Environment and Ecology 41(2B): 1263—1271, April—June 2023
ISSN 0970-0420

# Phytochemical analysis and Antioxidant Potency of Stem Bark of *Albizia lebbeck* (L.) (Siris)

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Received 12 January 2023, Accepted 24 April 2023, Published on 21 June 2023

## ABSTRACT

The goal of the current study was to analyze the phytochemical components and antioxidant potential of *Albizia lebbeck* stem bark extracts made from methanol, aqueous and ethyl acetate. The phytochemical analysis of *Albizia lebbeck* stem bark showed presence of phenolics, flavonoids and sugars. Total phenolics were highest in methanol extract 219.38±16.88 (mg GAE/g) followed by aqueous 161.99±1.12 (mg GAE/g) and ethyl acetate 9.75±0.24 (mg GAE/g). Similarly total flavonoids were highest in methanol extract 42.21±0.67 (mg CE/g) followed by aqueous 19.89±0.56 (mg CE/g) and ethyl acetate 6.70±0.25 (mg CE/g). In methanol, aqueous and ethyl acetate stem bark extracts, total sugars (13.52±0.34, 40.05±0.74 and 5.37±0.18 (mg/g), reducing sugars (10.51±0.14, 28.86±0.64 and 1.21±0.09 (mg/g) and non-reducing sugars (3.01±0.33, 11.19±0.53 and 4.16±0.26 (mg/g) respectively were reported. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, the antioxidant capacity of *Albizia lebbeck* stem bark extract was assessed. The results showed that the $IC_{50}$ values were 8.83, 15.12 and 936.71 g/mL, respectively. The total antioxidant capacity of methanol, aqueous and ethyl acetate extract of *Albizia lebbeck* stem bark extract using phosphomolybdenum assay was 222.33±6.42, 204.67±5.14 and 20.30±0.68 mg AAE/g respectively.

**Keywords** *Albizia lebbeck*, Phytochemicals, Phenolics, Flavonoids, Antioxidant capacity.

## INTRODUCTION

India is incredibly ethnically diverse and many of the indigenous cultures have however preserved their traditional understanding of the therapeutic value of the flora. In India, more than 8000 plant species are employed in traditional and modern medicine and 90–95% of the medicinal plants are collected come from the wild (Yadav *et al.* 2014). Numerous plants are exploited in medicine for curative or preventative purposes. Medicinal plants are believed to have healing properties because they contain substances that are useful for treating ailments, such as alkaloids, flavonoids, glycosides, vitamins, tannins and coumarins. These plant materials called phytochemicals. These organic compounds affect a person's body physiologically, interact with microorganisms to stop their growth at different stages of development and make the body free from disease (Mittal *et al.* 2014, Aggarwal *et al.* 2022). Phytochemistry is the study of plant-derived phytochemicals in the context of natural products. All components of the plant body spontaneously manufacture phytochemicals, which are bioactive compounds. In accordance with their

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roles in plant metabolism, phytochemicals are separated into two categories: Primary and secondary metabolites. Proteins, amino acids, carbohydrates, and chlorophyll are examples of primary metabolites, while phenols, flavonoids, tannins and other compounds are examples of secondary metabolites (Kennedy and Wightman, 2011, Devi *et al*. 2022, Nehra *et al.* 2022). Free radicals are thought to have a significant impact on human health, leading to disorders including diabetes, cancer, hypertension, and heart attacks. Exogenous antioxidant intake can aid the body's efficient scavenging of free radicals. Additionally, numerous studies have demonstrated that a higher dietary consumption of natural phenolics is associated with a decreased risk of coronary heart disease, a lower cancer mortality rate and a longer life expectancy. Furthermore, investigations have shown that these polyphenolic compounds have a number of health-related benefits, including antioxidant, anticancer, antiviral and anti-inflammatory effects. Butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ), two extensively used synthetic antioxidants, have raised questions about their safety, which has stimulated interest in natural antioxidants that occur in plants as secondary metabolites (Ghafar *et al*. 2010, Devi *et al*. 2021) Synthetic antioxidants typically have phenolic structures with varying degree of alkyl substitution, whereas natural antioxidants can be nitrogen compounds (alkaloids, amino acids, chlorophyll derivatives and amines), phenolic compounds (flavonoids, tocopherols, and phenolic acids), or carotenoids in addition to ascorbic acid. Numerous naturally occurring antioxidants, particularly flavonoids, have a variety of biological activities, including anti-inflammatory, antibacterial, antiviral, antithrombotic, anti-allergic and vasodilatory properties (Velioglu *et al.* 1998).

