In vitro antimicrobial and cytotoxicity screening of n-hexane, chloroform and ethyl acetate extracts of *Lablab purpureus* (L.) leaves

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ABSTRACT

In the present study the antimicrobial & cytotoxic activity of crude extracts (Chloroform, n-Hexane, Ethyl acetate) of leaves of Lablab purpureus L. were studied. Antimicrobial activity was tested against eleven important pathogenic bacteria including both gram positive and gram negative bacteria and three fungi. The bacteria are B. megaterium, B. subtilis, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Salmonella paratyphi, S. typhi, Shigella boydii, S. dysenteriae and Vibrio mimicus & V. parahemolyticus. Disc diffusion technique was used for invitro antibacterial and antifungal screening. Here kanamycin disc (30µg /disc) was used as standard for antibacterial study. The extracts showed antimicrobial activity against most of the bacterial strains with an average zone of inhibition of 8-20mm. The tested fungi are Saccharromyces cerevaceae, Candida albicans and Aspergillus niger. The extracts showed moderate to good antifungal activity with an average 9 -15 mm zone of inhibition. Among the three solvent extracts used, the most effective extract was found to be n-Hexane extract and maximum activity (20 mm, zone of inhibition) found against Staphylococcus aureus with Minimum inhibitory concentration (MIC) values of 64µg/ml. The maximum zone of inhibition for chloroform extract showed 17mm against Bacillus subtilis and E.coli with MIC 128µg/ml and 32µg/ml respectively. The maximum zone of inhibition for Ethyl acetate extract showed 17mm against Vibrio mimicus with MIC values of 64µg/ml .Cytotoxicity test was also studied by Brine Shrimp Lethality Bioassay and compare with LC₅₀ values of standard Vincristin sulphate as a positive control. The results illustrated significant cytotoxicity against A. salina, with LC_{50} 13.88µg/ml, 19.17µg/ml and 17.97µg/ml for n-Hexane, Chloroform and Ethyl acetate extracts respectively.

Keywords: Antimicrobial activity, Minimum inhibitory concentration(MIC), Pathogenic bacteria. Cytotoxicity.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and the original source of many important pharmaceuticals currently in use have been plants used by indigenous people (Balick et al., Besides small molecules from medicinal 1996). chemistry, natural products are still major sources of innovative therapeutic agents for various conditions. including infectious diseases (Clardy et al., 2004). Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethnomedicinal use (Verpoorte et al., 2005). Continuing search of new therapeutic agent is geared toward the discovery and development of novel chemical structures such as therapeutic antimicrobial agents, antioxidants, hypoglycemic agent. The ongoing problem of development of resistance to existing antibacterial agents and the dearth of good antifungal agents motivates this effort toward innovation (Silver and Bostian, 1990).Therapeutics of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious disease(Iwu *et al.*, 1999)The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Mitscher *et al.*, 1987). In the continuation of this strategy of new drug discovery we have studied the leaves of the plant *Lablab purpureus* for its antimicrobial and cytotoxic activity.

Lablab purpureus or hyacinth bean belongs to the family Fabaceae (alt. Leguminosae). It is a large twiner. Leaves are 3-foliolate; leaflets are 5-15 cm long, ovate, acute, base cuneate or deltoid. Leaves are emmenagogue and reputed alexipharmac; given in colic. Lablab purpureus has great potential as

