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ANTI-INFLAMMATORY, ANTIOXIDANT AND CYTOTOXICITY POTENTIAL OF METHANOLIC EXTRACT OF TWO BANGLADESHI BEAN *LABLAB PURPUREUS* (L.) SWEET WHITE AND PURPLE

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ABSTRACT

This study was subjected to investigate in vitro anti-inflammatory, antioxidant and cytotoxic properties of methanol extracts of two Bangladeshi bean pods namely *Lablab purpureus* L. sweet 'white' and 'purple'. Protease Inhibition method was used to determine *in-vitro* anti-inflammatory properties. DPPH free radical scavenging method, total flavonoids, Total antioxidants and reducing power of the two samples were assessed for antioxidant properties and cytotoxicity potentials were assessed by the brine shrimp lethality test. In in-vitro anti-inflammatory investigation there is a linear relation of %inhibition for the white bean pods which indicates having positive anti-inflammatory property. In DPPH test the lowest and highest IC50 values are 430.00µg/ml and 853.13µg/ml, with *L. purpureus* sweet 'purple' and *L. purpureus* sweet 'white' respectively and compared with standard ascorbic acid. In Cytotoxicity test LC_{50} value was found 960.06 µg/ml for *L. purpureus* (L.) sweet 'purple' and 66.5 µg/ml for *L. purpureus* sweet 'white', So *L. purpureus* sweet 'white' is more potent. The total Flavonoid contents of the test samples are 42.55±5.77 and 32.09±0.36 mg/g quercetin equivalents for 'white' and 'purple' respectively. *L. purpureus* (L.) Sweet White and Purple may posses' potent anti-inflammatory, powerful antioxidant as well as good cytotoxic potential.

INTRODUCTION: Phytochemicals are excessively found in plant kingdom [1]. In medicine about 50% drug used are of plant origin, in developing countries nearly 80% people depends on traditional plant based medicines for primary health care ,and only a small fraction of medicinal plants has been tested [2].

To solve the healthcare problems the attention to the medicinal plants are increasing world-wide. Plants are the origin of 25% of prescribed drugs in the world [3] and over 3000 species of plants have been reported to have anticancer properties [4].

Antioxidants are added to a variety of foods to prevent or deter free radical induced lipid oxidation [5]. If free radical production rate exceeds the normal capacity of the antioxidant defense mechanisms, substantial tissue injury results [6].

Continuous exposure to chemicals and contaminants leads to increase the free radicals amount and causes irreversible oxidative damage which includes biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to aging [7, 8].

Some studies have shown the positive correlation of the increased dietary intake of natural antioxidants with the reduced coronary heart disease and cancer mortality, as well as with longer life expectancy [9, 10]. Moreover, many polyphenolic compounds have shown many health benefiting bioactive properties like antioxidant, anticancer, antiviral, anti-inflammatory activities [11-13].

Among the south Asian countries Bangladesh has a rich and prestigious heritage of herbal medicines, out of 500 species of medicinal plants about 250 species are used for the preparation of traditional medicines in Bangladesh but majority of these plants have not yet undergone chemical, Pharmacological and toxicological studies to investigate their bioactive compounds [14]. Therefore much current research devoted to the Phytochemical screening for different types of biological activity.

L. purpureus sweet or Hyacinth bean is an important vegetable crop in Bangladesh and in winter an average yielding of 4.53 t of fresh pods per ha for a total yield of about 50000 t [15]. In Bangladesh Hyacinth bean is grown for fodder and cover crop, the leaves and seeds contain 20-28% protein, with well balanced amino acid composition [16]. The fresh pods and green seeds are eaten boiled or used in curries, mature seeds are used as pulses, often in soup; Sprouted seeds are occasionally sun-dried and stored for use as a vegetable [17]. The Lablab bean species are extremely diverse [18, 19].

