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Determination of *in vitro* antioxidant capacity of *Albizia lebbeck* Leaves

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Abstract
Potential research on natural products has expanded a wide popularity due to the potential of discovering bioactive molecules. The antioxidant properties confined in plants have been proposed as one of the tool for the observed beneficial properties for various diseased conditions. Therefore, the present study has been accomplished to phytochemical testing and evaluation of antioxidant capacity of 70% ethanolic extract of *Albizia lebbeck* Leaves (EEAL). The antioxidant property of 70% EEAL was tested by using reducing power and free radical (superoxide, hydroxyl and nitric oxide) scavenging models (*in vitro*). *Albizia lebbeck* ethanolic extract has shown dose dependent antioxidant activity in all the models of the study (i.e. 82.03%-reducing power, 79.12%-superoxide, 49%-hydroxyl scavenging activity at 100mcg concentration). The 70% EEAL possess significant antioxidant activity. The antioxidant property may be attributed to the polyphenolic compounds like flavonoids and tannins that are present in the 70% EEAL.

Keywords: *Albizia lebbeck,* free radical, antioxidant activity, phytochemical investigation

1. Introduction
Free radical and antioxidant system are present in the balance form in the human body. Free radicals are unpaired electrons and generated during various metabolic reaction, exposure to ionizing radiation and by the influence of many xenobiotics. Antioxidant systems scavenge/quench the free radical. Excess generation of free radical overtakes the antioxidant defense of the cells. This leads to various physiological disorders such as cancer, atherosclerosis and ageing [1]. Several antioxidant of plant origin are experimentally proved and used as effective protective agents against free radicals [2, 3].
Albizia lebbeck benth. (Mimosaceae) is a large, erect, unarmed and deciduous tree. Upon literature review it was found that, the leaves are used in opthalmia [4]. The bark is used in bronchial asthma & other allergic disorders [5]. The flowers are useful in chronic cough & bronchitis [6]. The seeds are aphrodisiac [4], useful in inflammation, scrofula, skin disease, leprosy, leucoderma, chronic catarrh, seminal weakness, ophthalmopathy & poisoning [5]. The leaves of the plant *Albizia lebbeck* are rich in flavon., echinocystic acid, β-sitosterol and vicenin II etc [5]. The modern literature revealed that the plant is reported to possess anti-flammatory [7], nootropic [8, 9], anxiolytic [9], anticonvulsant [10, 11], antifertility [12] and antidiarrheal activity (seed) [13]. The present study was undertaken with the aim to assess the antioxidant activity of *Albizia lebbeck* Leaves.

2. Material and Method
2.1 Plant Material & Preparation of 70% EEAL
The leaves of plant *Albizia lebbeck* were collected from fields of Anand, Gujarat in the month of December 2010. It was identified and authenticated by Prof. G.C. Jadeja, Dept of Agricultural Botany, Anand Agricultural University.
The leaves were shade dried at room temperature to maintain the phytoconstituents and pulverized. The 70% ethanolic extract (12.82%) was prepared by using 70% ethanol in a soxhlet apparatus after de-fatting with petroleum ether.
Preliminary phytochemical investigation showed the presence of flavonoids, saponins in 70% EEAL as shown in Table. No. 01.

2.2 Determination of Reducing power of 70 % ethanolic *Albizia lebbeck* extract:
The reducing power of 70% EEAL were determined according to the method of Oyaizu (Oyaizu, 1986)[14]. Different doses of 70% EEAL were mixed in 1 ml of distilled water so as to get 20µg-100µg concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700nm. The % reducing power was calculated by using the formula (Figure 1):

$$\% \text{ increase in absorbance} = \frac{\text{Test OD} - \text{control OD}}{\text{Control OD}} \times 100$$

2.3 Superoxide anion scavenging activity
Measurement of Superoxide anion scavenging activity of 70% EEAL was done by using the method explained by Nishimiki and modified by Ilhami *et al* [15]. About 1 ml of nitroblue tetrazolium (NBT) solution (156µM NBT in 100 mM

phosphate buffer, pH 7.4), 1 ml NADH solution (468µM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of 70% EEAL and standard in water was mixed. The reaction was started by adding 100µl of Phenazine methosulphate (PMS) solution (60µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance at 560 nm was measured against blank. The % inhibition of OD was calculated by using the formula (figure 2):

$$\% \text{ inhibition in absorbance} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