medicinal legume. Among the legumes, hyacinth bean constitutes an important source of therapeutic agents used in the modern as well as traditional systems of medicine (Morris, 1996, 1999, 2003). It carries tremendous healing potential. In fact, it is considered a multipurpose crop since it is used for food, forage, soil improvement, soil protection and weed control (Shivashankar and Kulkarni, 1989; Karachi, 1998; Morris, 1997, 2003; Pengelly and Maass, 2001; Maass, 2006). The young pods and tender beans are used as vegetables in India and tropical and warm temperate Asia. It is also been known for its use as a green manure and produces edible young pods, dried seeds, leaves and flowers (Morris, 1997, 2003). The seeds are used as a anthelmintic, laxative, diuretic, antispasmodic, aphrodisiac, anaphrodisiac, digestive, carminative, febrifuge and stomachic (Chopra et al., 1986: Kirtikar and Basu, 1995). Hyacinth beans contain fiber which is known to prevent cancer, diabetes, heart disease, obesity and is used a laxative (Beckstrom-Sternberg and Duke, 1994). Hyacinth bean contains the potential breast cancer fighting a flavonoid known as kievitone (Hoffman, 1995). The flavonoid, genistein found in hyacinth bean may play a role in the prevention of cancer (Kobayashi et al., 2002) and as a chemotherapeutic and/or chemo preventive agent for head and neck cancer (Alhasan et al., 2001). Tryrosinase (polyphenol oxidase) is present in plant tissue and is important in fruit and vegetable processing as well as storage of processed foods. Prevention of browning of foods, enzymatic or nonenzymatic, has long been a concern of food scientists (Matheis, 1987; Sanchez-Ferrer et al., 1995; Paul and Gowda, 2000). Hyacinth bean contains tyrosinase, which has potential for the treatment of hypertension in humans (Beckstrom-Sternberg and Duke, 1994). Fresh leaves pounded and mixed with lime are rubbed over ringworms to cure (Yusuf et al., 2009). There are very few reports regarding to its antimicrobial and cytotoxic effects using various parts of this plant. The present study was aimed to evaluate the antimicrobial and cytotoxic potentiality of different solvent extracts of Lablab purpureus leaves.

MATERIALS & METHODS

Plant material: Leaves of *Lablab purpureus* were collected in February 2011 from Mirpur Dhaka Bangladesh and authenticated at Bangladesh National Herbarium, where a voucher specimen no DACB 35515 has been deposited.

Extraction and isolation: The air-dried leaves (1kg) were finely pulverized and extracted by percolation with ethanol for seven days at room temperature. The extracts were filtered and concentrated under vacuum to obtain a crude extract of leaves. The extract was fractionated by the modified Kupchan partitioning method (Van Wagenen *et al.*, 1993) into n-hexane, chloroform and ethyl acetate. The fractioned extracts were concentrated under vacuum to obtain solid extracts.

Antimicrobial assay

Microorganisms: Antimicrobial activity was tested against *B. megaterium, B. subtilis, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Salmonella paratyphi, S. typhi, Shigella boydii, S. dysenteriae, Vibrio mimicus & V. parahemolyticus , Saccharromyces cerevaceae, Candida albicans* and *Aspergillus niger.* These microbial strains were isolated from clinical samples and obtained as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Determination of the diameters of inhibition zone: The leaves crude extracts were tested in vitro for antimicrobial activity by the standard disc diffusion method (Bauer et al., 1966, Rahman et al., 2008) bacteria. Solutions against the of known concentration (500µg/ 10µl) of the test samples were made by dissolving measured amount of the samples (50 mg) in 1 ml of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances (500µg/ disc) using micropipette and the residual solvents was completely evaporated. Discs containing the test materials were placed on to nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4°C) for 16 hours to allow maximum diffusion of the test materials and kanamycin. The plates were then incubated at 37°C for 24 hours to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean value was taken.

Antifungal screening: The n-hexane, chloroform and ethyl acetate extracts of leaves of *Lablab purpureus* were tested for their antifungal activity against the selected fungi by the standard disc diffusion method (Bauer *et al.*, 1966, Rahman *et al.*, 2008). In this antifungal screening each of the crude extract was used at a concentration of 500µg/disc. Following the above mentioned procedure,the antifungal activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

Minimum inhibitorv concentration (MIC) determination: The minimum inhibitory concentration of the leave extracts was determined against Escherichia coli, Bacillus subtilis, Vibrio mimicus, Staphylococcus aureus, Bacillus subtilis .The tests were carried out by serial dilution technique (Reiner, 1982). Nutrient broth was used as bacteriological media. Dilution series were set up with 2, 4, 8, 16, 32, 64, 128, 256, 512 µg/ml of nutrient broth medium. The tests were continued out in triplicates. The lowest concentration which did not show any growth of the tested microorganism after macroscopic evaluation was determined as the MIC.