Shivashankar and Kulkarni [20] described three groups among the cultivated plants based on relative position of seeds in the pods. Cultivar group lablab is widely distributed and in this study we have discussed about two different color lablab bean which are most commonly used in Bangladesh as local name Sheem as a vegetable, the objective of this study is to find out the scientific basis which will accelerate the medicinal use of bean not only in Bangladesh but also world-wide.

MATERIALS AND METHODS:

Collection and Preparation of the Plant material: The two Beans, *L. purpureus* sweet 'white' and *L. purpureus* sweet 'purple' were collected in December 2010 from Savar kancha Bazar, Dhaka, Bangladesh. In the local area (Savar) these beans are known as Nal-dog sada sheem and Nol-dog kuli sheem. The samples were identified by experts in Bangladesh National Herbarium, Mirpur, and Dhaka. The accession numbers are: *L. purpureus* sweet 'white'-34994; L. purpureus sweet 'purple' -34995.

Extraction of the Plant material: The fresh beans were washed with distilled water, minced into small pieces, the tested materials were sun dried for seven days and ground. Extraction was performed at room temperature. About 200 g of dried, ground plant material were soaked in 98% methanol for 48 h, stirring a sterilized glass rod, filtered, and the solvent removed in vacuum using a rotary evaporator at a temperature below 40°C. Extracts were stored in sample glass ware in the refrigerator prior to use.

Chemicals and drugs: DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid) and ferric chloride were obtained from Sigma Chemical Co. USA; ascorbic acid was from SD Fine Chem. Ltd. India, ammonium molybdate from Merck, Germany.

In-vitro Anti-inflammatory Test: In-vitro Anti-inflammatory (Protease inhibition) Test was performed as described [21] by 1ml of extract solution transfer from the mother test tube to the respective marked test tube.0.03mg (2 or 3 finepart) of trypsin is added in each test tube. Then 1ml of 25mM tris HCl buffer (pH-7.4) is added. 1ml of 0.8% (w/v) case in solution is added. The mixture is incubated for an additional 20 minutes.2ml of HclO4 (70%) is also added to terminate the reaction. Cloudy suspension is centrifuged at 3000 rpm for 30 minutes. At last the absorbance of supernatant was taken at 280nm against buffer as blank.

Antioxidant Activity Test:

Determination of Total Phenolic Content: The total phenolic content of extracts was determined using Folin-Ciocalteu method [22]. The extracts were oxidized with Folin-Ciocalteu reagent and ware neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 60 min. using gallic acid as standard total phenolic content was expressed as mg GA equivalent/gm of extract.

Determination of Total Flavonoids Content: The flavonoids content was determined using a method as described by Kumaran and Karunakaran [23] using quercetin as a reference compound. 1 mg of plant extract in methanol was mixed with 1ml aluminium trichloride in Ethanol (20 mg/ml) and a drop of acetic acid, and then diluted with Ethanol to 25 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 mg of plant extracts and a drop of acetic acid, and then diluted to 25 ml with ethanol. The absorption of standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions.

Determination of Total Antioxidant Capacity: The antioxidant activity of the extracts of *L. purpureus* ware evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* [24]. The assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95o C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (Shimadzu, UV-150-02) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

DPPH Radical Scavenging Activity: The free radical scavenging capacity of the extracts was determined using DPPH [25, 26]. A methanol DPPH solution (0.004% w/v) was mixed with serial dilutions (0 to 500 µg) of *L. purpureus* extracts and after 10 min; the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a standard. The inhibition curve was plotted and IC50 values were calculated.

Reducing Power: The reducing power of *L. purpureus* extractives was determined according to the method previously described by Oyaizu [27]. Different concentrations of *L. purpureus* extracts in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as a reference standard. Phosphate buffer (pH) was used as blank solution.

Cytotoxic Activity:

Brine Shrimp Lethality Bioassay: Cytotoxic activity of the plant extracts was determined by brine shrimp lethality bioassay method [28]. It is a recent development in the assay procedure of bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds [29]. The assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacterial toxins, pesticides and cytotoxicity testing of dental materials. The eggs of Brine Shrimp ware hatched in a tank at a temperature around 37^0C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). With the help of a pasteur pipette nauplii were exposed to different concentrations of the extracts.