*Albizia lebbeck*, commonly known as Shirisha, Siris and Shris in Hindi, is an unarmed deciduous tree belonging to family-Fabaceae (Singh and Agrawal 2018). *Albizia lebbeck* can grow to a height of 30 m and a diameter of 1 m, but it often reaches about 15-20 m tall and has a diameter of 50 cm. Its bark is reddish brown with rough, fissured edges. Orwa *et al*. (2009). Stem bark phytochemicals present in plant extract are responsible for its aphrodisiac, anti-inflammatory, anti-allergic, anti-anaphylactic, anti-asthamatic, anti-histaminic, analgesic, antioxidant, immunomodulatory, anticonvulsant and anti-spermatogenic properties. The tree parts are used as a traditional remedy for lung and stomach tumours, boils, cough, eye problems and illness (Gupta *et al.* 2005). The current study objectives were to identify the phytochemical components and evaluating the antioxidant potential of *Albizia lebbeck* stem bark extracts in methanol, aqueous, and ethyl acetate. In this study, the stem bark was found to be rich source of polyphenolics, flavonoids and sugars. The abundance of phenolics and flavonoids, which was measured using the DPPH free radical scavenging assay and the phosphomolybdenum assay, was attributed to the stem bark's strong antioxidant potential.

**Fig. 1.** *Albizia lebbeck* (Siris).

## MATERIALS AND METHODS

### Collection of plant material

Stem bark of *Albizia lebbeck* (Fig. 1) were collected from Research farm, Department of Forestry, CCS Haryana Agricultural University Hisar, Haryana. Before processing, the plant materials were kept under the shade at room temperature. The proposed studies were conducted in the Department of Chemistry, CCS Haryana Agricultural University Hisar, Haryana.

### Preparation of methanol, aqueous and ethyl acetate extract of *Albizia lebbeck* stem bark

In a thimble made of Whatman No. 1 filter paper, 10 grams of powdered *Albizia lebbeck* stem bark samples were collected. This thimble was placed in a Borosil

soxhlet configuration using a 500 mL flask with a round bottom. The solvents (methanol, aqueous, and ethyl acetate) were added in amounts of about 300 mL up to 1.5 syphons. As a result, the powdered stem bark sample was percolated using the soxhlet equipment employing methanol, aqueous, and ethyl acetate as the solvent. After completing seven to eight cycles with methanol, aqueous and ethyl acetate as solvents, the process was maintained for 5-6 hrs using a siphon mechanism. Each filtered solvent's volume was measured after extraction. The total phenolic content, total flavonoids, total sugars, reducing sugars, non-reducing sugars, DPPH free radical scavenging activity and total antioxidant capacity were all determined using these extracts.

**Quantitative analysis of phytochemicals**

*Determination of total phenolic content*

The total phenolic content in the extracts of *Albizia lebbeck* stem bark was determined using the standard curve Folin Ciocalteu method (Singleton and Rossi 1965) by using Gallic acid as standard. 1.0 mL of each extract of *Albizia lebbeck* stem bark was taken in a test tube diluted with respective solvent. Additionally, 1 mL of 1 mol/L Folin-Ciocalteu reagent and 2 mL of $Na_2CO_3$ (20%, w/v) were mixed, and the volume was increased to 10 mL using distilled water. After standing for 8 minutes, the mixture was centrifuged at 6000 rpm for 10 minutes. The absorbance of the supernatant solution was measured at 730 nm against a prepared blank using a UV-Vis Double Beam Spectrophotometer (Model UV 1900, Shimadzu). Similarly, blank prepared but instead of extracts it contains the respective solvent. The concentration of total phenolic content in each extract was calculated from regression equation obtained from the standard curve of gallic acid and represented as milligrams of gallic acid equivalent per gram (mg GAE/g).