Cytotoxicity screening: Cytotoxic activity of the plant extracts was determined by brine shrimp lethality bioassay method (Meyer *et al.*, 1982). This method detect the lethality of crude extracts on *Artemia salina*, used as a convenient monitor for the screening. The eggs of Brine Shrimp (*A. salina*) were hatched in a tank in artificial seawater (3.8% NaCl solution) at a temperature around 37° C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii.

Preparation of Test Groups: The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. For the experiment, the test samples (extracts) were prepared by dissolving them in DMSO (not more than 50 μ l in 5 ml solution) and sea water to attain concentrations of 20, 40, 60, 80 and 100 μ g/ml. A vial containing 50 μ l DMSO diluted to 5ml was used as a control. Standard Vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. Each test tube contained about 5 ml of seawater and 10 shrimp nauplii.

Counting of nauplii: After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. The rate of mortality of nauplii was found to be increased in concentration of each of the samples. From this data,

the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

Lethality concentration determination: The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values were obtained from the best-fit line plotted concentration verses percentage lethality. Vincristin sulphate was used as a positive control in the bioassay.

RESULT

Result of Antimicrobial screening: The extracts of the *L. purpureus* leaves were screened against eleven human pathogenic bacteria to check antibacterial activities by disc diffusion method .The extracts showed variable zone of inhibition against tested pathogenic bacteria which was shown in table 1.

All the extracts showed significant antimicrobial activity. The n -hexane extract of *L. purpureus* leaves showed moderate to maximum antibacterial activity against all tested pathogenic bacteria. This extract showed very good antimicrobial activity against the Gram-(-) *Escherichia coli* (18 mm) and the Gram-(+) *Bacillus subtilis* (18mm) ,*Staphylococcus aureus* (20mm) bacteria with an average zone of inhibition of 8-20mm.

The chloroform extract showed moderate to good antibacterial activity against all tested pathogenic bacteria with an average zone of inhibition of 8-17 mm. This extract exhibited very good antimicrobial activity against *Bacillus subtilis* (17mm) and *Escherichia coli* (17mm).

Whereas an average zone of inhibition of 9-17 mm, against all the tested bacteria was showed by ethyl acetate extract. The maximum zone of inhibition exhibited against *Vibrio mimicus* (17mm). The minimum zone of inhibition showed 9 mm against *Shigella boydii.*

Result of antifungal screening: The results of the extracts displaying antifungal effect against tested fungus are shown in Table 2. All the extracts showed mild to moderate activity against the fungus. The exhibited diameter of zone of inhibitions were 12mm, 12mm, 13mm against *Aspergillus niger*, 9mm, 9mm, 10mm against *Sacharomyces cerevaceae* and 12mm, 10mm, 15mm against *Candida albicans* for the n –hexane, Chloroform and ethyl acetate extracts respectively

Test Organism	Diameter of zone of inhibition (in mm)			
	Chloroform extract(500µ/disc)	Ethyl acetate extract(500µ/disc	n-hexane extract(500µ/disc)	Kanamycin (30µ/disc)
	Gram (+) ve bacte	eria		
Bacillus subtilis	17	12	18	30
Bacillus megaterium	10	13	15	32
Bacillus cereus	8	11	15	33
Staphylococcus aureus	13	12	20	29
Sarcina lutea	12	11	14	31
		Gram (-) ve bacteria		
Shigella dysenteriae	13	10	11	25
Salmonella paratyphi	8	10	10	31
Vibrio mimicus	12	17	14	30
Shigella boydii	11	9	8	30
Escherichia coli	17	14	18	22
Vibrio parahaemolyticus	11	10	15	32

Table 1: In vitro antibacterial activity of different extracts of Lablab purpureus leaves

Table 2: In vitro antifungal activity of Lablab purpureus leaves extracts

	Diameter of zone of inhibition				
Test organisms	Chloroform extract (500µ/disc)	Ethyl acetate extract (500µ/disc)	n-hexane extract (500µ/disc)		
Aspergillus niger	13	12	12		
Sacharomyces cerevaceae	10	9	9		
Candida albicans	15	10	12		

