In vitro antioxidant activity and polyphenolic content of bark extracts of plant *Albizia lebbeck* (L.) Benth.

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Abstract-
Background: The study consists of estimation of Total Flavonoid Content, Total Phenolic Content and Assessment of in vitro Antioxidant Activity of Extracts of bark of plant *Albizia lebbeck* (L.) Benth. Leaves by using various in-vitro methods.
Materials and Methods: In this study the Total Flavonoid Content, Total Phenolic Content, Antioxidant activity of aqueous, methanolic and hydroalcoholic extract of bark of the plant *Albizia lebbeck* (L.) Benth. was evaluated by Folin–Ciocalteau reagent, Aluminium chloride, 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging, Nitric oxide radical scavenging assay, Hydrogen peroxide and reducing assay methods and compared.
Result and Discussion: The result indicated that methanolic extract of the leaves of plant exhibited potent antioxidant activity as compared to hydroalcoholic and aqueous extract with reference to Ascorbic acid (Vitamin C) as standard.
Conclusion: The present estimation and assessment showed that the plant *Albizia lebbeck* (L.) Benth. used as potential antioxidant activity with maximum concentration of Total Flavonoid and Total Phenolic Content.

***Keywords: Albizia lebbeck* (L.) Benth., Antioxidant, Total Phenolic, Total Flavonoid, DPPH scavenging activity, Nitric oxide scavenging activity, Reducing power, Hydrogen peroxide etc.**

INTRODUCTION:

Free radicals are naturally occurring by-products of our own body metabolism. Free radicals are electrically charged molecules that attack various cells, tissues, organs, tearing through impermeable cellular membranes to react with the nucleic acids, proteins and enzymes present in the body (1). Free radicals can cause the lipid peroxidation in foods which leads to their deterioration (2). Oxidation is known as to be the major cause of foods and materials degradation (3).

Lots of research work are clearly showed that free radicals would damage nearby structures including DNA, proteins and lipids. Radical scavenging antioxidant substances are particularly important in antioxidative-defence which are protecting the cells from the injury of free-radical (4).

Plants are very good source of biologically active compounds known as phytochemicals. The phytochemicals are found to be act as potent antioxidants by scavenging free radicals and may have important therapeutic potential for free radical associated disorders (5). It is well known that free radicals which are the major causes of various chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer. Therefore, it is very important to find out antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants (6).

Reactive oxygen species (ROS) like as superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are generated as by products of biological reaction or from exogenous factors (7). *In vivo*, some of these Reactive oxygen species (ROS) are play an important role in cell metabolisms like as energy production, phagocytosis and intercellular signaling (8). However, generally these Reactive oxygen species (ROS) are produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic reactions which have a wide variety of pathological effects like as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases (9-11).

Antioxidants are substances which are provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking (12). There are many substances from natural sources have been shown to contain antioxidant and they are under the study. Antioxidant compounds like as Phenolic acids, polyphenolic substances and flavonoids which scavenge the free radicals like as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (13). Ethnomedical literature has reveals that the large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There are many plants that have been found to possess strong antioxidant activity (14).

Albizia lebbeck (L) Benth . (Family – Fabaceae) is commonly known as Lebbeck Tree, siris tree in English; shiris Tree, siris in Hindi. A large, erect, unarmed, deciduous, spreading tree. *A. lebbeck* (L.) Benth. Deciduous tree, growing to 30 m tall in native forests. In open situations, trees develop a spreading, sometimes multistemmed habit, to 25 m tall and 30 m across, with low branching. It can develop root suckers, and produces dense coppicing from cut stumps (15).

Bark used as astringent, acrid, bitter, sweet, expectorant, aphrodisiac, depurative, ophthalmic, tonic (16), diseases of the gum and toothache (17), weakness, cures diseases of blood, anthelmintic, itching, skin disease, piles, deafness, scabies, syphilis and boils (18) helminthes infections, bronchitis, dental infections, leprosy, antidiarrheal activity and paralysis (19) pruritus, eczema, paralysis and worm infestation (20). Flower used as Asthma (21) bronchitis and chronic cough (22) chronic catarrh, inflammation, poisoning, skin diseases, opthalmopathy, leprosy (23). Leaves used as Antiseptic, anti-cancer activity, anti- tubercular, antimicrobial, anti-

protozoal, anti- dysenteric and antifertility (24). The seed oil is applied topically to cure leucoderma, astringent, aphrodisiac (25) diarrhoea and piles (26).

This research paper reports total flavonoid, total phenolic and antioxidant potential of methanolic, hydroalcoholic and aqueous extracts of bark of the plant *Albizia lebbeck* (L.) Benth. evaluated by Folin–Ciocalteau reagent, Aluminium chloride, 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging, Nitric oxide radical scavenging assay, Hydrogen peroxide and reducing assay methods.