*Determination of total flavonoids*

Standard analysis of total flavonoids was done by aluminum chloride colorimetric assay (Ribarova and Atanassova 2005). Each *Albizia lebbeck* stem bark extract, 1.0 mL, was added to a test tube along with 4 mL of double-distilled water, 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ after 5 minutes. Immediately, 2 mL of 1 M NaOH was added and the volume was increased to 10 mL using double-distilled water. After thoroughly combining the solution, its absorbance at 510 nm was measured in comparison to a blank made using a UV-Vis Double Beam Spectrophotometer (Model UV 1900, Shimadzu). Similarly, blank prepared but instead of standard solution of catechin it contains double distilled water. The total flavonoids concentration in extract was calculated from the regression equation obtained from the standard curve of catechin and expressed in milligrams (mg CE/g) of catechin equivalents per gram.

*Determination of total sugars*

The estimation of total sugars was done using a modified version of Dubois *et al.* (1956). A test tube contained 1.0 mL of each *Albizia lebbeck* stem bark extract that had been diluted with distilled water. Next, 2.0 mL of phenol solution (2%, w/v) and 5.0 mL of concentrated sulphuric acid were added. Acid was poured directly on the solution. About 30 minutes, test tubes were allowed to cool and using UV-Vis double beam Spectrophotometer (Model UV 1900 Shimadzu), absorbance of the solution was measured at 490 nm against a blank prepared. Similarly, instead of extracts blank contains the respective solvent. The total sugars concentration in extract was calculated from the regression equation obtained from the standard curve of D-glucose and expressed as milligrams per gram (mg/g).

*Determination of reducing sugars*

For the estimation of reducing sugars, Somogyi (1952) further modified the Nelson (1944) method. Each *Albizia lebbeck* stem bark extract was diluted with distilled water to 1.0 mL in a test tube. After that, 1 mL of distilled water and 1 mL of alkaline copper reagent were added, mixed thoroughly, and covered with aluminium foil. The mixture was then heated in a hot water bath for 20–25 minutes. After allowing the boiling tubes to cool to room temperature, 1.0 mL of the arsenomolybdate reagent was added, properly mixed, and the reaction mixture was diluted with dis-

tilled water to a volume of 10.0 mL. The absorbance of the solution was measured at 520 nm using a UV-Vis spectrophotometer (Model UV 1900 Shimadzu) in comparison to a blank that was made in a similar manner but used the appropriate solvents rather than extracts. The concentration of reducing sugars in the acetone extract was determined using the D-glucose standard curve, and the results were expressed in milligrams per gram (mg/g).

*Determination of non-reducing sugars*

The non-reducing sugar was estimated as the difference between the concentration of total sugar and that of reducing sugar.

Non-reducing sugar = Total sugar – Reducing sugar

**Evaluation of antioxidant capacity of different extract of *Albizia lebbeck* stem bark**

*DPPH free radical scavenging activity*

*Albizia lebbeck* stem bark extracts in methanol, aqueous, and ethyl acetate solvents were measured for dry mass. The evaluation of DPPH free radical scavenging activity used the Hatano *et al.* (1988) method. To measure antioxidant activity, 0.2 mL of extracts (at various concentrations) were combined with 3.0 mL of 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH, 0.1 mM in 100% methanol) and vigorously stirred for 5 minutes. A DPPH stock solution was prepared in a 50/50 mixture of methanol and water to test the antioxidant activity of various concentrations of water extracts. The rest of the procedure was the same. As a control, 0.2 mL of each solvent was used instead of extract. After 30 minutes of incubation in the dark at room temperature, the absorbance of the sample and control was measured at 517 nm using a UV-VIS spectrophotometer (Model UV 1900, Shimadzu) against a blank containing the respective solvent. A graph was created by plotting the extract concentration (x-axis) against the percent DPPH free radical scavenging activity (y-axis). The quadratic regression equation ($y = ax^2+bx+c$) was then developed with the aid of Origin. The equation $y = ax^2+bx+c$ changed to $ax^2+bx+c = 0$ when $y = 50\%$ was added. The equation $ax^2+bx+c= 0$ was used to drive the $IC_{50}$ using the following formula :

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Where, $x = IC_{50}$ (µg/mL)

The formula used to determine the percentage of DPPH scavenged (% DPPH*sc) was

$$\%\ DPPH^*\ sc = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ = Absorbance of control
$A_{sample}$ = Absorbance of sample

*Phosphomolybdneum assay*

Modified phosphomolybdenum method (Prieto *et al*. 1999) was used to determine the total antioxidant capacity of extracts of *Albizia lebbeck* stem bark. Three mL of phosphomolybdenum reagent were added to glass vials containing one mL of each extract and the mixture was then thoroughly mixed before being sealed. They were incubated at 95°C for 90 minutes.

