

***In vitro* Antioxidant and Antibacterial Activity of *Albizia lebbek* (L) Benth Stem Bark**

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Abstract	Article Information
<p>Nature provides various remedy for the management of different diseases since the existence of mankind. 80% of the world population still relies on nature as a main source of remedies for primary health care. The main aim of the present study was to evaluate <i>in vitro</i> antibacterial and antioxidant activity and preliminary phytochemical screening of the methanolic extract of <i>Albizia lebbek</i> (L) Benth stem bark. Preliminary phytochemical screening showed the presence of alkaloids, saponins, glycosides, phytosterols, flavonoids and tannins. The antioxidant activity of the stem bark extract was studied using DPPH free radical scavenging assay. Extract was shown to display a dose dependent free radical scavenging effect. In disc diffusion method, the methanol extract showed considerably good antibacterial activity against Gram positive and Gram negative bacteria.</p>	<p>Article History: Received : 14-04-2015 Revised : 13-06-2015 Accepted : 15-06-2015 Keywords: <i>Albizia lebbek</i> Disc diffusion method DPPH *Corresponding Author: Biniam Paulos E-mail: biniamku@gmail.com</p>

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INTRODUCTION

Nature has been a source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries. Plants have formed the basis of traditional medicine systems that have been the way of life for thousands of years. Traditional medicine has been the main source for a large majority of people in Ethiopia for treating health problems including infectious and non-infectious diseases (Teklehaimanot and Gidey, 2007).

Albizia lebbek (L) Benth. belongs to the family *Fabaceae/ Mimosoideae* is a common plant as a shade tree grown in different parts of the globe including Africa and the Caribbean. The plant is a deciduous tree which may reach 25 m, usually 8–14 m; trunk often short, crown low and spreading. Leaves are compound, pairs of pinnae, 3–11 pairs of leaflets, each oblong, tip rounded, usually 2–3 cm. The flowers are green to yellow; fragrant brush heads on a stalk, short lived. The fruits are shiny yellow to brown pods in clusters decorate the tree for a long time, each pod up to 30 cm long, bulging over seeds, the seeds and pods "chatter" in the wind (Orwa *et al.*, 2009).

The plant is used traditionally for treating various kinds of ailments. Bark is used in toothache, piles, diarrhea and diseases of the gum. Decoction of the leaves and barks are used to treat bronchial asthma and other allergic disorders (Bobby *et al.*, 2012). Bark of the plant is used in the management of Salmonellosis among Segen people in their folk medicine. Various studies indicated that

different parts but stem bark showed different activities (Padamanabhan *et al.*, 2013). The main aim of the present study was to investigate *in vitro* antioxidant and antibacterial activity of methanolic stem bark extracts of *A. lebbek* against clinical isolates of bacteria and evaluating its *in vitro* antioxidant activity.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

Leaves, flowers and bark of *A. lebbek* were collected from Birisho Mountain near to Segen River, SNNPR, Ethiopia and identified by Mr. Melaku Wondafersh, Botanist at National Herbarium in Addis Ababa University. The shaded dried stem bark was grinded by using pestle and mortar to attain the average powder size. Powdered plant material was macerated in conical flask with methanol (70%) and left for 48 hours to prepare total plant extract. The extract was filtered using Whatman No. 1 filter paper and evaporated to dryness (Naik *et al.*, 2015). The extracts were kept in stoppered sample vials at 4°C until they were used.

Preliminary Phytochemical Analysis of Stem Bark Extract

Phytochemical analysis of the methanol extract of stem bark was carried out using standard methods. The plant materials were checked for the presence of active constituents like alkaloids, glycosides, flavonoids, tannins, fixed oils and fats, resins and phytosterols (Evans, 2002).

Antibacterial Activity of Stem Bark Extract

Stock cultures of test bacterial strains namely *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* were sub-cultured on nutrient agar slant and incubation was done at 37°C for 24 hours to obtain fresh cultures. 5ml of sterile distilled water was used to make suspension. The overnight growth of the test organisms was emulsified in sterile distilled water and the suspension was adjusted to match the 0.5 McFarland's standard. The test bacteria were inoculated on agar plates using sterile cotton wool swabs. 1mg of extract was dissolved in 1ml of the appropriate solvent and 10µl of the extract was impregnated onto 6mm sterile filter paper disk and air dried. The disks were placed aseptically onto the inoculated plates and the plates were incubated at 37°C for 24 hours. After incubation, inhibition zone (diameter) was measured in millimeters and recorded against the corresponding concentrations (Hendriksen, 2002). Positive controls were set using standard antibiotics (Gentamycin (10µg/disc), Ciprofloxacin (20µg/disc), and Chloramphenicol (30µg/disc) while negative controls were set using disk impregnated with extraction solvent.

Determination of Minimum Inhibitory Concentration (MIC)

Broth dilution method was used to evaluate MIC. Serial dilution of extract at concentration of 10, 1, 0.1, 0.01 mg/ml was made in test tubes containing sterile nutrient broth. Culture of test bacteria was inoculated into each test tube. The test tubes were then incubated at 37°C for 24 hours. The MIC was recorded as the lowest extract concentration demonstrating no visible growth as compared to the control broth tube. All the experiments were done in triplicates and average results were recorded (Reynolds *et al.*, 2003).

