International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2013; 3(2): 93-101

Research Article

EVALUATION OF ANTI OXIDANT ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *ALBIZIA LEBBECK* (L.) BENTH

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(Received: 15 April 2013; Accepted: 21 April, 2013; Published: 28 April, 2013)
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Abstract: *Albizia lebbeck* (L.) Benth belonging to the family Fabaceae is a medicinal plant associated with diverse biological activities like antimicrobial, analgesic, anti-inflammatory, anti diarrhoeal, immunomodulatory, anti-arthritic, anti-ashmatic, anticonvulsant nootropic etc. reported by various researchers. In the presented study ethanolic root extract of the plant was evaluated for its *in-vitro* anti-oxidant potential by DPPH free radical scavenging activity, Nitric oxide scavenging activity, Hydrogen peroxide scavenging activity and reducing power assay taking ascorbic acid as the standard. The extract exhibited antioxidant activity possessing IC_{50} values (H_2O_2 Scavenging Activity), 345.94(Nitric Oxide Scavenging Activity), 945.76 (DPPH Free Radical Scavenging Activity). In ferric reducing assay the absorbance were found to increase with increase in concentration of the extract revealing reducing power.

Key**words:** *Albizia lebbeck,* Antioxidant activity, ethanolic root extract, DPPH, Nitric oxide scavenging, Hydrogen peroxide, Reducing power.

INTRODUCTION

Albizia lebbeck is native to India, Pakistan, Thailand, Nepal, Bangladesh, Indonesia, Australia, Malaysia and naturalized in many other tropical and subtropical areas. Literatutre survey revealeg that various parts of this plants are associated with diverse number of biological activities like Antibacterial and Antifungal Activity of pods, seeds, flowers and roots[1], Antibacterial activity of leaves[2], Antidiarrhoeal activity of stem bark[3], Analgesic and Anti-Inflammatory of bark[4-5], Immunomodulatory activity of leaves and bark[6], Anti-Arthritic activity of bark[7], Anti-asthmatic activity of stem bark[8], Antioxidant potential of pods and seeds[9], Anticonvulsant Activity of leaves[10], Mast Cell Stabilization Activity of bark and leaves[11], Nootropic activity of leaves[12], Antifertility activity of pods[13], Anti-histaminic activity of bark[14] etc. As the root part is not reported for anti arthritic activity, it was planned to evaluate the in-vitro antioxidant activity of the ethanolic extract of the root part.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) includes free radicals and other non-radical reactive derivatives. Reactivity of radicals is generally stronger than non-radical species though radicals are less stable[15]. ROS and RNS includes radicals such as hydroxyl (OH•), superoxide (O_2•−), peroxyl (RO_2•), alkoxyl (RO•), hydroperoxyl (HO_2•), nitrogen dioxide (NO_2•) nitric oxide (NO•) and lipid peroxyl (LOO•); and non radicals like hydrogen peroxide (H_2O2), nitrogen dioxide (NO2•) hypochlorous acid (HOCl), ozone (O3), singlet oxygen , peroxynitrate (ONOO−), nitrous acid (HNO_2), lipid peroxide (LOOH), dinitrogen trioxide (N_2O_3) 1[6].Free radicals are the species capable of independent existent that contains one or more unpaired electrons in its outer shell. Free radicals having single electron in their outer shell and becomes more reactive. They are unstable and try to become stable, either by accepting or donating an electron [17]. The free radicals are generated in living systems as a part of the normal physiological process [18]. Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress[19-20]. The ROS and free radicals mediated reactions are involved in various pathological conditions such as anaemia, asthma, arthritis, inflammation, neurodegeneration, cancer, mutagenesis, Alzheimer's, AIDS, ageing process and perhaps dementia, malaria[21-23].

Antioxidants are the substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance[24]. Plants are the potential source of natural antioxidants. The scientific reports and experimental studies have shown that plants contain a large variety of phytochemicals that have antioxidant property[25]. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants[26]. Examples include, carotenoids, flavonoids, tocopherols (delta> gamma> beta> alpha), Beta carotene, Lycopene, Nordihydroguaretic Acid, Sesamol, Gossypol, Anthocyanins, Catechins, Ellagic acid, Lutein, Resveratrol, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocotrienols etc., are some of the antioxidants produced by the plants[27].