**Table 1.** Phytochemicals in methanol, aqueous and ethyl acetate extract of the *Albizia lebbeck* stem bark.

| Phytochemicals | Methanol | Aqueous | Ethyl acetate |
|---|---|---|---|
| Total phenolic content (mg GAE /g) | 219.38 ±16.88 | 161.99 ± 1.12 | 9.75 ± 0.24 |
| Total flavonoids (mg CE/g) | 42.21 ± 0.67 | 19.89 ± 0.56 | 6.70 ± 0.25 |
| Total sugars (mg/g) | 13.52 ± 0.34 | 40.05 ±0.74 | 5.37 ± 0.18 |
| Reducing sugars (mg/g) | 10.51 ±0.14 | 28.86 ±0.64 | 1.21 ±0.09 |
| Non-reducing sugars (mg/g) | 3.01 ± 0.33 | 11.19 ± 0.53 | 4.16 ± 0.26 |

**Table 2.** DPPH free radical scavenging activity of methanol, aqueous and ethyl acetate extract of the *Albizia lebbeck* stem bark.

| Sl. No. | Conc (µg/mL) | DPPH free radical scavenging activity (%) | | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| | | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | |
| 1 | Aqueous extract | 91.82 | 77.15 | 36.45 | 21.78 | 12.14 | 4.91 | 15.12 |
| 2 | Methanol extract | 92.53 | 79.41 | 62.72 | 45.81 | 33.67 | 24.43 | 8.83 |
| | | 2000 | 1500 | 800 | 400 | 200 | 100 | |

**Table 2.** Continued.

| Sl. No. | Conc (µg/mL) | DPPH free radical scavenging activity (%) | | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| | | 2000 | 1500 | 800 | 400 | 200 | 100 | |
| 3 | Ethyl acetate extract | 89.89 | 73.05 | 49.39 | 34.64 | 21.80 | 13.63 | 936.71 |

Then, after allowing the vial contents to cool, they were measured at 695 nm using a Shimadzu UV-VIS Double Beam Spectrophotometer (Model UV 1900) in comparison to a blank. The same procedure was used to create a blank, but this time the proper solvent was used in place of the extract. The relationship between absorbance and ascorbic acid concentration (µg/mL) was plotted using a standard curve.

## RESULTS AND DISCUSSION

### Quantitative analysis of phytochemicals

*Albizia lebbeck* stem bark extracts were subjected to quantitative analysis for a variety of phytochemicals, including total phenolic, total flavonoid, total sugar, reducing sugars, and non-reducing sugars. The results of the analysis are shown in Table 1.

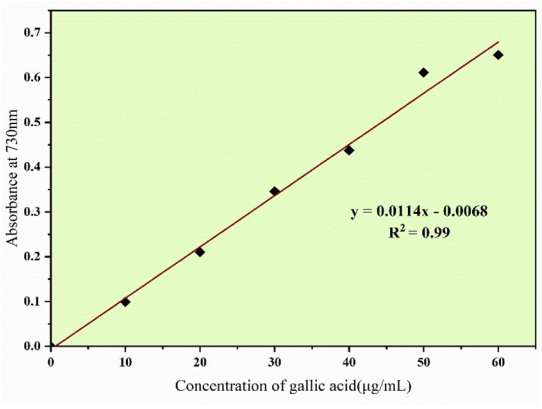


**Fig. 2.** Standard curve of total phenolic content using standard as gallic acid.

*Total phenolic content*

The total phenolic content in methanol, aqueous and ethyl acetate extract of *Albizia lebbeck* stem bark was calculated using a standard curve with gallic acid as the reference. In the plant extracts, all the phenolic compounds are oxidized by Folin-Ciocalteu reagent. After oxidation of the phenols, the reagent is reduced to a mixture of blue oxides of tungsten ($W_2O_3$) and molybdenum ($Mo_8O_{23}$) from the mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$). In the region of 730 nm, maximum absorption of blue coloration is produced and the quantity of phenolic compounds present are directly proportional to it. Fig. 2 showing standard curve of total phenolic content using standard as gallic acid. The absorbance and the amount of gallic acid were linearly related, according to the regression equation. Using the regression equation ($y = 0.0114x - 0.0068$, $R^2 = 0.99$), the total phenolic content in the methanol, aqueous and ethyl acetate extracts of *Albizia leb-*