MATERIALS AND METHODS

Plant Collection

The barks of the plant *Albizia lebbeck* (L.) Benth. were collected from local area of Dhule district, Maharashtra, India. The selected plant was authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L. K. Dr. P. R. Ghogrey Science College, Dhule, Maharashtra. Leaves were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Preparation of Plant extract

The leaves of plant were dried at room temperature and transformed to coarse powder. The powder of leaves were extracted with three solvents i.e methanol, water and water-ethanol separately by Soxhlet extraction method. Finally, the extract was evaporated and dried under vacuum to obtain thick sticky extract.

Chemicals

2-2 diphenyl-1 picryl hydrazyl (DPPH), Methanol, Sodium nitroprusside, Sulphanilamide, Potassium ferricyanide, Trichloroacetic acid, Ascorbic Acid, Ferric chloride, N-(1- naphthyl) ethylenediamine dihydrochloride), Hydrogen Peroxide solution, Phosphate Buffer and all other reagents were of analytical grade.

Instrument

Shimadzu UV – visible spectrophotometer

Determination of Total Phenolics Content (27)

The Total soluble phenolics in the extracts were determined with Folin–Ciocalteau reagent according to the method reported by Singletion *et al.,* (1999) using gallic acid as a standard phenolic compound. About 500µl (20mg/ml) of plant sample was added to 25ml of distilled water and 1ml of Folin-Ciocalteu reagent (1:10). Then this mixture was kept at room temperature for 3 minutes, after then 1.5ml of 2% sodium bicarbonate was added, soon after vortexting the reaction mixture for 1 hour at room temperature, the absorbance was measured at 760nm. All the tests were performed in triplicates and the results were averaged. The concentration of total phenolic compounds in methanolic leaf extracts was determined as microgram of gallic acid equivalent by using an equation that was obtained from the standard gallic acid graph (10- 100 µg/ml).

Determination of Total Flavonoid Content (28)

The aluminium chloride colorimetric assay was used for total flavonoids determination, as described by Zhishen *et al.* (1999). 100µl (20mg/ml) of the extract was mixed with 2.5 ml of distilled water and 300µl of 5% sodium nitrate. Then, it was incubated at room temperature for 5 minutes and 300µl of 10% aluminium chloride, 2ml of 1M sodium hydroxide and 1ml of distilled water were added. Then, absorbance of the reaction mixture was measured at 512nm, along with the standard, quercetin and blank. The total flavonoids content was determined as microgram, quercetin equivalent by using the standard, quercetin graph, obtained by comparing the calibration curve prepared from a reference solution containing quercetin (10- 100µg/ml).

DPPH radical scavenging assay (29)

The antioxidant activity of the methanolic, aqueous and hydroalcoholic extracts of dried bark of the plant *Albizia lebbeck* (L.) Benth. was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH carried out by using the method of Molyneux. About 1 ml of 100 µM DPPH solution in methanol, equal volume of the extract in methanol of different concentrations of the extract in methanol was added and incubated in dark for 30 min and 1ml of methanol served as control. The change in colour was observed in terms of absorbance using a spectrophotometer at 517 nm. The different concentrations of ascorbic acid were used as reference compound. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage Inhibition:-} \quad \frac{\text{Absorbance Control} - \text{Absorbance test}}{\text{Absorbance Control}} \times 100$$

Nitric oxide radical scavenging assay (30)

Nitric oxide radical scavenging activity was measured spectrophotometrically according to the method described by Govindharajan *et al*. When sodium nitroprusside was mixed with aqueous solution at physiological pH, suddenly it generates nitric oxide, which reacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Nitric oxide scavengers compete with oxygen leading to reduced production of nitrite ions. About 1 ml of Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the methanolic, aqueous and Hydroalcoholic extract (25 - 200 µg/ml) in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25°C for 2 h. After incubation 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride]. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-(1- naphthyl) ethylenediamine dihydrochloride) was measured spectrophotometrically at 546 nm. Control tube was maintained with all chemicals excluding *Albizia lebbeck* (L.) Benth. extract. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula

$$\text{Percentage Inhibition:-} \quad \frac{\text{Absorbance Control} - \text{Absorbance test}}{} \times 100$$

Absorbance Control

Hydrogen peroxide radical scavenging activity analysis

Hydrogen peroxide radical scavenging activity was determined by the method of Ruch et al. (1984) (31). Hydrogen peroxide (H_2O_2) is a biologically important oxidant because of its ability to generate the hydroxyl radical which is extremely potent. The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. Its very short half-life, however, restricts its diffusion capability and its potency (32, 33). A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of the methanolic, aqueous and hydroalcoholic extracts of bark of the plant *Albizia lebbeck* (L.) Benth. (25-200 µg/ml) in phosphate buffer were added to a H_2O_2solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of *Albizia lebbeck* (L.) Benth. and Ascorbic acid as standard compound was calculated as

H_2O_2 radical scavenging activity (%) = [{Ao – A1/Ao}] ×100.

Where, Ao is the absorbance of the H_2O_2,

A1 is the absorbance of the presence of the extract in H_2O_2 solution (34, 35).