It is widely accepted that a plant-based diet with high intake of fruits, vegetables, and other nutrient-rich

plant foods may reduce the risk of oxidative stress-related diseases[28-29]. Understanding the complex role of diet in such chronic diseases is challenging since a typical diet provides more than 25,000 bioactive food constituents[30], many of which may modify a multitude of processes that are related to these diseases. Many synthetic antioxidants are currently in use; nevertheless, there is a growing evidence of consumer preference for natural antioxidants because of their potentially lower toxicity.

All plants produce a diverse number of secondary metabolites. One of the most important groups of the metabolites is phenolic compounds. Phenolics are the compounds characterized by at least one aromatic ring (C6) containing one or more hydroxyl groups. They are mainly synthetized from cinnamic acid, which is formed from phenylalanine by the action of L-phenyloalanine ammonia-lyase[31].

Phenols are divided into several groups, distinguished by the number of constitutive carbon atoms in conjunction with the structure of the basic phenolic skeleton (simple phenols, benzoic acids, phenylopropanoids and flavonoids)[32-33]. Phenols are one of the major groups of non-essential dietary components that have been associated with the inhibition of atherosclerosis and cancer, as well as for age-related degenerative brain disorders[34-35]. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers[36].

As the root part of *Albizia lebbeck* (L.) Benth is not reported for antioxidant activity, it was planned to evaluate the in-vitro antioxidant activity of the ethanolic extract of the root part. As phenolic compounds are associated with antioxidant activity, it was planned to determine the total phenolic content of the ethanolic root extract.

MATERIALS AND METHODS

Collection of Plant Material and Authentfication

The plant part was collected from the roadside of Ananthagiri Village, Nalgonda, Andhra Pradesh and was authentified Bby Dr. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

Drying and Extraction

The root part was dried under shade, powdered and extracted with ethanol by hot continuous Soxhlet extraction process.

Chemicals

The all chemicals used were of analytical grade and were supplied from Aman Scientific Products, Vijayawada.

Instruments

The instruments facility of Anurag Pharmacy College was utilized.

Evaluation of Antioxidant Activity

DPPH free radical scavenging activity

Principle

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay evaluates free radical scavenging activity by measuring the color change that occurs when a DPPH radical is quenched by a free radical scavenger that donates a hydrogen atom.

Method[37]

The ethanolic plant extract was tested for the DPPH free radical scavenging activity according to the method of Pan *et al*.[37] with minor modification. 0.2 mL of the extract solution in ethanol (95 %) at different concentrations was added to 8 mL of 0.004 % (w/v) stock solution of DPPH in ethanol (95 %). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV–Visible spectrophotometer. As a positive control, synthetic antioxidant gallic acid was used. All determinations were performed in triplicate. The DPPH radical scavenging activity (*S%*) was calculated using the following equation:

$$S\% = [\,(A_{control} - A_{sample})\,/A_{control}\,]\times 100$$

where $A_{control}$ = absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} = absorbance of the test sample.

Nitric oxide scavenging activity assay

Principle

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat.[38] Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities[39]. Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases[40]. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases.

Method[38]

2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25oC for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a

spectrophotometer. The nitric oxide radicals scavenging activity was calculated as

$$S\% = [\,(A_{control} - A_{sample})\,/A_{control}\,]\times 100$$

where $A_{control}$ = absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} = absorbance of the test sample.

Hydrogen peroxide scavenging assay

Principle

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate[41].

Method[42]

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the method of Ruch [42]. Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following equation.

$$S\% = [\,(A_{control} - A_{sample})\,/A_{control}\,]\times 100$$

where $A_{control}$ = absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} = absorbance of the test sample.

Reducing power assay

Principle

Although a reductant is not necessarily an antioxidant, an antioxidant is commonly a reductant[43]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[44]. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[45-46].

Method[47]

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu[6] The extract (0.75 mL) at various concentrations was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (1%, w/v), followed by incubating at 50^{o}C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

RESULTS

Results of Total Phenolic content of EEAL

The Total Phenolic of EEAL root was estimated using standard Gallic acid equivalent of phenols. The various concentration of Gallic acid (10-200 µg/ml) calibration curve was plotted using Microsoft Office Excel 2007 and the results were given in Table 1 and in Figure 1. The total phenolic contents for EEAL was obtained for 500 µg/ml of extract from calibration curve of gallic acid and the results obtained are given in Table 2. The Total phenolic content for EEAL were calculated using standard calibration curve ($y=0.007x+ 0.056$, $R^2=0.995$) and was found to be 110.5. All the readings are taken for thrice.

