In-vitro potential antimicrobial and antioxidant activities of *Lablab niger* leaves

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ABSTRACT

Purpose: In this studies investigate potential in-vitro antimicrobial activity as well as antioxidant activities of methanol extracts and different fractions of *Lablab niger* leaves. **Methods:** The carbon tetrachloride and chloroform soluble fractions exhibited significant antibacterial activity, whereas methanolic extract and pet-ether soluble fractions showed mild to moderate antimicrobial activity against the tested microorganisms. **Results:** Total phenolic content (expressed as gallic acid equivalent) ranged from 5.65 to 75.91 mg per gm of dried extract. In DPPH free radicals assay, carbon tetrachloride (CTCSF) and aqueous soluble fractions (AQSF) exhibited significant free radical scavenging activity with IC_{50} value of 6.67 µg/ml and 6.73 µg/ml, respectively. **Conclusions:** These finding for the first time indicate that *L. niger* leaves could be an vital sources of natural antimicrobial and antioxidant activities.

Key Words: *Lablab niger*; antimicrobial activity; DPPH; total phenolic content.

1. INTRODUCTION

Lablab niger, commonly known as niger bean, is a climbing herb belonging to the family of Fabaceae, formerly Leguminosae. It is a summer growing annual or short-lived perennial fodder legume sown for grazing and conservation in tropical environments with a summer rainfall[1,2]. Its 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[2]. Flowers are white or pink, fascicled on nodes of lax racemes. Pods are compressed, tipped with the hooked persistent base[3]. To the best of our knowledge, no intensive scientific work on this plant has been reported yet. Considering the importance of antimicrobial agents and antioxidants of natural origin we investigated antimicrobial activity and antioxidant potential (in terms of total phenolic content amd DPPH activity) of the crude methanol extract of *Lablab niger* leaf and its aqueous and organic solvent soluble fractions for the first time and report the results of our preliminary investigations in this study.

2. MATERIALS AND METHODS

2.1. PLANT MATERIALS

The leaves of *Lablab niger* were collected from Mirpur, Dhaka, Bangladesh in November 2012. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no.38616). The sun dried and powdered leaves (500 gm) of *L. niger* 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[4] and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

2.2. ANTIMICROBIAL ACTIVITY

The antimicrobial test was performed by the disc diffusion method [5] against eleven bacteria and three fungi (Table-1) collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The bacterial or fungal suspensions were used to inoculate Petri plates (90 mm) and wells (6 mm) were filled with the extracts (400 µg/disc). Standard disc of Ciprofloxacin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The plates were incubated at 37°C for 24 hours. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition (mm) at the end of the incubation period [6].

2.3. TOTAL PHENOLIC COMPOUND ANALYSIS

Total phenolic content of extracts *L. niger* leaves was measured by the reported method involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard[7]. In brief, 0.5 ml of extract solution (2 mg/ml) in water, 2.5 ml of Folin -Ciocalteu reagent and 2.0 ml of sodium carbonate (7.5 % w/v) solution were added. After 20 minutes of incubation at room temperature the absorbance was measured at 760 nm. Total phenolics were quantified by calibration curve and were expressed as mg of GAE (gallic acid equivalent) / gm of the dried extract.

2.4. FREE RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of the crude methanol extract (ME) was evaluated by the reported method using 1,2-diphenyl-2-picrylhydrzzyl (DPPH)[8,9]. Ascorbic acid (ASA) was used as positive control. About 2.0 ml of a methanol solution of the sample (extracts or control) at different concentrations (1.0-500.0 µg/ml) were mixed with 3.0 mL of a DPPH solution (20 µg/ml of methanol). After 30 min reaction at room temperature in dark place, the UV absorbance of DPPH radical was measured at 517 nm using a spectrophotometer. Inhibition of free radical DPPH in percentage (I%) was calculated as follows:

I%=(1- absorbance of sample/absorbance of control)X100

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted by inhibition percentage against extract concentration.

2.5. STATISTICAL ANALYSIS

The data was expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the positive control. P values <0.005 were considered to be statistically significant.

3. RESULTS

3.1. ANTIMICROBIAL ACTIVITY

Antimicrobial activities of the plant extract against eleven bacteria and three fungi were compared with the standard antibiotic ciprofloxacin by measuring the zone of inhibition diameter (mm). The inhibitory effects of methanol extract and different fractions against different microorganisms are presented in Table 1. Carbon tetrachloride (CTCSF) and chloroform soluble fractions (CSF) produced zone of inhibitions ranging 8.33- 20.66 mm and 7.33-22.33 mm, respectively, in comparison to that of 40.3-56.6 mm of standard ciprofloxacin. So, CTCSF and CSF exhibited significant antimicrobial activity against all tested organisms. Methanol extract (ME) and pet-ether soluble fractions (PESF) demonstrated mild antimicrobial activity against some of tested organisms.