Reducing power assay (36)

The reducing power was determined according to the method of Berker *et al*. The methanolic, aqueous and hydroalcoholic extracts of bark of the plant *Albizia lebbeck* (L.) Benth. (25-200 µg/ml, 2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 min. After the addition of 2.5 ml of 10% trichloroacetic acid the reaction mixture was centrifuged at 3000 rpm for 10 min. About 5 ml of the upper layer was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power.

Statistical analysis

All the assays were carried out in triplicate and each experiment was independently repeated at least three times, through which means and standard deviations (SD) were generated. The results are presented as the average and standard error of three experiments. The data was analyzed by using Sigma plot 10.0.

Table 1. Total Phenolic Content present in extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Plant Extracts	Absorbance mean ± SEM (n=3)	Total Phenolic content (mg gallic acid equivalent/g of dried extract)
Aqueous	0.148±0.004	49.33±0.75
Methanolic	0.278±0.003	92.66±1.06
Hydroalcoholic	0.196±0.002	64.33±0.94

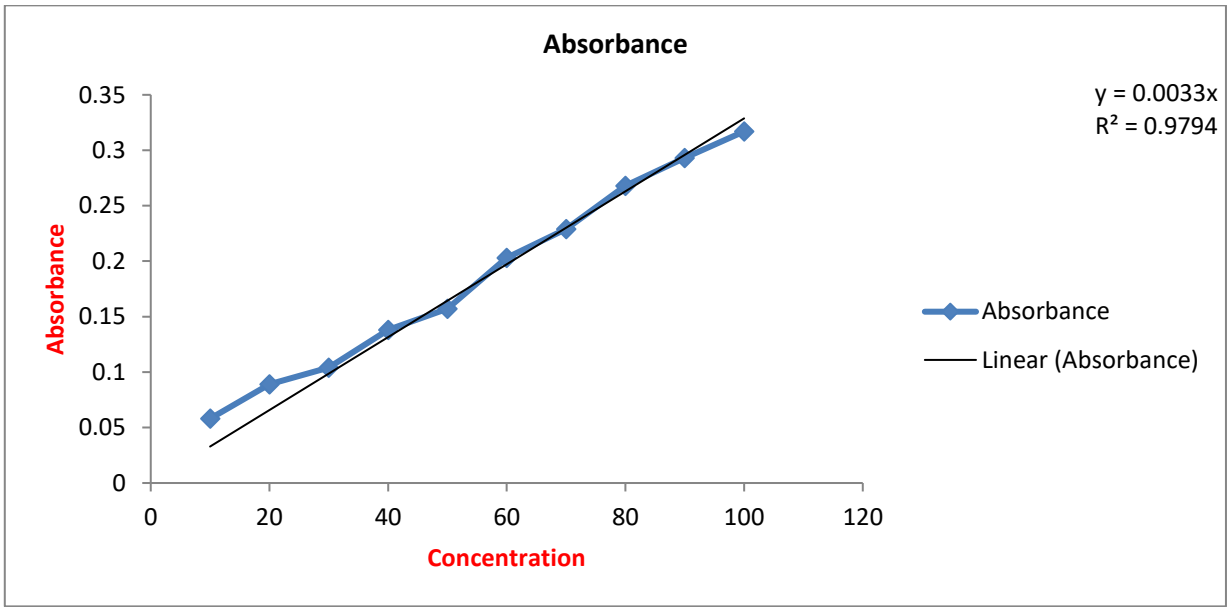

Figure 1. Calibration curve of Gallic acid

Table 2. Total Flavonoid Content present in extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Plant Extracts	Absorbance mean ± SEM (n=3)	Total flavonoid (mg querecetin equivalent/g of dried extract)
Aqueous	0.228±0.002	56.25±0.51
Methanolic	0.329±0.003	81.75±0.83
Hydroalcoholic	0.256±0.002	64.00±0.57