DPPH (1,1-diphenyl-2-picryl hydrazyl) Radical Scavenging Activity of Stem Bark Extract

DPPH radical scavenging activity of the exudate and isolated compound was determined by the method described by Cuendet *et al.* (1997), where 50 µL of various concentrations (400, 200, 100, 50 µg/ml) were mixed with 5 ml of 0.04% (w/v) DPPH solution in methanol. The mixture was incubated for 30 min in dark place. Ascorbic acid was used as positive controls. The decrease in the solution absorbance was measured at 517nm on spectrophotometer (Jenway Model 6500, England) by using blank DPPH as a control after 30 min of incubation. This test was being done in triplicates. The DPPH scavenging activity (%) was calculated based on the following formula:

$$\text{Radical scavenging activity (\%)} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100,$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the plant sample. The percentage of scavenging of the plant extract was compared with positive controls.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The percentage yield of methanol extract of stem bark of *A. lebbeck* was 12%. The chemical tests were performed for testing different chemical groups

in methanol extract of *A. lebbeck*. The stem bark methanolic extract showed presence of glycoside, tannins, and flavonoids, alkaloids and tannins (Table 1). Phytochemical investigation of different parts of the plant also indicated the presence of different primary and secondary metabolites (Padamanabhan *et al.*, 2013).

Table 1: Phytochemicals in methanolic extract of stem bark

Phytochemical	Result
Alkaloids	+
Saponin	+
Glycosides	+
Phytosterols	+
Fixed oils	-
Resins	-
Flavonoids	+
Tanins	+

'+' Presence; '-' Absence

DPPH Free Radical Scavenging Activity of Bark Extract

DPPH assay is one of the simple and widely used free radical scavenging assays. The scavenging of DPPH radicals by antioxidants is due to their hydrogen or electron donating ability. In alcoholic solution, DPPH gives a strong absorption band at 517 nm. When the odd electron becomes paired off in the presence of a scavenger, the absorption reduces and the DPPH solution is decolorized as the color changes from deep violet to light yellow. The degree of reduction is indicative of the radical scavenging (antioxidant) power of the substances (Sudharshan *et al.*, 2010; Naik *et al.*, 2015). In the present study, methanolic bark extract of *A. lebbeck* showed free radical scavenging activity in a dose dependent manner (Figure 1). The IC_{50} value of the extract was 156µg/ml. However, ascorbic acid (reference antioxidant) showed potent scavenging activity with IC_{50} value of 4.6µg/ml. Different studies also indicated the in vitro antioxidant activity of different parts of the plant (Zia-Ul-Haq *et al.*, 2013). Recent studies have shown that plants and plant based formulations can reduce the risk of chronic diseases produced due to oxidative stress. Such protective effects may be attributed to the presence of natural antioxidants such as polyphenolics and flavonoids. The antioxidant activity of the extract might be associated with the presence of phenolic compounds (Braca *et al.*, 2002; Demiray *et al.*, 2009; Rakesh *et al.*, 2013).

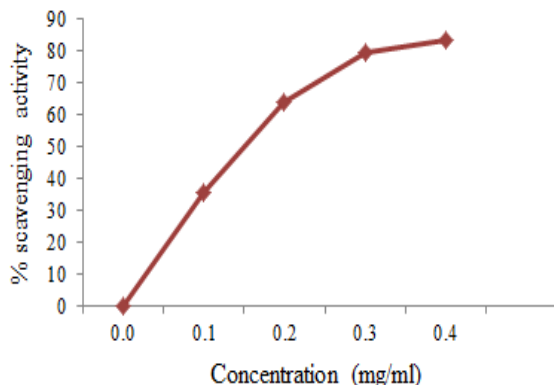


Figure 1: Scavenging of DPPH radicals by different concentrations of bark extract

Antibacterial Activity of Stem Bark Extract

Methanol extract was screened for the antibacterial activity against the selected clinical isolates. The present study results demonstrated that methanolic extract of *A. lebbeck* conferred the better spectrum activities that inhibited the growth of all test bacteria with different zones of inhibition (Table 2). Marked inhibitory activity was observed against *S. typhi* followed by *E. coli* and *S. aureus*. When compared to commercially available antibacterials, methanol extract of *A. lebbeck* stem bark was found to show greater inhibition against *S. aureus*. However, it was found to exhibit moderate activity against *E. coli* and *S. typhi*. In addition, it was observed that the antimicrobial activity of antibiotics was higher against the gram-negative organism as compared to the gram-positive. The antibacterial activity of the different parts of the plant has also been mentioned in different findings (Shahid and Firdous, 2012; Bobby *et al.*, 2012). MICs are considered the gold standard for determining the susceptibility of organisms to antimicrobials. MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definite answer when a borderline result is obtained by other methods of testing or when diffusion methods are not appropriate (Andrews, 2001). In the present study, the MIC values of the methanol extract against *S. aureus*, *E. coli* and *S. typhi* was found to be 0.01mg/ml. These values are indicative of antimicrobial activity of the extract.

Table 2: Antibacterial activity of bark extract of *A. lebbeck*

Test sample	Zone of Inhibition (mm)		
	<i>S. aureus</i>	<i>S. typhi</i>	<i>E. coli</i>
Methanol extract	16	19	18
Ciprofloxacin	0	21	15
Chloramphenicol	0	22	21
Gentamycin	0	21	21
Methanol	0	0	0

CONCLUSION

The presence of various secondary metabolites in *A. lebbeck* is indicated in phytochemical screening. Methanolic extract stem bark of *A. lebbeck* showed *in vitro* antioxidant and antibacterial activity. However, further bioassay guided isolation of compounds and toxicity studies must be done to assure the traditional claim on the plant.

Conflict of Interest

The authors declare there is no conflict of interest.

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