Vol.2.Issue.1.;2014

Bulletin of Pharmaceutical and Medical Sciences (BOPAMS)
A Peer Reviewed International Journal http://www.bopams.com

RESEARCH ARTICLE

E-ISSN 2341-4103

PHYTOCHEMICAL SCREENING AND BIOLOGICAL STUDIES ON ROOT BARK OF LEBBEK TREE [*Albizia lebbeck (L.) Benth*]

BALABHADRA KRUPA KARUNA VANI

Lecturer in Chemistry, SVKP College, Markapur, Prakasam Dist. AP., India

Article Info
Received:02/1/2014
Revised on: 21/02/2014
Accepted on: 03/03/2014

ABSTRACT: A systematic method for the extraction and identification of the natural products in *Albizia lebbeck* (L.)Benth plant root bark was developed. The identification of the natural products was obtained in n-hexane/ethyl acetate (60:40 v/v) extract by using GC/MS method. Ten major compounds are found in the root bark extract. The conjoint compounds that found in these extracts are: 2-Methoxy-4-vinyl phenol, 3,5-di-tert-Butyl-4-hydroxy benzaldehyde, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 6-Octadecenoic acid,(Z)-, 9,12-Octadecadienoic acid, ethyl ester, Phenol, 2,6-, dimethoxy-, 9-Octadecenoic acid ethyl ester, tridecanoic acid methyl ester, Furfural, Phenol, 2-methoxy-. Different percent compositions for the major components were observed in the root bark extract. Furthermore, extract was tested for its antimicrobial activity using the dilution broth method, exhibiting moderate inhibition of human pathogenic bacteria. This study forms a basis for the biological characterization and importance of the compounds identified and creates a platform to screen many bioactive components to treat many diseases.

Key Words: *Albizia lebbeck* (L.)Benth plant root bark, soxhlet extraction, n-hexane/ethyl acetate/GC-MS method, antimicrobial study

Introduction

Medicinal plants are best owed with large number of pharmaceutically useful compounds which can be studied for investigation of new drugs for many serious diseases like cancer, tumours etc. Natural products, which come out from medicinal plants are important for pharmaceutical research and for drug development as a sources of therapeutic agents. At presents the demand for herbal or medicinal plant products has increasing significantly [1].

Albizia lebbeck (L) Benth (Family-Mimosoideae) (figure 1)]is an important anti-poisoning herb of Ayurveda. Its use is even indicated in snake bite poisoning. It grows into a big tree, usually found in road sides of Southern India. According to Ayurveda Bark, seeds, leaves and flowers of *Albizia lebbeck* are used for medicinal purposes [2].

A. lebbeck is a member of this genus and used in folk medicine to treat inflammatory conditions as asthma, arthritis, burns allergic rhinitis, bronchitis and leprosy [4] and it have been claimed to be useful in treatment of Alzheimer`s and Parkinson`s diseases [3]. Moreover the extracts of *A. lebbeck* exhibited versatile biological effects as antioxidants [4], hepatoprotective, cardiotonic, lipid-lowering, hypoglycemic activities [6,7] antihistaminic [3] and antimicrobial [7]. Literature survey on *A. lebbeck* revealed the presence of sterols and triterpenes [8], phenolic compounds, flavonoids [9], isoflavone [10], alkaloids [11], miscellaneous compounds [12] and saponins [13]. But there is no report about *A. lebbeck* plant root bark powder growing in south India [14], this prompted us to investigate this plant. The present work deals with the isolation and identification of phytochemical constituents in the root bark were isolated using n-hexane/ethyl acetate as solvents for the first time from *A. lebbeck* root bark and tested for its anti-microbial activity against two important pathogenic bacteria

Figure 1: Schematic representation of *Albizia Lebbeck* L root bark

Experimental

MATERIALS AND METHODS

***Chemicals*:** All Chemicals used in the entire study were AR grade obtained from SD fine, Merck chemicals, India, Pvt Ltd.,

***Plant Material*:** Fresh roots of *A. lebbeck* root bark in bulk collected in the month of May 2012 from agricultural fields of local area of Tenali revenue subdivision, Andhra Pradesh. 30x10 cm roots were collected and separated root bark manually, cut in to small pieces (figure 1), washed and dried in sunlight for one month completely to eliminate surface moisture. Then roots packed into envelop and kept in oven at 55°C temperature for further dryness. Dried material was grinded separately in a mortar obtained fine powder and sieved; which was then kept in plastic bags for further use.

Preparation of plant extract: The dry root bark powder material of *A. lebbeck* passed through sieve (100). The coarse powdered drug (200grams) was extracted in Soxhlet apparatus for 48 h with n-hexane and ethyl acetate (60:40) combination extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky brown residue (10.5 gm)

***GC-MS Analysis*:** GC-MS analysis of each extract sample was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with Elite-I, fused silica capillary column (30mm x 0.25mm 1D x 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1mL/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 72 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Table 2 & Figure 2, b).