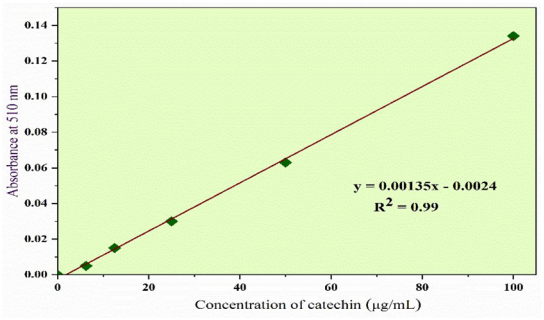


**Fig. 3.** Standard curve of total flavonoids using catechin as a standard.

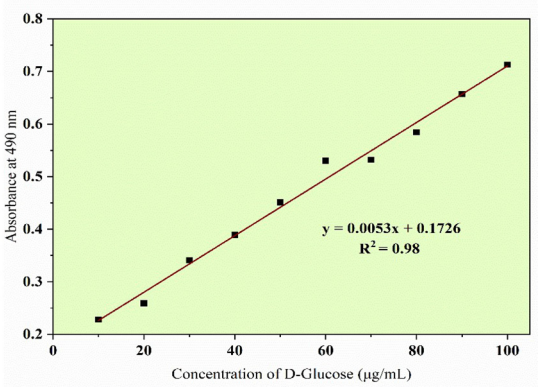


**Fig. 4.** Standard curve of total sugars using. D-glucose as standard.

*beck* stem bark was calculated to be 219.38±16.88, 161.99±1.12 and 9.75±0.24 mg GAE/g, respectively.

*Total flavonoids*

Using catechin as a reference, the total flavonoids in the *Albizia lebbeck* stem bark extract in methanol, aqueous, and ethyl acetate were calculated (Fig. 3). $AlCl_3$ forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols, according to the main principle of the aluminium chloride colorimetric assay. Additionally, by reacting with $AlCl_3$, the orthodihydroxyl groups of the A and B ring flavonoids also produce acid labile complexes.

Fig. 3 showed standard curve of total flavonoids using catechin as a standard. A linear relationship between the absorbance and the concentration of catechin was shown by the regression equation. Using the regression equation (y = 0.00135x - 0.0024, $R^2$= 0.99),

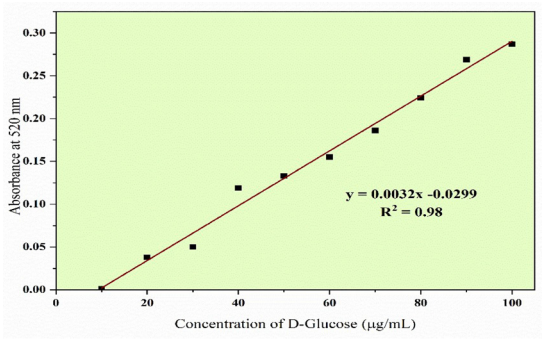


**Fig. 5.** Standard curve of reducing sugars using D-glucose as standard.

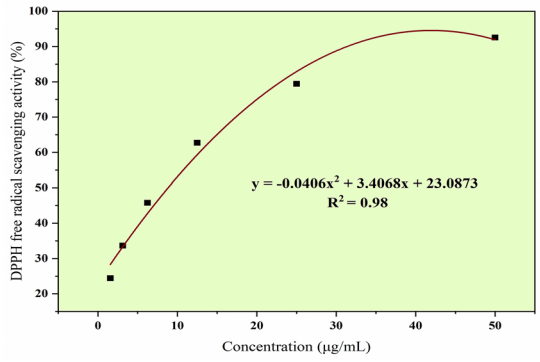


**Fig. 6.** Quadratic regression equation for $IC_{50}$ value (µg/mL) of methanol extract of stem bark of *Albizia lebbeck*.

the total flavonoid content of the *Albizia lebbeck* stem bark extract was calculated to be 42.21±0.67, 19.89±0.56 and 6.70±0.25 mg CE/g, respectively.