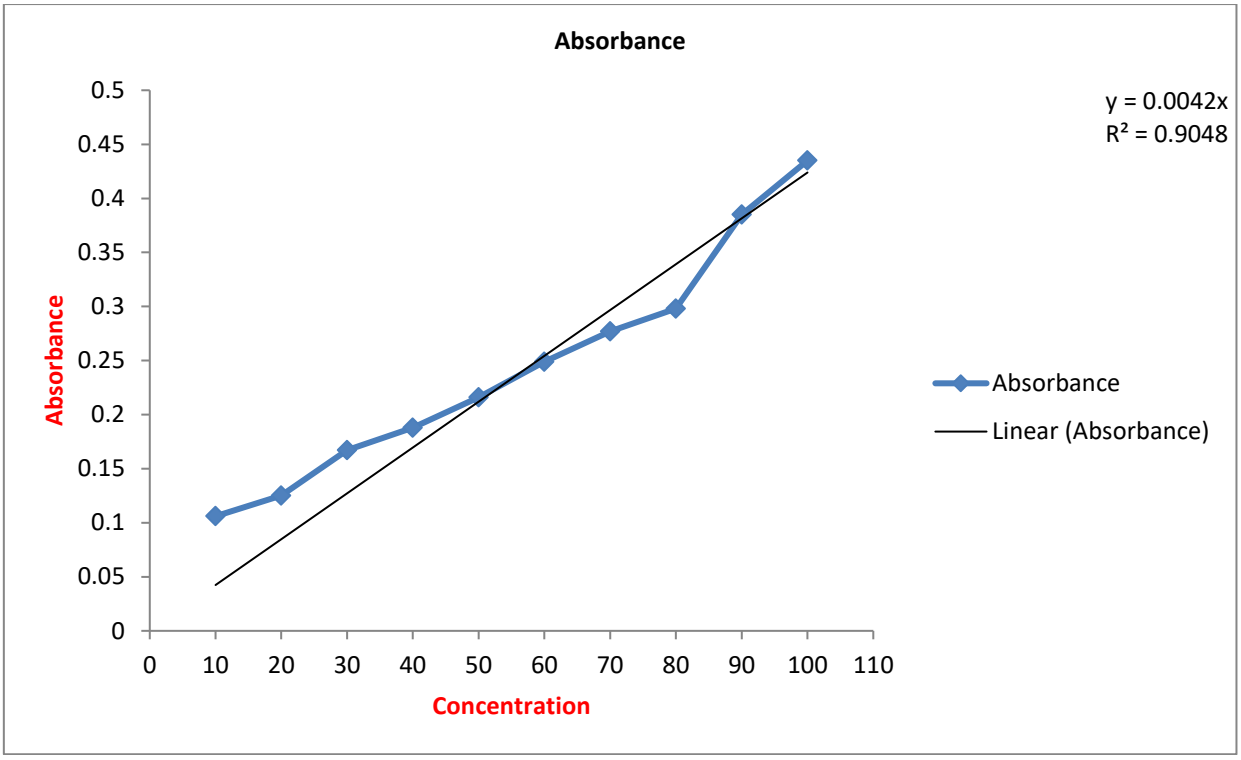

Figure 2. Calibration curve of Querecetin

Table 3. DPPH free radical scavenging activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard ASC	MET	HAL	AQE
25	19.71±0.26	14.22±0.24	5.64±0.19	2.7±0.18
50	30.1±0.27	20.31±0.11	9.37±0.14	4.48±0.19
75	44.36±0.26	32.28±0.18	20.96±0.15	13.89±0.11
100	59.57±0.21	49.25±0.21	33.97±0.26	26.48±0.18
125	76.81±0.45	59.46±0.22	47.21±0.18	39.58±0.15
150	86.22±0.25	65.46±0.11	58.73±0.28	57.92±0.26
175	88.31±0.14	81.55±0.25	77.28±0.11	68.11±0.22
200	96.97±0.45	89.56±0.24	82.47±0.23	74.88±0.17
IC50	92.93	109.64	126.90	143.26