Table 1: Results for construction of gallic acid standard curve

Sl. No.	Concentration (µg/ml /ml)	Absorbance (Mean ±SD)
1	10	0.083±0.002
2	50	0.463±0.01
3	100	0.803±0.02
4	200	1.486±0.04

Figure 1: Construction of gallic acid standard curve

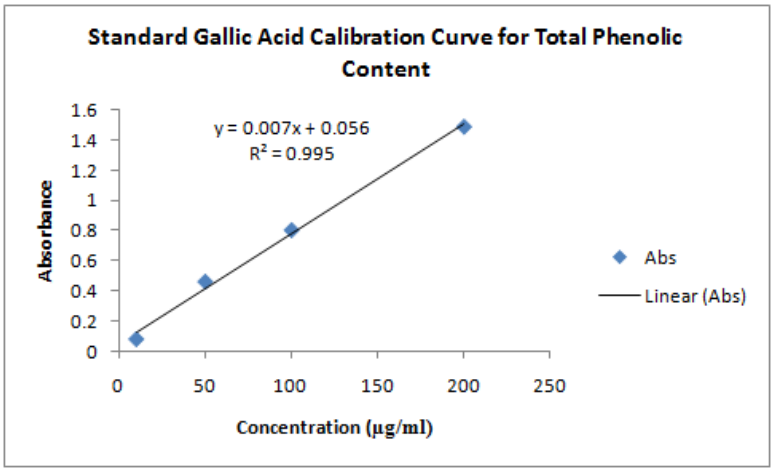


Table 2: Total Phenolic contents EEAL

Extract	Concentration	Total Phenolic Content (mg/g GAE)
EEAL	500(ug/ml)	110.5

Results of Antioxidant activity

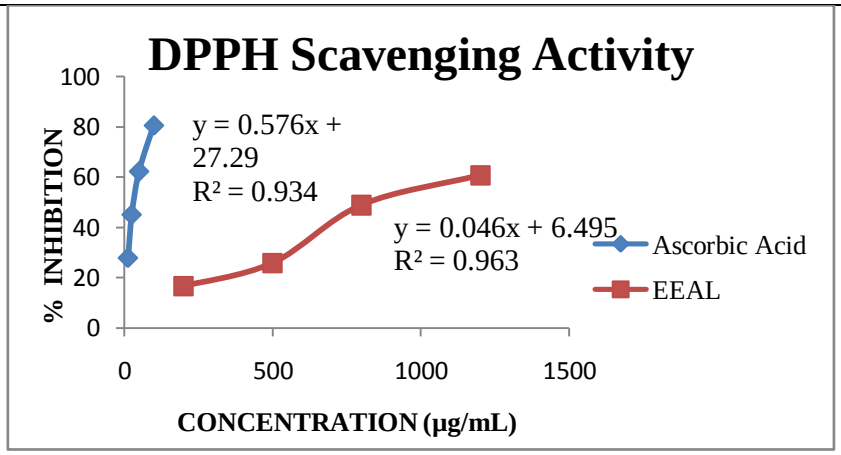
DPPH free radical scavenging activity

The DPPH radical scavenging activity of EEAL root was evaluated and compared with Ascorbic acid and the results are given in Table 3. The % inhibition at various concentration (200-1200 µg/ml) of EEAL root as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Figure 2 using Microsoft Office Excel 2007. The IC_{50} values are calculated from graph and were found to be 38.36 µg/ml (Ascorbic acid) and 945.76 (EEAL).

Table 3: Results of DPPH scavenging activity

Sl. No.	Concentration (ug/ml)	% Inhibition (Mean±SD)	IC_{50} (µg/ml)
Std (Ascorbic Acid)			38.36
1	12.5	27.95± 2.46	
2	25	45.16±1.61	
3	50	62.36±1.93	
4	100	81.72±2.46	
EEAL			945.76
1	200	16.66± 1.09	
2	500	25.80± 3.22	
3	800	48.92± 2.46	
4	1200	60.75± 2.46	