3.2. TOTAL PHENOLIC COMPOUND ANALYSIS

The amount of total phenol in all the partitionates of *L. niger* extract was measured by Folin-Ciocalteu reagent in term of gallic acid equivalent. The amount of total phenolic content ranged from 5.44 to 75.25 mg of GAE / gm of extractives of *L. niger* (Figure 1). Among all extractives of *L. niger* leaves, the highest phenolic content was found in CTCSF (75.25 mg of GAE / gm of extractives) followed by PESF (55.25 mg of GAE / gm of extractives). Significant amount of phenolic compounds were also present in CSF (26.56 mg of GAE / gm of extractives), ME (20.43 mg of GAE / gm of extractives) and AQSF (5.44 mg of GAE / gm of extractives).

3.3. FREE RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of methanol extract and different fractions of *L. niger* leaves was assessed by using DPPH free radicals. The results revealed that that carbon tetrachloride (CTCSF) and aqueous soluble fractions (AQSF) exhibited highly significant free radical scavenging activity with IC_{50} value of 6.5 µg/ml & 6.7 µg/ml, respectively which were comparable with the IC_{50} value of reference ASA (Figure 2). Chloroform soluble fractions (CSF) and pet-ether soluble fractions (PESF) also significantly scavenged free radicals with IC_{50} value of 11.8 µg/ml & 22.6 µg/ml, respectively.

4. DISCUSSION

In the present study, the leaf extracts and fractions of *L. niger* were evaluated for *in vitro* antimicrobial and antioxidant activities. Our results demonstrated that the fractions CTCSF and CSF showed significant antimicrobial activity against all tested organisms. The inhibitory effect against both gram-positive bacteria and gram-negative bacteria indicates that the plant extracts may have a broad spectrum antimicrobial activity. It can be assumed that the varied antimicrobial activities of the plant could be mainly due to the presence of different quantities of lead antimicrobial compounds in leaf extracts and different fractions of *L. niger*.

Plants produce several secondary metabolites that include phenolics, flavones, flavonoids, flavonols, coumarins, alkaloids, tannins, lectins, polypeptides and other compounds which support the plant defence against the microorganisms[10]. These compounds have also been reported in previous studies to possess antioxidant, anti-inflammatory and antimicrobial properties[10-13]. This study indicates that the fraction (CTCSF) which contained

highest phenol compounds also exhibited highest antimicrobial activity. This further supports that the antimicrobial activities of the plant extract of the present study are likely to be due to the high phenolic content.

It is also well documented that different classes of polyphenols, especially flavonoids are mostly responsible for many antioxidant effects of plant foods and medicinal plants[14-16]. The results of this study reveals that carbon tetrachloride soluble fraction (CTCSF) possessing highest phenol content also exhibited highest free radical scavenging activity. This further supports the anti-oxidant activity of the plant extracts is due to a high content of phenolic compounds. Such antioxidant-based drug products may be helpful for the treatment and prevention of complicated diseases like atherosclerosis, diabetes, stroke, Alzheimer's disease, and cancer [17].

5. CONCLUSIONS

It can be concluded that the alcoholic extracts of *L. niger* leaves and its carbon tetrachloride and chloroform soluble fractions possess significant antimicrobial and antioxidant potentials. Results of the present study suggest that the plant can serve as a natural source to develop antimicrobial and antioxidant agents. Further studies will be conducted for isolation and purification of the active principles of the plant responsible for these effects.

6. CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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Table-1

Antimicrobial activity of methanol extracts and different fractions of *L. niger*.

Test microorganisms	Diameter of zone of inhibition (mm) *				
	ME	PESF	CTCSF	CSF	Ciprofloxacin
Gram positive bacteria					
Bacillus megaterium	7.66±0.58	-	17.33±0.58	7.33±0.58	40.3±1.55
Bacillus subtilis	-	7.66±0.58	15.66±0.58	8.66±0.58	41.6±2.35
Staphylococcus aureus	-	-	8.33±0.58	10.66±0.58	46.3±1.24
Sarcina lutea	-	-	10.33±1.52	10.33±1.52	50.3±0.89
Gram negative bacteria					
Escherichia coli	10.66±0.58	8.33±0.58	17.33±0.58	10.33±0.58	46.3±.94
Pseudomonas aeruginosa	7.66±0.58	-	10.66±0.58	10.33±0.58	44.0±2.8
Salmonella paratyphi	-	10.66±0.58	11.33±0.58	14.66±0.58	56.6±6.23
Shigella boydii	9.33±0.58	-	20.66±0.58	19.33±0.58	48.0±2.82
Shigella dysenteriae	9.66±0.58	-	12.33±0.58	12.66±0.58	48.3±5.32
Vibrio mimicus	10.33±0.58	-	19.66±0.58	22.33±1.53	42.3±3.68
Vibrio parahemolyticus	9.66±0.58	-	12.33±0.58	9.66±0.58	46.3±.94
Fungi					
Candida albicans	14.33±0.58	-	15.66±0.58	13.33±0.58	41.6±2.35
Aspergillus niger	9.33±0.58	-	19.33±0.58	19.66±0.58	40.3±1.55
Sacharomyces cerevacae	7.66±0.58	-	16.66±0.58	12.33±0.58	43.66±0.58

Values are expressed as mean ± SEM of 3 replicates.