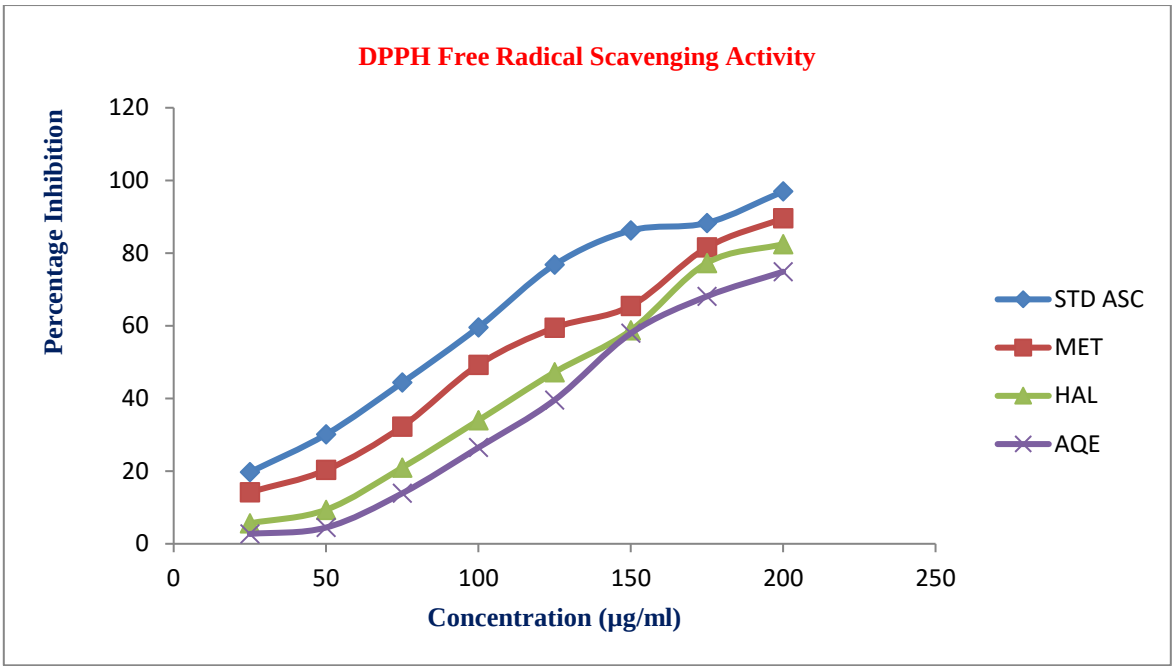

Figure 3. DPPH free radical scavenging activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Table 4. Nitric oxide free radical scavenging activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard Ascorbic Acid	MET	HAL	AQE
25	18.23±0.09	15.57±0.22	8.38±0.27	4.35±0.10
50	35.38±0.11	24.66±0.10	17.11±0.10	10.24±0.10
75	48.9±0.19	38.71±0.15	32.96±0.25	24.7±0.10
100	64.46±0.1	48.21±0.15	38.07±0.10	34.08±0.18
125	76.41±0.13	56.54±0.17	48.57±0.10	44.98±0.18
150	82.8±0.1	70.4±0.21	58.08±0.06	54.48±0.21
175	89.21±0.06	80.78±0.14	72.18±0.10	67.88±0.10
200	95.44±0.13	89.9±0.36	80.5±0.10	75.32±0.07
IC50	92.42	107.99	125.62	136.98

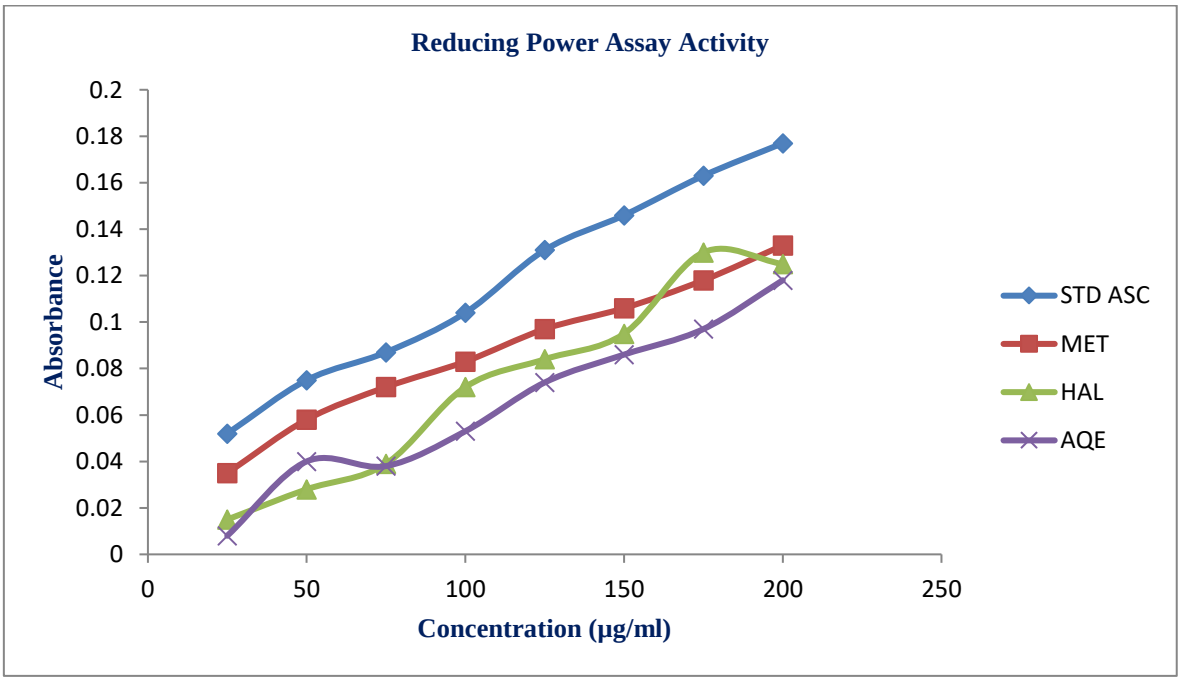

Figure 4. Nitric oxide free radical scavenging activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Table 5. Hydrogen peroxide scavenging activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard Ascorbic Acid	MET	HAL	AQE
25	14.69±0.21	9.44±0.18	6.2±0.01	1.75±0.18
50	30.18±0.15	18.48±0.19	14.38±0.13	09.01±0.15
75	41.49±0.12	27.38±0.20	21.78±0.23	15.68±0.16
100	55.59±0.31	37.27±0.31	35.18±0.15	23.11±0.16
125	63.2±0.18	49.51±0.18	44.5±0.29	34.4±0.24
150	77.53±0.31	65.14±0.13	58.53±0.18	49.62±0.27
175	85.97±0.08	75.91±0.52	72.58±0.15	58.52±0.19
200	94.13±0.12	84.55±0.23	79.57±0.22	71.59±0.14
IC50	99.80	120.19	130.20	158.73