Preparation of Test Groups: For the experiment, 20 mg of extract were dissolved in 1ml of DMSO and adjusted up to 20 ml by 3.8% NaCl. Then the solutions of varying concentrations (500, 200, 100, 50, 20, 10, 5, 1 µg/ml) were obtained by serial dilution technique. Each test tube contained about 5 ml of sea water with 10 shrimp napulli.

Counting of Nauplii: The test tubes were kept at room temperature for about 24 hours and then, percent of mortality of napulli was counted with the help of a magnifying glass. The rate of mortality of nauplii was found to be increased in concentration of each of the samples. The median lethal concentration (LC50) was determined using the probit analysis method described by Finney [30], as the measure of toxicity of the plant extract.

RESULTS AND DISCUSSION:

***In-vitro* Anti-Inflammatory Activity Test:** Anti-inflammatory property is shown in **figure 1** and it shows that with concentration the absorbance is decreased it means %inhibition is increased with concentration and there is a significant anti-inflammatory effect.

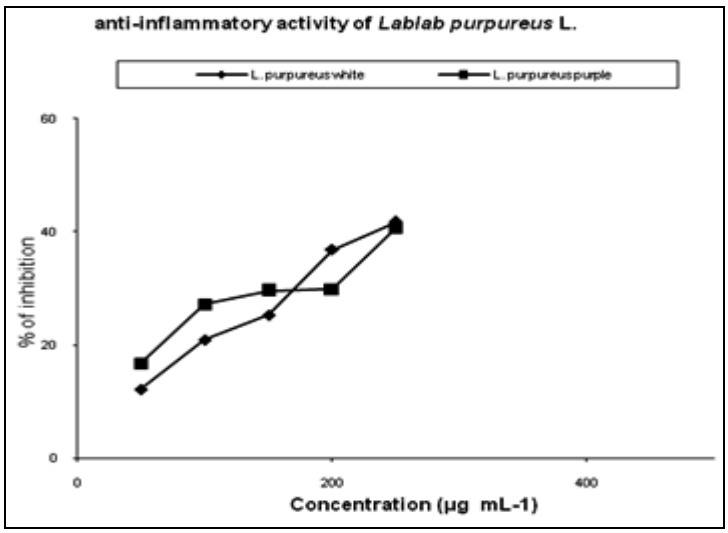

FIGURE 1: ANTI-INFLAMMATORY TEST OF METHANOL EXTRACT OF *LABLAB PURPUREUS* L.

It can be inferred from the **figure 1** that there is a significant anti- inflammatory activity compared with the results found by Kumarappan *et al*. [21]. However, animal study and other studies are necessary for its activity and also there is a need to elucidate its mechanism/s of anti– inflammatory action.

Total Flavonoid and Total Antioxidant Activity: The total flavonoid and total antioxidant contents of Lablab purpureus L. were expressed in quercetin and ascorbic acid equivalents respectively that are shown in **Table 1**.

The Total Antioxidants capacity of the samples was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The total antioxidants capacity of the test samples were calculated using the standard curve of Ascorbic acid (y=0.004x+0.150; R^2=0.887). The maximum total flavonoids (42.55±5.77 mg/g) was shown by Lablab purpureus (L.) sweet 'white' and The minimum was shown by Lablab purpureus (L.) sweet 'purple' (32.09±0.36 mg/g).