2.4 Hydroxyl radical scavenging activity

Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation, assay of Halliwell and Gutteridge [16]. In 50mM phosphate buffer (pH 7.4), 1 mM deoxyribose, 0.2 mM phenyl hydrazine hydrochloride were prepared. 0.6ml of 1mM deoxyribose and 0.4ml of 70% EEAL and standard were taken. 0.6 ml phosphate buffer was added to make reaction solution 1.6ml. After 10 min incubation 0.4ml of 0.2 mM phenyl hydrazine was added. Incubation was terminated after 1 hr and 4 hrs and 1 ml each of 2.8% TCA and 1% (w/v) thiobarbituric acid were added to the reaction mixture and heated for 10 mins in a boiling water bath. The tubes were cooled and absorbance was taken at 532 nm (figure 3 & 4)

2.5 Nitric oxide (NO) radical scavenging activity

Nitric oxide (NO) radical were generated from sodium nitroprusside solution at physiological pH. Sodium nitroprusside (1ml of 10mM) were mixed with 1ml of 70% ethanolic extract of leaves of *Albizia lebbeck (Linn)* Benth different concentration (20-100 µg/ml) in phosphate buffer (pH 7.4). The mixture was incubated at 25°C for 150 min. To 1 ml of the incubated solution, 1ml of Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm [17].

3. Statistical analysis

Results were expressed as mean of # SEM (n=3). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparisons test. P value less than 0.05 was considered to be statistically significant ($p<0.05$).

4. Results and Discussion

The antioxidant activity of 70% EEAL showed dose dependent antioxidant activity. The 70% EEAL showed 82.03% reducing power, 79.12% super oxide anion, 49.00% hydroxyl and 45.90% nitric oxide radical scavenging activities at 100 mcg concentrations which are comparable to that of Sodium meta-bisulfate 25 mcg.

The method of Oyaizu (1986) was applied for the analysis of the reducing power. We measured the change of absorbance that accompanies the Fe^{3+} to Fe^{2+} transformations [14,15] in the presence 70% EEAL at 700 nm. The reducing power capacity of the extract may serve as a significant indicator of its potential antioxidant activity as shown in Figure No.01.

In the Superoxide anion scavenging activity, superoxide anions are produced from dissolved oxygen by PMS/NADH coupling reaction. This reaction reduces yellow dye (NBT) to produce the blue formation that is measured at 560 nm. Antioxidants are able to inhibit the blue NBT formation [18, 19]. The decrease of absorbance at 560 nm with extracts indicates the consumption of superoxide anion in the reaction mixture as discussed in Figure No.02.

In biochemical systems, superoxide radical and H_2O_2 react together to form the hydroxyl radical, $OH^{\bullet}$ this can attack and destroy almost all known biochemical [20]. Phenylhydrazine when added to erythrocyte hosts cause peroxidation of endogeneous lipids and alteration of membrane fluidity. This peroxidation damage to erythrocytes is probably initiated by active oxygen species like $O_2^{\bullet}$, $OH^{\bullet}$ and H_2O_2 which are generated in solution from auto-oxidation of phenyl hydrazine. Decrease in absorbance is indicating the increase in the hydroxyl free radical scavenging activity as shown in Figure No.03 and Figure No. 04.

In the Nitric oxide radical scavenging activity, nitric oxide reacts with oxygen to generate nitrite and peroxynitrite anions, which act as free radicals [17]. The 70% EEAL competes with oxygen to react with nitric oxide and thus inhibits the generation of the anions. The scavenging of nitric oxide by the extract as shown in Figure No.05 was concentration dependent.

In conclusion, the present study demonstrates that 70% EEAL possesses antioxidant activity. This property may be attributed to the antioxidant principles of plant, namely tannins and flavonoids. Further investigation is going on to isolate, characterize and screen the active principles that possess antioxidant property.