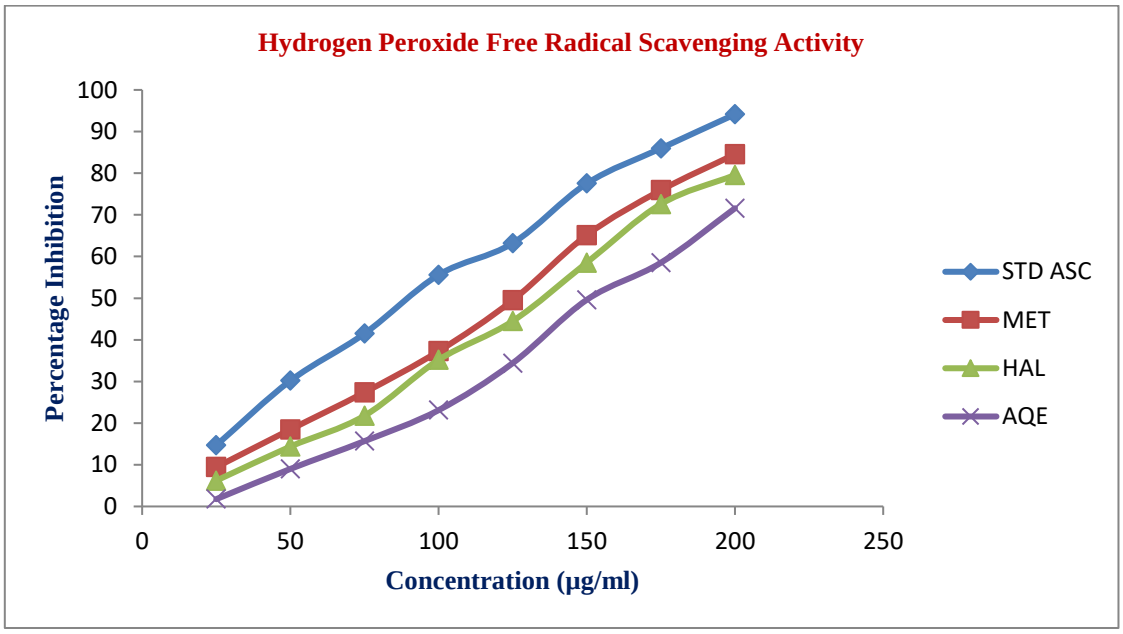

Figure 5. Hydrogen peroxide free radical scavenging activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Table 6. Reducing Power Assay activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

Concentration (ug/ml)	Absorbance (Mean ± SEM) (n=3)			
	Standard ASC	MET	AQE	HAL
25	0.051±0.0022	0.034±0.0012	0.014±0.0018	0.007±0.0008
50	0.074±0.0014	0.057±0.0018	0.027±0.0005	0.03±0.0008
75	0.086±0.0022	0.071±0.0012	0.038±0.0012	0.039±0.0014
100	0.103±0.0018	0.082±0.0006	0.071±0.0018	0.052±0.003
125	0.13±0.0021	0.098±0.0018	0.082±0.0018	0.073±0.0015
150	0.144±0.0016	0.108±0.0012	0.093±0.0015	0.085±0.0018
175	0.161±0.0009	0.119±0.0003	0.12±0.0005	0.096±0.0012
200	0.175±0.0016	0.132±0.0012	0.124±0.0018	0.119±0.0016