TABLE 1: TOTAL FLAVONOID AND TOTAL ANTIOXIDANT CONTENTS OF METHANOL EXTRACT OF *LABLAB PURPUREUS*

Sample	Total Flavonoid content (mg/g quercetin equivalents)	Total Antioxidant capacity (mg/g ascorbic acid equivalents)
L. purpureus sweet 'white'	42.55±5.77	46.89±9.02
L. purpureus sweet 'purple'	32.09±0.36	58.88±5.77

Values are the mean of duplicate experiments and represented as mean ± SD

Different studies suggest that different types of polyphenolic compounds such as flavonoids, phenolic acids which are found in plants have multiple biological effects, including antioxidant activity [31]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1 g was daily ingested from a diet rich in fruits and vegetables [32, 33]. The maximum Total antioxidant activity (58.88±5.77mg/g) was shown by Lablab purpureus (L.) sweet 'purple' and The minimum was shown by Lablab purpureus (L.) sweet 'white' (46.89±9.02 mg/g).

DPPH Radical Scavenging Activity: The results of DPPH free radical scavenging activity on the two crude methanol extracts and of ascorbic acid (standard) are shown in **figure 2**, where increasing the concentration of the extract, activity was found to increase. The maximum radical scavenging activity (IC50 value, 853 µg/ml) was shown by *L. purpureus* sweet 'white' and The minimum radical scavenging activity was shown by *L. purpureus* sweet 'purple' (IC50 value of 430 µg/ml). These results are not comparable to ascorbic acid.

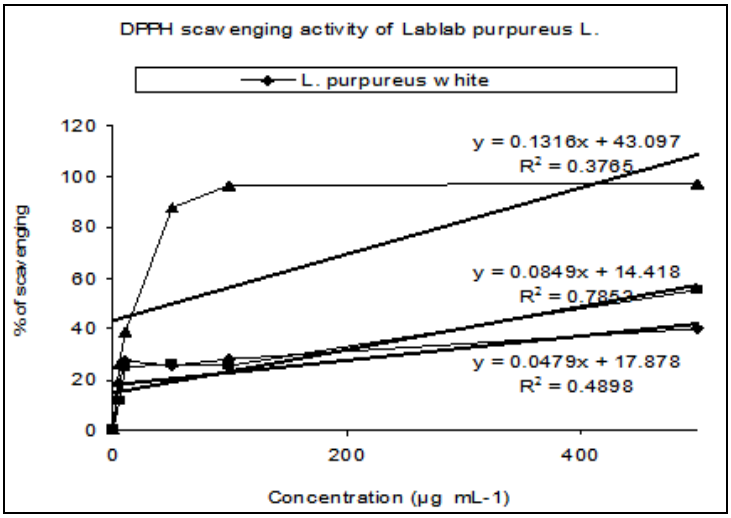

FIGURE 2: DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT *OF L. PURPUREUS.*

Reducing power capacity: By using the potassium Ferricyanide reduction method, the reductive capabilities of the plant extracts was identified in comparison with ascorbic acid which demonstrated at **figure 3**. The reducing power of the extracts was moderately strong while increasing dose it shows little increment.

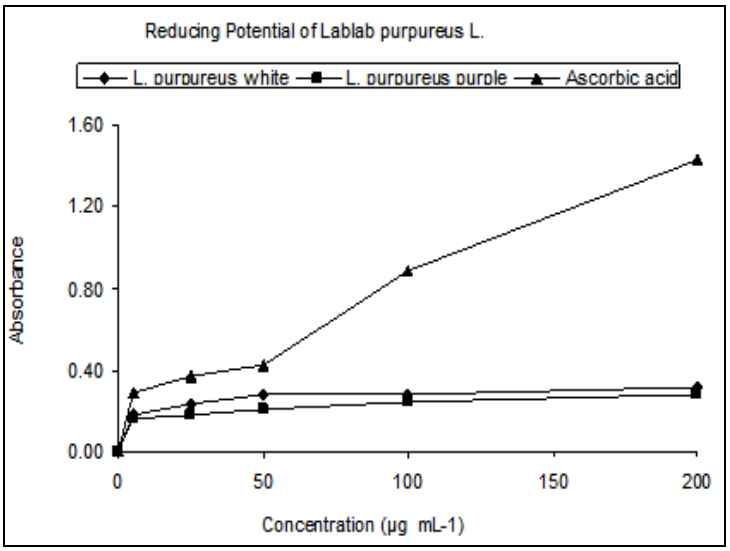

FIGURE 3: REDUCING POWER OF THE CRUDE PLANT EXTRACTS OF *L. PURPUREUS* AND ASCORBIC ACID
Values represents duplicate experiments and expressed as mean ± SD