*Total sugars*

The total sugars in the *Albizia lebbeck* stem bark methanol, aqueous, and ethyl acetate extract were calculated using D-glucose as a reference (Fig. 4). The phenol sulphuric acid method is based on the idea that glucose is dehydrated to hydroxymethyl furfural in a hot acidic medium for total sugar analysis. A yellow-brown product is produced after reaction with phenol and maximum absorption occurs at 490 nm.

The absorbance and the amount of D-glucose were linearly related, according to the regression equation. Using the regression equation (y = 0.0053x + 0.1726, $R^2$= 0.98), it was determined that the total

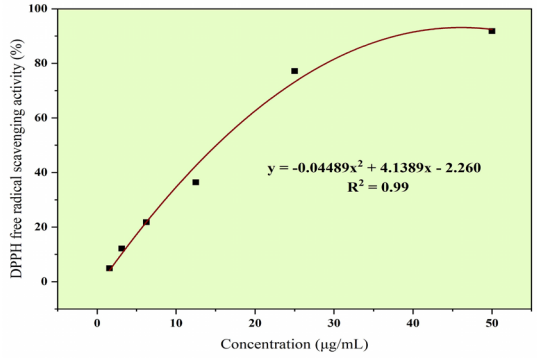


**Fig. 7.** Quadratic regression equation for $IC_{50}$ value (µg/mL) of aqueous extract of stem bark of *Albizia lebbeck*.

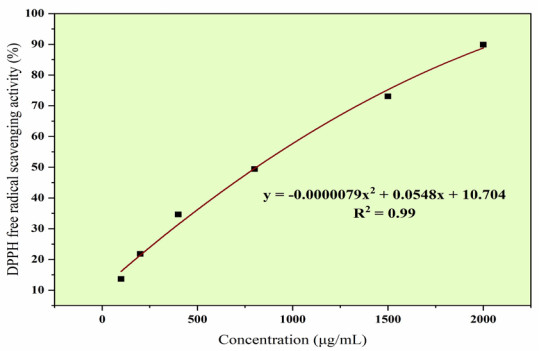


**Fig. 8.** Quadratic regression equation for $IC_{50}$ value (µg/mL) of ethyl acetate extract of stem bark of *Albizia lebbeck*.

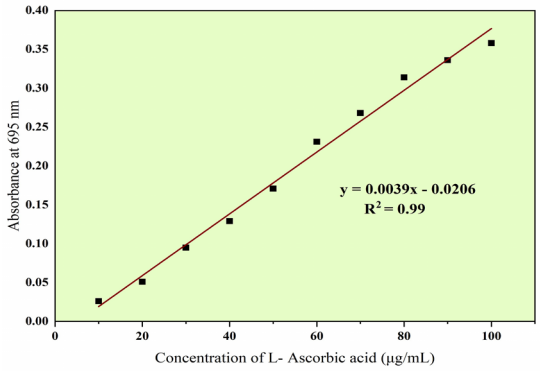


**Fig. 9.** Standard curve for total antioxidant capacity using ascorbic acid as standard.

sugar content in the methanol, aqueous and ethyl acetate extracts of *Albizia lebbeck* stem bark were 13.52±0.34, 40.05±0.74, and 5.37±0.18 mg/g, respectively.

*Reducing sugars*

Reducing sugars in the *Albizia lebbeck* stem bark methanol, aqueous, and ethyl acetate extract were calculated using D-glucose as a reference (Fig. 5). According to basic principle, when reducing sugars are heated with alkaline copper tartrate, cupric ions are reduced to the cuprous state, which leads to the production of cuprous oxide. Further, when cuprous oxide is treated with arsenomolybdic acid, molybdic acid is reduced to molybdenum blue in color. The colored developed is spectrophotometrically.

The absorbance and the amount of D-glucose were linearly related, according to the regression equation. Using the regression equation ($y = 0.0032x - 0.0299$, $R^2 = 0.98$), the amount of reducing sugar in the methanol, aqueous, and ethyl acetate extracts of *Albizia lebbeck* stem bark was calculated to be 10.51±0.14, 28.86±0.64 and 1.21±0.09 mg/g, respectively.