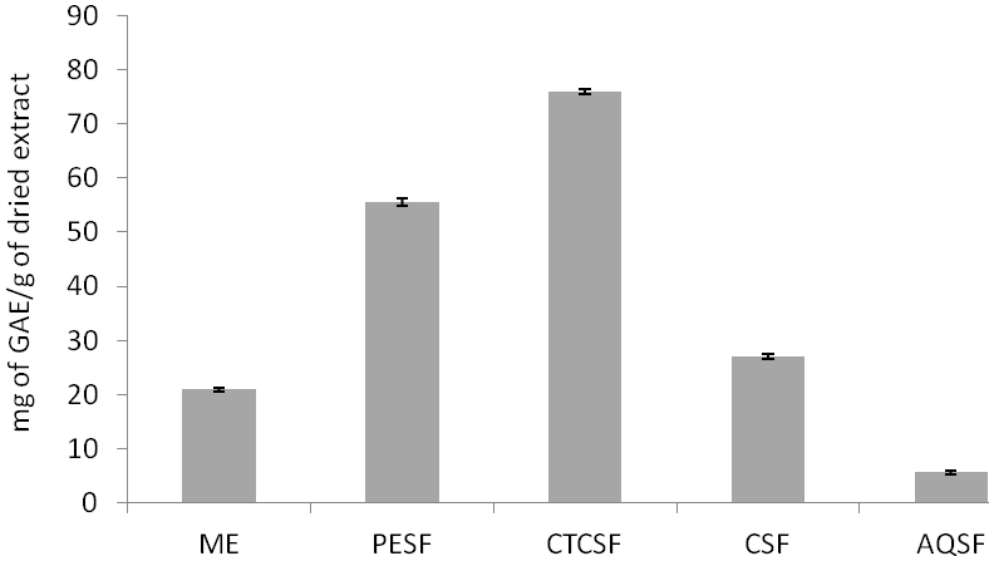

Figure 1. Total phenolic content of methanolic extract and different fractions of *L. niger*. ME = methanolic extract; PESF = pet-ether soluble fraction; CTCSF = carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = aqueous soluble fraction of the methanolic extract of *L. niger.* Values are expressed as mean ± SEM. of 3 replicates.

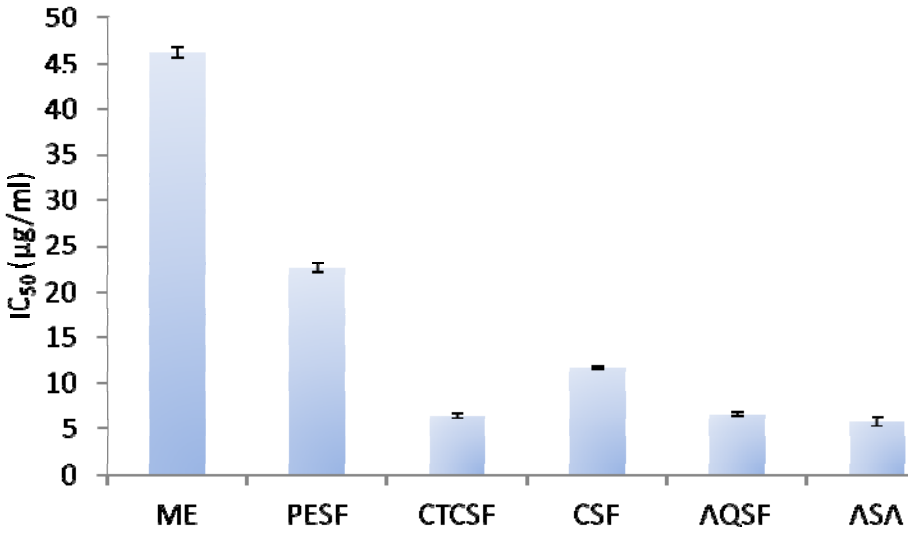

Figure 2. Free radical scavenging activity of the standard and fractions of *Lablab niger* leaves. ME = methanolic extract; PESF = pet-ether soluble fraction; CTCSF = carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = aqueous soluble fraction of the methanolic extract of *L. niger*; ASA= Ascorbic acid. Values are expressed as mean ± SEM. of 3 replicates. In all cases $P < 0.005$ as compared with the standard.