Figure 2: DDPH scavenging activity of Ascorbic acid and EEAL



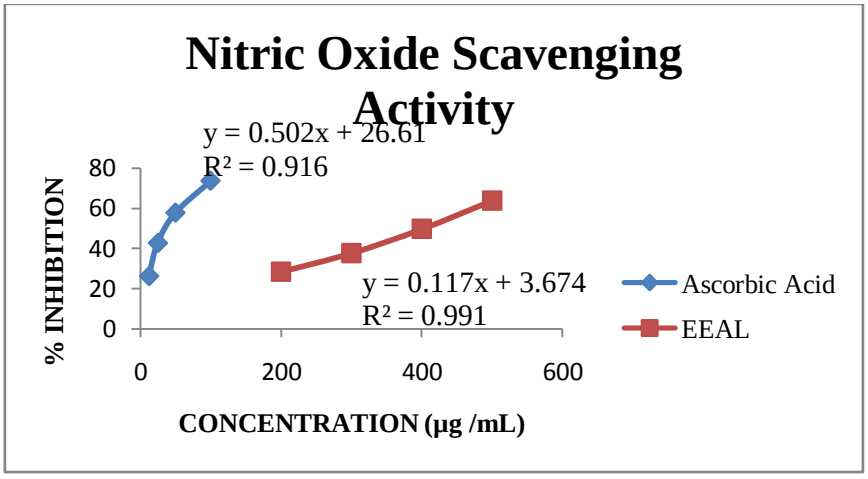
Results of Nitric Oxide scavenging activity

The nitric oxide scavenging activity of EEAL root was evaluated and compared with Ascorbic acid and the results are given in Table 4. The percentage inhibition (% inhibition) at various concentration (200-500 µg/ml) of EEAL as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Figure 3 using Microsoft Office Excel 2007. The IC_{50} values are calculated from graph and were found to be 46.59 µg/ml (Ascorbic acid) and 395.94 (EEAL).

Table 4: Results of Nitric Oxide scavenging activity

Sl. No.	Concentration (µg/ml)	% Inhibition (mean ±SD)	IC_{50} (µg/ml)
Std (Ascorbic Acid)			46.59
1	12.5	26.53±2.96	
2	25	41.66±1.00	
3	50	57.45±1.36	
4	100	73.68±1.97	
EEAL			395.94
1	200	28.5±2.31	
2	300	37.72±2.31	
3	400	49.68±1.00	
4	500	63.8±2.38	

Figure 3: Nitric Oxide scavenging activity of Ascorbic acid and EEAL



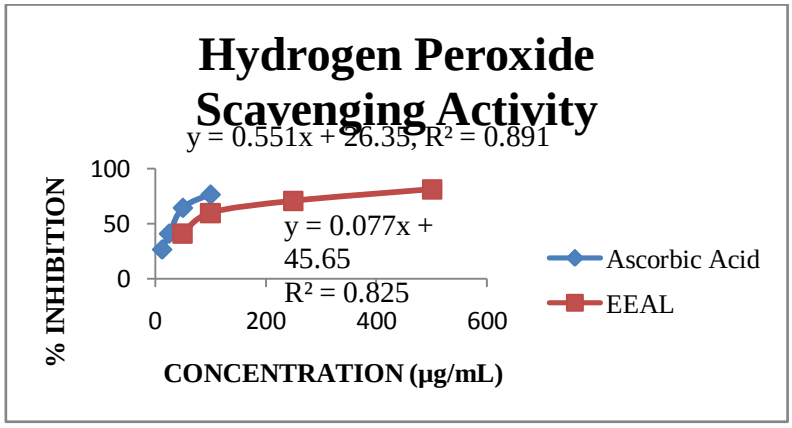
Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of EEAL root was evaluated and compared with Ascorbic acid and the results are given in Table 5. The percentage inhibition (% inhibition) at various concentration (200-500 µg/ml) of EEAL as well as standard Ascorbic acid (12.5-100 µg/ml) were calculated and plotted in Figure 4 using Microsoft Office Excel 2007. The IC_{50} values are calculated from graph and were found to be 42.92 µg/ml (Ascorbic acid) and 56.47 (EEAL).