*Non-reducing sugars*

The non-reducing sugars were calculated as the difference between total sugar and reducing sugar concentrations. According to the findings, the amounts of non-reducing sugar (mg/g) in the stem bark of *Albizia lebbeck* extracted in methanol, aqueous and ethyl acetate were 3.01±0.33, 11.19±0.53 and 4.16±0.26 mg/g, respectively.

**Antioxidant capacity of different extract of *Albizia lebbeck* stem bark**

*DPPH free radical scavenging activity*

Antioxidants inhibit the oxidation of essential biological macromolecules by preventing the growth of the oxidative chain reaction. Due to the drawbacks of synthetic antioxidants, researchers concentrated on obtaining natural antioxidants. A hydrogen donor combines with DPPH (2,2-diphenyl-1-picrylhydrazyl), a purple stable free radical. The molecule of DPPH possesses delocalized spare electrons throughout, which prevent dimerization and also give the compound its color, with an absorption peak in the UV-Vis spectrum at about 517 nm. The DPPH radical reacts to make the reduced form of DPPH, or hydrazine form, which turns the substance from purple to pale yellow. The content of antioxidants has an impact on how much purple color fades. The degree of solution discoloration as a result revealed scavenging. The $IC_{50}$ value was used to express antioxidant activity. Quadratic regression equation for $IC_{50}$ value (µg/mL) of methanol extract of stem bark of *Albizia lebbeck* shown in Fig. 6.

The $IC_{50}$ is the concentration of antioxidants needed to reduce the initial DPPH radical by 50% and were assessed for methanol, aqueous, and ethyl acetate extract based on the percentage of DPPH free

radical scavenged (Table 2). *Albizia lebbeck* stem bark extract was reported to have an $IC_{50}$ value of 8.83, 15.12 and 936.71 µg/mL in methanol, aqueous and ethyl acetate respectively. Quadratic regression equation for $IC_{50}$ value (µg/mL) of aqueous and ethyl acetate extract of stem bark *Albizia lebbeck* shown in Figs. 7– 8 respectively.

*Phosphomolybdneum assay*

The total antioxidant capacity in the methanol, aqueous, and ethyl acetate extract of *Albizia lebbeck* stem bark were calculated using ascorbic acid as a reference (Fig. 9). Reduction of Mo (VI) to Mo (V) occurs due to the presence of antioxidants. In an acidic solution, Mo (V) reacts with the phosphate group of sodium phosphate to form green colored phosphomolybdenum complex. The reaction is highly time-dependent and is monitored using a spectrophotometer at $\lambda_{max}$. Standard curve for total antioxidant capacity using ascorbic acid as standard shown in Fig. 9. The absorbance and ascorbic acid concentration were shown to be linearly related by the regression equation. Using the regression equation ($y = 0.0039x - 0.0206$, $R^2 = 0.99$), the total antioxidant capacity in the methanol, aqueous, and ethyl acetate extracts of *Albizia lebbeck* stem bark was calculated to be 222.33±6.42, 204.67±5.14 and 20.30±0.68 mg AAE/g, respectively.

## CONCLUSION

The present study indicated the presence of phytochemicals in methanol, aqueous and ethyl acetate extract of *Albizia lebbeck* stem bark which is responsible for scavenging of oxidative stress inducing species. The quantitative analysis of phytochemicals and antioxidant capacity would be helpful in understanding the pharmacological actions of *Albizia lebbeck* stem bark. It is concluded that stem bark extract prepared in methanol solvent has highest amount of total phenolics, flavonoids and antioxidant activity followed by aqueous and ethyl acetate solvent. Hence, methanol extract is found to be best among three solvents for phenolics, flavonoids and antioxidant activity of stem bark. As *Albizia lebbeck* stem bark are rich source of phytochemicals so its applications in the medicinal, pharmaceutical and nutraceutical fields need to be explored in the future. However, more investigation is required to identify the precise substances that make up the antioxidant system and create applications for the pharmaceutical and food industries.

## ACKNOWLEDGMENT

We are grateful for the research facilities provided by Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India.

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