Result of Minimum inhibitory concentration (MIC): We have tested the MIC values for the extracts that showed excellent or good antibacterial activity. The results of MIC of n-hexane extract were 64 μ g/ml against *Staphylococus aereus* and 128 μ g/ml against *Escherichia coli and Bacillus subtilis*. The Chloroform extract of *lablab purpureus* showed excellent MIC 32 μ g/ml against *Escherichia coli and* MIC 128 μ g/ml against *Bacillus subtilis*. The ethyl acetate extract showed MIC 64 μ g/ml against *Vibrio mimicus*.

Result of cytotoxicity screening: Following the procedure of Meyer, the lethality of the extracts of *L.pupureus* to brine shrimp was determined on *A. salina* after 24 hours of exposure of the samples and the positive control, vincristine sulphate. The results of the different extracts of *L.pupureus* (% mortality at different concentrations and LC ₅₀ values) were shown in Fig. 1. The percent mortality increased with an increase in concentration. The crude n-hexane, chloroform and ethyl acetate extract showed better cytotoxic activity with LC ₅₀ values of 13.88µg/ml, 19.17µg/ml, 17.97µg/ml in comparision with

vincristine sulphate as standard whose LC_{50} value is 7.55µg/ml.

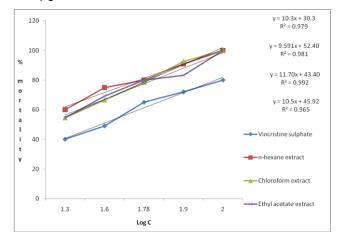


Fig 1: Determination of LC_{50} values for standard and crude n-hexane, chloroform and ethyl acetate extracts of *Lablab purpureus* leaves from linear correlation between logarithms of concentration versus percentage of mortality.

DISCUSSIONS

Antimicrobial potential: In the present study the result of antimicrobial activity reveals that all the extracts showed significant antimicrobial activity towards both bacteria and fungi suggested the presence of antimicrobial compounds. Among the three extracts of *L. purpureus*, the n-hexane extract exhibited more antibacterial potential than the chloroform & ethyl acetate extracts. Evidence showed that Leaves contain sterols (including cholesterol and its derivatives), fatty acids, palmitic, palmitoleic, linoleic and linolenic acids. A pyridine alkaloid, trigonelline and sterols have been isolated from tissue cultures of leaves.

In case of antifungal activity all the three extract showed almost same potential.

The MIC results of the extracts against selected strains were linear with the antimicrobial potential.

Cytotoxic activity: From the results of the brine shrimp lethality bioassay it can be well predicted that all the crude extracts have considerable cytotoxic potency. Among the three fractions, n-hexane extract showed significant cytotoxicity than ethyl acetate and chloroform extracts of *L. purpureus leaves*. Previous phytochemical screening indicated the presence of alkaloids and flavones, which have been shown to possess cytotoxic activity, may be responsible in part for the antitumour effect on Ehrlich ascites carcinoma

(Brown ,1980) . The variation in results may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the different solvent crude extracts. The possible mechanism of cytotoxicity of *L. purpureus* against brine shrimp nauplii due to poisonous effect on cell mitosis.

CONCLUSIONS

Extensive practice of Traditional and alternative medicine has regained public attention over the past 20 years as this type of medicine is easily accessible in some regions. A postulation denoted that by 2010, at least two-thirds of Americans will opt for alternative therapies (Zhao *et al.*, 1992). The non prescription use of medicinal plants is cited today as an important health problem, in particularly their toxicity to the kidney (Humber, 2002). So, if the plant extract found to show significant antimicrobial activities must take into account acceptable levels of toxicity.

The results obtained in our present study indicated that the crude extracts of *L. purpureus* leaves has got profound cytotoxic and antimicrobial effect and may have potential use in medicine. This novel finding will aid us to conduct pharmacological studies to understand the underlying possible mechanisms of the observed activities as well as bioactivity guided isolation and characterization of leading compounds in due course.

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