Table 5: Results of Hydrogen Peroxide scavenging activity

Sl. No.	Concentration (µg/ml)	% Inhibition (mean ±SD)	IC_{50} (µg/ml)
Std (Ascorbic Acid)			
1	12.5	26.51±0.28	42.92
2	25	40.90±1.21	
3	50	64.39±1.23	
4	100	76.51±2.08	
EEAL			
1	200	40.90±2.27	56.49
2	300	59.59±1.15	
3	400	70.49±2.21	
4	500	81.06±2.27	

Figure 4: Hydrogen Peroxide scavenging activity of Ascorbic acid and EEAL



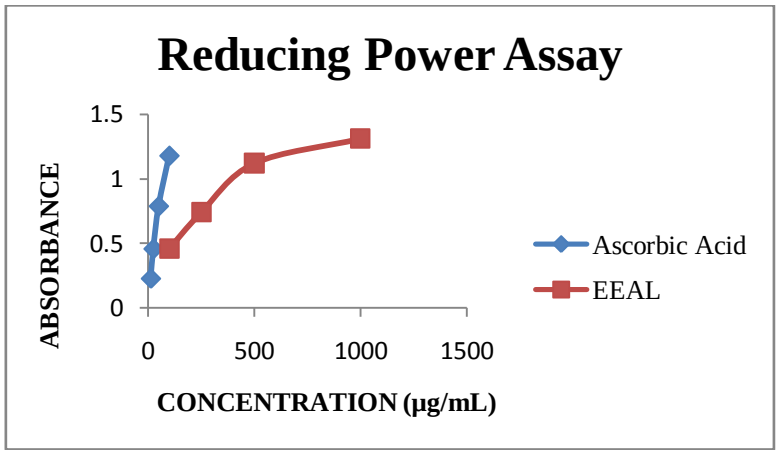
Reducing power assay

The reducing abilities of EEAL root was evaluated and compared with Ascorbic acid and the results are given in Table 6. The mean of three absorbance at various concentration (50- 500 µg/ml) of EEAL as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Figure 5 using Microsoft Office Excel 2007. The reductive capabilities were found to increase with increasing of concentration of EEAL as well as standard ascorbic acid.

Table 6: Results of Reducing power Assay

Sl. No.	Concentration (µg/ml)	% Inhibition (mean ±SD)
Std (Ascorbic Acid)		
1	12.5	0.23±0.03
2	25	0.46±0.02
3	50	0.79±0.02
4	100	1.18±0.03
EEAL		
1	50	0.47±0.01
2	100	0.65±0.02
3	250	0.95±0.02
4	500	1.05±0.04

Figure 5: Reducing Power of Ascorbic acid and EEAL



DISCUSSION

Polyphenolic compounds are present in the ethanolic root extract of *Albizia lebbeck (L.) Benth.* It is well known that flavonoids and polyphenols are natural antioxidants but have also been reported to significantly increase SOD, glutathione and catalase activities. Furthermore it was shown that these compounds act as promoters for SOD, catalase and glutathione and cause the expression of SOD, glutathione and catalase[48].

So, the extract was planned to be evaluated for in-vitro antioxidant activity. The *in-vitro* antioxidant potential of the EEAL was evaluated by DPPH free radical scavenging activity, Nitric oxide scavenging activity, Hydrogen peroxide scavenging activity. Moreover the Ferric reducing power assay was also carried out which indirectly suggest the anti-oxidant potential of the concerned extract. The studies were carried out taking ascorbic acid as the standard antioxidant which is also a natural antioxidant. The results of antioxidant activity by DPPH free radical scavenging activity, Nitric oxide scavenging activity and Hydrogen peroxide scavenging activity were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. Concentration dependent effects were observed in each case i.e; higher concentrations were found to exhibit higher % inhibition in each protocol of the antioxidant study. The graphs were constructed by taking % inhibition along the X-axis and various concentrations were taken along the Y-axis. The IC_{50} value (50% inhibition) of the EEAL and the standard ascorbic acid were determined in all the studies. With reference to the observed IC_{50} value of EEAL, the antioxidant potential was found to be highest in case of hydrogen peroxide scavenging activity and it had the following order-

H_2O_2 Scavenging Activity (IC_{50} -) > Nitric Oxide Scavenging Activity (IC_{50} -345.94) > DPPH Free Radical Scavenging Activity (IC_{50} -945.76)

The ferric reducing assay of the EEAL suggested that it has the potential to reduce the ferric form. The absorbance value was found to be increased with increase in concentration of the extract.

CONCLUSION

From the presented study it can be concluded that the ethanolic foot extract of *Albizia lebbeck* (L.) Benth possess antioxidant activity. The further study on this plant might provide the isolation of some active constituents endering the antioxidant potential.

Acknowledgement

The authors are very thankful to the management of Anurag Pharmacy College for providing the necessary facilities for carrying out the investigational studies.

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