

Phytochemical screenings, thrombolytic activity and antimicrobial properties of the leaf extracts of *Lablab purpureus*

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Abstract

In this present study, the leaves extracts of *Lablab purpureus* were subjected to the thrombolytic activities were assessed by using human erythrocyte and the results were compared with standard streptokinase (SK). On the other hand, leaves extracts of *L. purpureus* revealed moderate antibacterial activity against some microorganisms used in the screening. Preliminary phytochemical investigation suggested the presence of reducing sugar group, tannins, saponins and alkaloids.

Key words: thrombolytic activity, *Lablab purpureus*, antimicrobial activity.

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Introduction

Lablab purpureus commonly known as the hyacinth bean, Indian bean, Egyptian bean, a species of bean in the family Fabaceae, is widespread as a food crop throughout the tropics, especially in Africa, India, Bangladesh, and Indonesia. A traditional food plant in Africa, this little-known vegetable has potential to improve nutrition, boost food security and foster rural development and support sustainable land care.

The wild forms of lablab are believed to have originated in India (Deka and Sarkar, 1990) and were introduced into Africa from southeast Asia during the eighth century (Kay, 1979). Presently, lablab is common in Africa, extending from Cameroon to Swaziland and Zimbabwe, through Sudan, Ethiopia, Uganda, Kenya and Tanzania (Skerman et al., 1991).

Lablab is a summer growing annual or short-lived perennial fodder legume sown for grazing and conservation in tropical environments with a summer rainfall. It is a vigorously trailing, twining herbaceous plant, resistant to disease and insect attack (Milford and Minson, 1968; Cameron, 1988). Stems are trailing to upright, reach to 3 m in length and are robust. Leaves are large and trifoliate, with the leaflets having a broad ovate-rhomboid shape measuring 7 to 15 cm long. The dorsal side of the leaf is smooth with the underside being hairy (Cameron, 1988).

Lablab has the capability of being an outstanding resource for tropical agricultural systems and in improving human food and animal feedstuffs (Pengelly and Lisson, 2002). When used as human food it is eaten as green pods or mature seeds and the leaves as vegetables. It is also used as animal feed, where it is cut as hay or mixed with other feed as silage (Maundu et al., 1999).

As a part of our continuing studies on medicinal plants of Bangladesh (Hossain et al., 2012; Shahriar et al., 2012(a); Shahriar et al., 2012(b); Shahriar et al., 2012(c); Shahriar and Kabir, 2011; Shahriar, 2010), the organic soluble materials of the leaves extracts of *Lablab purpureus* were evaluated for phytochemical screenings, anti-thrombolytic activity, cytotoxic properties, activities for the first time.

Materials and Methods

Plant materials: The leaves of *Lablab purpureus* were collected from Mirpur, Dhaka, Bangladesh, in May 2010. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no 35475).

The sun dried and powdered plant parts (500 gm) of *Lablab purpureus* was successively extracted in a soxhlet extractor at elevated temperature using 200 ml of distilled ethanol (40-60)°C which was followed by n-hexane and carbon tetrachloride. All extracts were filtered individually through filter paper and poured on petri dishes to evaporate the liquid solvents from the extract to get dry extracts. The dry crude extracts were weighed and stored in air-tight container with necessary markings for identification and kept in refrigerator (0-4)°C for future investigation.

Preliminary phytochemical screening: One gram of the ethanol extract was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents (Harbone, 1998; Kokate, 2001).

Streptokinase (SK): Commercially available lyophilized Altepaste (Streptokinase) vial (Beacon pharmaceutical Ltd.) of 15, 00,000 I.U., was collected and 5 ml sterile distilled water was added

and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for *in vitro* thrombolysis.

Blood sample: Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Thrombolytic activity: The thrombolytic activity of all extracts was evaluated by the method developed by Daginawala (2006) and slightly modified by Kawsar *et al.* (2011) using streptokinase (SK) as the standard.

Antimicrobial activity: The antimicrobial screening, which is the first stage of antimicrobial drug discovery, was performed by the disc diffusion method (Ayafor, 1972) against some gram positive and gram negative bacteria and also against fungi (Table 2) collected as pure cultures from the department of microbiology, University of Dhaka, Bangladesh. Standard disc of Kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm (Bauer *et al.*, 1966).

Results and Discussion

Thrombolytic activity: As a part of discovery of cardio-protective drugs from natural sources the extractives of *Averrhoa bilimbi* were assessed for thrombolytic activity and the results are presented in Table 1. Addition of 100µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed 93.29% lysis of clot. At the same time, distilled water was treated as negative control which exhibited negligible lysis of clot (4.74%). In this study, the carbon tetrachloride soluble fraction (CCSF) exhibited highest thrombolytic activity (51.60%).

Table 1: Thrombolytic activity of different fractions of *Lablab purpureus*

Sample	Thrombolytic Activity (% of lysis)
SK	93.29%
Water	4.74%
EF	41.81%
HSF	22.47%
CCSF	51.60%

SK= Streptokinase, EF= ethanolic fraction, HSF= n-hexane soluble fraction and CCSF= carbon tetra chloride soluble fraction of the leaves extracts of *L. purpureus*

Antimicrobial activity: The crude extract and its different partitionates when subjected to antimicrobial screening at 400 µg/disc revealed antimicrobial activity against the tested microorganisms having the zone of inhibition ranging from 8 to 14 mm (Table 2).

Table 2: Antimicrobial activity of *Lablab purpureus*

Test microorganisms	Diameter of zone of inhibition (mm)			
	HSF	CCSF	EF	Kanamycin
Gram positive bacteria				
<i>Bacillus cereus</i>	-	9	9	40
<i>Bacillus megaterium</i>	-	9	9	41
<i>Bacillus subtilis</i>	7	9	8	41
<i>Staphylococcus aureus</i>	7	11	9	40
<i>Sarcina lutea</i>	-	10	8	40
Gram negative bacteria				
<i>Escherichia coli</i>	-	9	8	42
<i>Pseudomonas aeruginosa</i>	-	10	8	40
<i>Salmonella paratyphi</i>	7	10	8	40
<i>Salmonella typhi</i>	-	9	8	40
<i>Shigella boydii</i>	-	10	8	40
<i>Shigella dysenteriae</i>	-	10	8	40
<i>Vibrio mimicus</i>	7	10	8	40
<i>Vibrio parahemolyticus</i>	7	10	8	40
Fungi				
<i>Candida albicans</i>	-	10	10	41
<i>Aspergillus niger</i>	-	9	10	41
<i>Sacharomyces cerevacae</i>	-	14	10	41

EF= ethanolic fraction, HSF= n-hexane soluble fraction and CCSF= carbon tetra chloride soluble fraction of the leaves extracts of *L. purpureus*.

Preliminary phytochemical screening: In preliminary phytochemical screening, the methanol extract of *Lablab purpureus* demonstrated the presence of alkaloids, saponins, tannins and reducing sugar group (Table 3).

Table 3: Analysis of phytochemical in the methanol extract of *Lablab purpureus*

Phytochemicals	Result
Alkaloids	+
Saponins	+
Tannins	+
Reducing Sugar group	+

+ = Presence.

Conclusion

It can be concluded that the extracts of the *Lablab purpureus* can be used to design different antimicrobial agents as well as thrombolytic agent due to its moderate antimicrobial activity. Further work is needed to isolate the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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