5. Tables and Figures

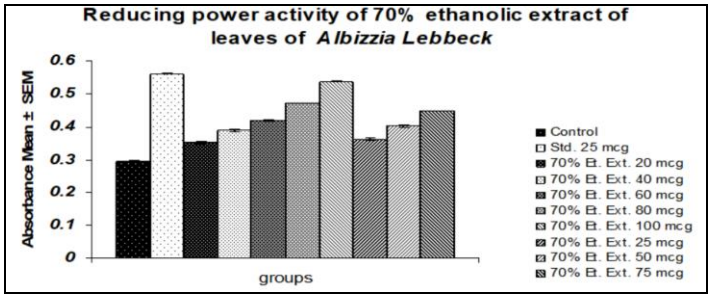

Fig 1: Reducing power of 70% ethanolic extract of *Albizia lebbeck*

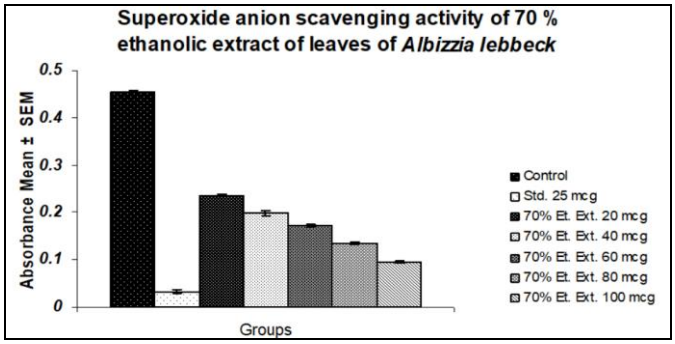

Fig 2: Superoxide anion scavenging activity of 70% ethanolic extract of *Albizia lebbeck*

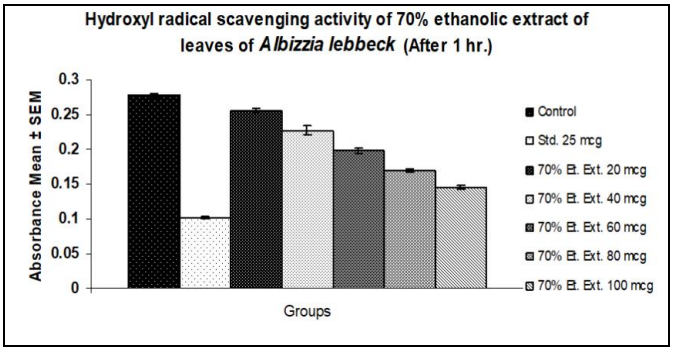

Fig 3: Hydroxyl Radical scavenging activity of 70% ethanolic extract of *Albizia lebbeck* after 1hr

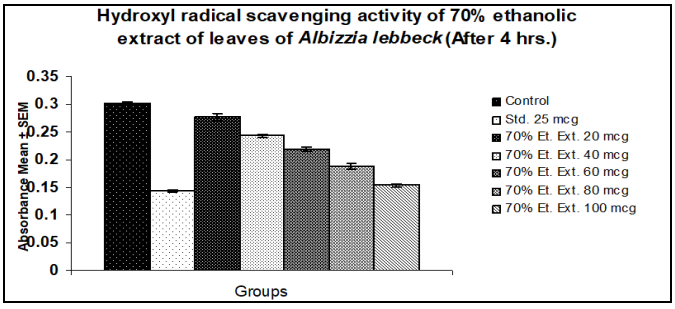

Fig 4: Hydroxyl Radical scavenging activity of 70% ethanolic extract of *Albizia lebbeck* after 4hr

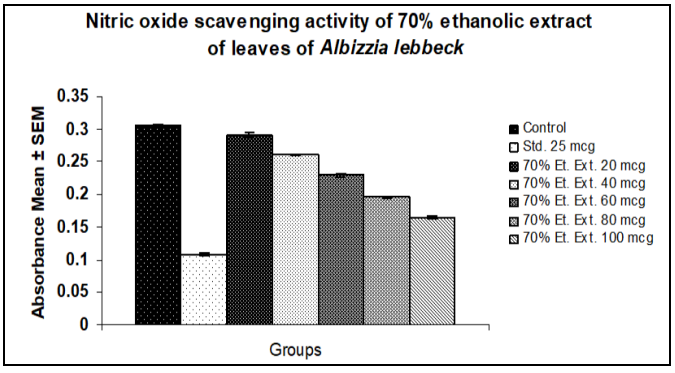

Fig 5: Nitric oxide radical scavenging activity of 70% ethanolic extract of *Albizia lebbeck*

Table 1: Preliminary Phytochemical Investigation of *Albizia lebbeck* leaves extract

Phyto-constituents	Observations
Flavonoids	+
Quinones	+
Saponins	+
Terpenoids	+
Anthraquinones	+
steroids	+

6. Acknowledgements

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