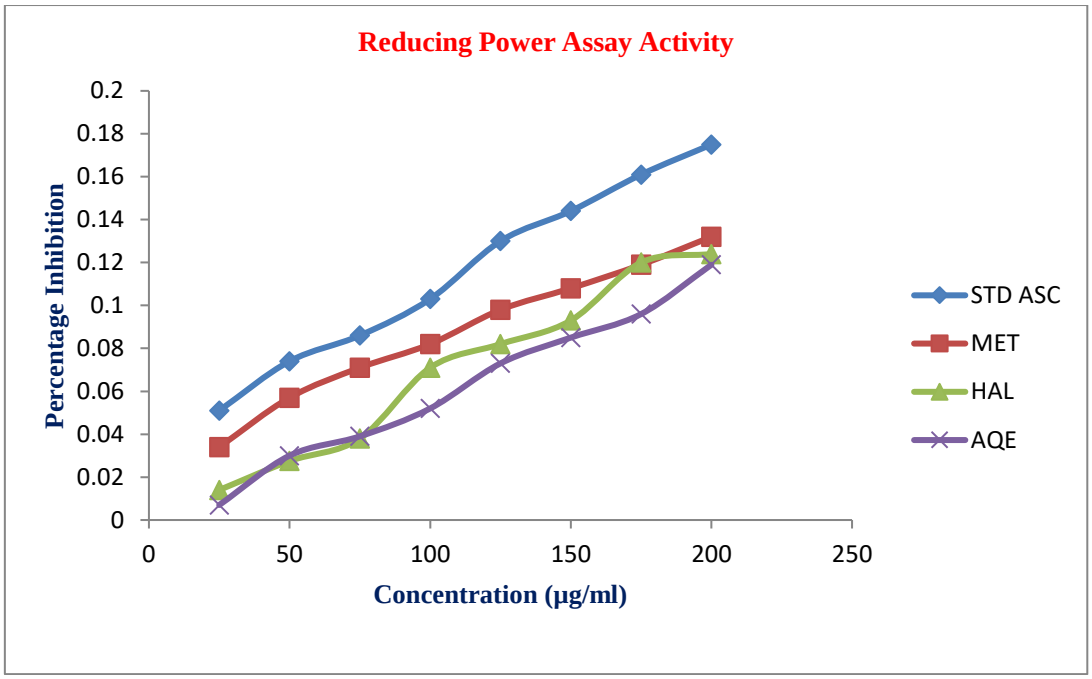

Figure 6. Reducing Power Assay activity of extracts of bark of plant *Albizia lebbeck* (L.) Benth.

RESULT

Total Phenolics Content

The Folin-Ciocalteu method is sensitive to reducing compounds, polyphenols there by producing blue colored complex. The quantative phenolics estimation was performed at 760 nm by change in intensity of Folin-phenolic compounds complex. In methanolic, hydroalcoholic and aqueous extracts of bark of plant *Albizia lebbeck* (L.) Benth., the total phenolic content was found to be 92.66 µg/ml, 64.33 µg/ml and 49.33µg/ml respectively in terms of gallic acid equivalent (Table No.1 and Figure No. 1). In addition it has been determined that the highest extraction yield was found in leaf extract.

Total Flavonoids Content

The aluminium chloride forms acid stable complexes with the C-4 keto group and either with C-3 or C-5 hydroxyl group of flavones and flavonols. Aluminium chloride also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids. In methanolic, hydroalcoholic and aqueous extracts of bark of plant *Albizia lebbeck* (L.) Benth., the total flavonid content was found to be 81.75 µg/ml, 64 µg/ml and 56.25 µg/ml respectively in terms of quercetin equivalent (Table No. 2 and Figure No. 2).

DPPH radical scavenging activity:-

The DPPH assay is purely based on the assumption that an antioxidant serve as a hydrogen donor and thus reduces the DPPH free radicals (the color turns from purple to yellow). This assay is known as a basic and quick tool to carry out evaluation of antioxidant activity of plant extracts. The antioxidant potency of a compound is relative to loss of DPPH free radicals that can be quantified through a decrease in the maximum absorption of DPPH at 517 nm. In this study, results showed that all plant extracts had significant levels of radical scavenging activity in a dose dependent manner (Table No. 3 and Figure No. 3). The DPPH-derived IC_{50} values of plant extracts are also illustrated in Table No.3 The methanolic, hydroalcoholic and aqueous exract of plant inhibited DPPH upto 89.56 %, 82.47% and 74.88% at concentration 200 ug/ml. Amongst the plant extracts of *Albizia lebbeck*, methanolic extract *was* found to be the most potent DPPH scavengers, as they could inhibit DPPH free radicals up to 89.56% at 200ug/ml concentration compared to the rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 96.97 % of DPPH scavenging activity.

Nitric oxide radical scavenging assay:-

Nitric oxide is an unstable free radical which involved in many biological processes and associated with several diseases. It react with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be act as toxic and inhibition of over production is an important goal. In this study, results showed that all plant extracts had significant levels of radical scavenging activity in a dose dependent manner (Table No. 4 and Figure No. 4). The Nitric oxide-derived IC_{50} values of plant extracts are also illustrated in Table 4. The methanolic, hydroalcoholic and aqueous extract of plant inhibited Nitric oxide upto 89.9%, 80.5% and 75.32%, at concentration 200ug/ml.. Amongst the plant extracts of *Albizia lebbeck*, methanolic extract was found to be the most potent Nitric oxide scavengers, as they could inhibit Nitric oxide free radicals up to 89.9% at 200 ug/ml concentration compared to the rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 95.44% of Nitric oxide scavenging activity.

Hydrogen peroxide scavenging activity

Scavenging of H_2O_2 by extracts may be attributed to their polyphenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The ability of plant extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch, where they are compared with that of tocopherol as standard (Ruch et al., 1984). The plant extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner (Table No. 5 and Figure No. 5). Although hydrogen peroxide itself is not

very reactive, but sometimes it can cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removal of H_2O_2 is very important living systems. The Hydrogen peroxide -derived IC_{50} values of plant extracts are also illustrated in Table 5. The methanolic, hydroalcoholic and aqueous exract of plant inhibited Nitric oxide upto 84.55%, 79.57% and 71.59% at concentration 200ug/ml. Amongst the plant extracts of *Albizia lebbeck*, methanolic extract *was* found to be the most potent Hydrogen peroxide scavengers, as they could inhibit Hydrogen peroxide free radicals up to 84.55% at 200ug/ml concentration compared to the rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 94.13% of Hydrogen peroxide scavenging activity.