Brine Shrimp Lethality Assay: The Methanolic extract of L. purpureus sweet 'white' and L. purpureus sweet 'purple' was tested for Brine shrimp lethality bioassay by using brine shrimp nauplii and DMSO as a solvent. Control was used to see whether DMSO had any effect on brine shrimp lethality or not. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality. For the extract, the number of nauplii died and percent mortality was counted. The results are shown in the following **table 2**.

TABLE 2: RESULTS OF BRINE SHRIMP LETHALITY ASSAY FOR *L. PURPUREUS* SWEET *'WHITE'AND* 'PURPLE'
Table 1

Conc. (C) µg/ml	Log C	No. of naupli taken	No. of naupli dead		% mortality		Probit		LC_{50} µg/ml	
			white	purple	White	purple	white	purple	white	purple
1	0	10	1	1	10	10	3.72	3.72	66.5	960.06
5	0.699	10	1	2	10	20	3.72	4.16		
10	1	10	3	2	30	20	4.48	4.16		
20	1.301	10	2	2	20	20	4.16	4.16		
50	1.699	10	3	2	30	20	4.48	4.16		
100	2	10	4	3	40	30	4.75	4.48		
200	2.301	10	9	5	90	50	6.28	5		
500	2.699	10	10	10	100	100	-	-		

Each of the test samples showed different mortality rates at different concentrations, the percentage mortality increased with an increase in concentration. 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 crude extracts. **Figure 4** indicates plot of probit and predicted regression line of *L. purpureus* from where the LC_{50} values were calculated. All of the extracts showed 100% mortality to brine shrimp at 500 µg/mL concentration. LC50 values are 66.5 µg/ml to 960.06 µg/mL for *L. purpureus* sweet 'white' and *L. purpureus* sweet 'purple' respectively, which indicates moderate cytotoxic effect of two bean pods.

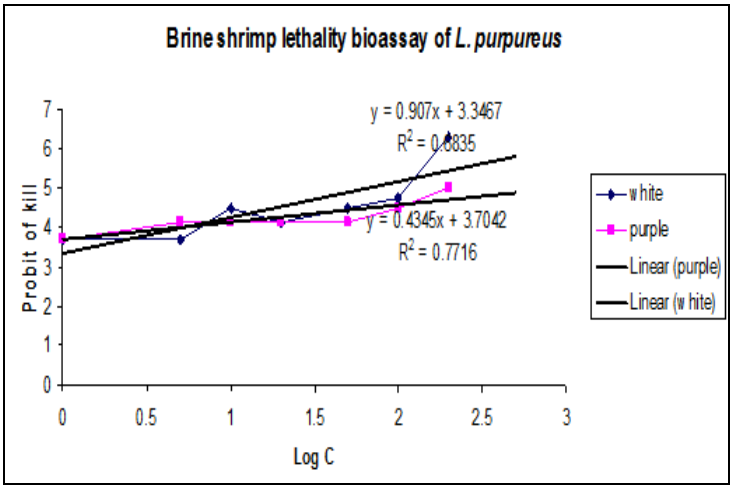

FIGURE 4: PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE OF *L. PURPUREUS*

CONCLUSION: In conclusion, it was observed from the present study that *L. purpureus* sweet 'white', *L. purpureus* sweet 'purple' have significant anti-inflammatory activity as well as are potential source of natural antioxidants while *L. purpureus* sweet 'white' has the potential cytotoxicity where the percentage mortality increased with an increase in concentration. But further pharmacological studies are required to be undertaken to understand the underlying possible mechanisms of the observed activities as well as need to isolate, purify, Characterize active phytochemicals responsible for these bioactivities.

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