that flavonoids have profound stabilizing effects on lysosomes both in-vitro and in-vivo experiments[22-24], while tannin and saponins have the ability to bind cations and able to stabilize erythrocyte membrane[25-26]. The present investigation suggests that the membrane stabilizing activity of *lablab purpureus* leaves plays a significant role in anti-inflammatory activity may be due to its high flavonoids or tannin content. It can be concluded that some extractives of *lablab purpureus* showed significant clot lysis activity. These herbal preparations may be incorporated as a thrombolytic agent for the improvement of the patients suffering from atherothrombotic diseases. This is a preliminary study and the extract should thoroughly be investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

4. Conclusions

In conclusion, the results of the present study indicate that the MeOH extract and its various fractions exhibit interesting antioxidant cytotoxic, as well as thrombolytic properties, and also show potent antibacterial activity with moderate toxicity. The different extractives of *L purpureus* leaves moderately protect the lysis of human erythrocyte membrane induced by hypotonic solutions as well as head induced lyses which confirms that the plant has potent membrane stabilizing activity as it stabilized the membrane of RBCs. These results of the investigation do not reveal that which chemical compound is responsible for aforementioned activity. To explore the lead compounds liable for aforementioned activity from this plant are in progress.

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