Reducing Power Assay

Reducing capability of an antioxidant substance can be assessed using its ability to convert Fe^{3+} to Fe^{2+}. Intensity of Perl's Prussian blue color caused by this reduction is measured at 700 nm. Higher absorbance indicates higher reducing power. The reducing power of the compound can be contributed to its antioxidant potency. The reducing power assay of the plant extracts of plant *Albizia lebbeck* were tested in this study illustrated in Table No. 6 Figure No. 6. The findings revealed that the values of reducing power of the plant extracts of *Albizia lebbeck* were functions of their concentrations. In this study, results showed that all plant extracts had significant levels of Reducing Power activity in a dose dependent manner. At concentration 200ug/ml, methanolic, aqueous and hydroalcoholic extract had reducing power values of 0.132, 0.124, 0.119 as compare to standard Ascorbic acid 0.175. At this concentration, methanolic extract showed a remarkable reducing power that was significantly greater than those of the hydroalcoholic and aqueous extract as compared to standard ascorbic acid.

DISCUSSION

Polyphenolic compounds are known as powerful chain breaking antioxidant (37). They are important constituents in plant because of their scavenging ability, which is because of their hydroxyl groups (38).

Flavonoids are called as polyphenolic compounds, which exhibit several biological effects such as antioxidant, antiulcer, anticancer, anti-inflammatory, hepatoprotective, antiallergic, antiviral activities (39). Flavonoids are capable of scavenging the reactive O_2 species because of their phenolic hydroxyl groups and therefore called as potent antioxidants (40).

In the present study several biochemical constituents and free radical scavenging activities of extracts of *Albizia lebbeck* were evaluated. Free radicals are involved in several disorders like inflammation, cancer, neurodegenerative diseases and AIDS. Antioxidant activity due to their scavenging activity and they are useful for the control and management of these diseases. DPPH is a stable free radical which is a sensitive way to determine the antioxidant property of plant extracts (41, 42).

The DPPH method as antioxidant activity was evidently introduced nearly 50 years ago by Blois and is used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant potency. The parameter IC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) (43).

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons and other cells. It is involved in the regulation of various physiological processes. Excess concentration of Nitric oxide is always associated with several diseases. Nitric oxide is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citralline with the formation of Nitric oxide via a five electron oxidative reaction. These compounds are responsible for altering the structural and functional behavior of many cellular components (44).

The degree of inhibition of the Nitric oxide free radicals was found to be increased in increasing concentration of the *Albizia lebbeck* extracts, this indicates that the extract may contain compounds capable of inhibiting the generation of nitric oxide and offers scientific evidence for the use of *Albizia lebbeck* in the treatment of various diseases. The antioxidant principles present in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. It is to be noted that *Albizia lebbeck* greater inhibition comparative to other plant extracts but less than ascorbic acid which has shown good inhibition of Nitric oxide.

Hydrogen peroxide is a weak oxidizing agent and because of its ability to penetrate biological membranes, once inside the cell it can probably react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (45).

All the extracts of *Albizia lebbeck* when added to the reaction mixture scavenge hydroxyl radicals in a concentration dependent manner. The scavenging activity of the hydroxyl radicals may be due to the presence of polyphenolic compounds in the extracts which can donate electrons to H_2O_2, thus neutralizing it to water.

The reducing capacity of a extract may act as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, degradation of peroxides, reducing capacity and radical scavenging (46).

CONCLUSION

Nature for all time acts as a source of medicinal agents for thousands of years and an impressive number of modern drugs are isolated from natural sources, many based on their use in traditional medicine. Higher medicinal plants are act as a sources of medicinal compounds, which have continued to play a leading role in the maintenance of human health since ancient times. Present study showed that polyphenolic content in the methanolic bark extract of *Albizia lebbeck* is high and these extracts exhibited strong antioxidant activity compared to that of the hydroalcoholic, aqueous and with reference to standard compound. The results would help to determine the potency of the methanolic, hydroalcoholic and aqueous extract from plant *Albizia lebbeck* as a potential basis of natural antioxidants. Presence of sufficient amount of polyphenolic and flavonoid compounds may account for this fact. So these findings of present study suggested that the plant *Albizia lebbeck* have a potential source of natural antioxidant. Further studies are suggested for the isolation and characterization of antioxidant compounds from plant *Albizia lebbeck*. It will also leads to *in vivo* studies are needed for understanding their mechanism of action as antioxidants.

ACKNOWLEDGEMENT

The Corresponding author is very grateful to Principal Dr. Rajendra D. Wagh, DCS's A.R.A. College of Pharmacy, Nagaon, Dhule, for providing necessary facilities, support and proper guidance throughout the antioxidant activity.

CONFLICTS OF INTEREST

None. The authors declare no conflict of interest.

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