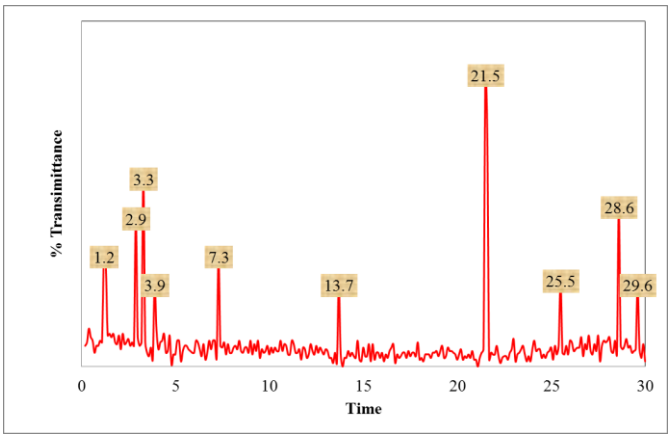


Figure 2: GC-MS spectra of n-hexane/ethyl acetate (60:40 v/v) root bark extract

Anti-Bacterial Activity by Disc Diffusion Method [16]

Preparation of Inoculum: *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* strains were used. 50ml of nutrient broth was prepared in 100ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37°Cfor 24 hours for organism to grow.

Preparation of Media: 200ml of nutrient agar media (NAM) was prepared and the pH was maintained at 7.0 to 7.2.

Pour Plate Method: 1ml of prepared inoculum was poured in sterile Petri dish & then 15 ml of NAM was poured in it & allowed to solidify.

Disc Diffusion Method: After solidification the disc of whatman 42 filter paper imbibed with 20 µl plant extracts were carefully placed with the help of forceps at the centre of the Petri dish and then kept in incubator for 24hrs.

Measurement of Zones: With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured

Preliminary phytochemical screening: root powder extract was subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in them.

[1]. Alkaloids: The 0.25 g of root extract was diluted to 5 mL of 2 N HCl. Aqueous layer formed was decanted and then it was added with one or a few drops of Mayer's reagent. Formation of precipitate or turbidity formed indicates the presence of alkaloids.

[2]. Aminoacids: The 1% ninhydrin was added to the 2 mL of the test solution. Formation of blue or violet color indicates the presence of aminoacids.

[3]. Coumarins: A 10% NaOH was added with the each of the test solution. Formation of yellow color indicates the presence of coumarins.

[4]. Glycosides: To the 2 mL of test solution, 2 mL of glacial acetic acid added with one drop of ferric chloride and 1 mL of concentrated H_2SO_4. Mix well and the formation of brown greenish ring indicates the presence of glycosides.

[5]. Phenols: Few drop of 5% ferric chloride solution was added to the test solutions. Formation of intense blue color indicates the presence of phenols.

[6]. Quinones: A few drops of concentrated H_2SO_4 were added with the 0.5 g of test solution. Formation of red color indicates the presence of quinones.

[7]. Reducing sugars: To the 2 mL of the test solution, 2 mL of Fehling's solution and 3 mL of distilled water was added and boiled for 2 min. The formation of reddish orange color indicates the presence of reducing sugars.

[8]. Sugars: The 0.5 g of each extract was added with very small quantity of anthrone and a few drops of concentrated H_2SO_4 and heated. Formation of green or purple color indicates the presence of sugars.

[9]. Saponins: The 2 mL of each test solution was added with H_2O and shook. Formation of foamy lather indicates the presence of saponins.

[10]. Terpenoids: The 2 mL of test solution was added with 2 mL of chloroform and a 3 mL of conc. H_2SO_4 mixed well and the formation of reddish brown indicates the presence of terpenoids.

[11]. Tannins: A 2 mL of each test solution was added with distilled H_2O and a pinch of lead acetate, formation of white precipitate indicates the presence of tannins.

Results and discussion

A. lebbeck plant root bark was screened for the preliminary phytochemical analysis and it was summarized in the Table 1. Phenolic compounds were present in major proportions. Aminoacids, coumarins and Sugars compounds were completely negligible in the extract.

Table 1: Display the presence/ absence of different phytochemicals in the root bark extracts of *A. lebbeck*

Functional Group	n-Hexane/ Ethyl Acetate
Alkaloids	+
Aminoacids:	-
Coumarins	+
Glycosides	+
Phenols	+
Quinones	+
Reducing sugars	+
Sugars	+
Saponins	-
Terpenoids	+
Tannins	+

GC–MS chromatogram of the ethanol extract of root of *A. lebbeck* L. root bark clearly shows 10 peaks (figure 2) indicating the presence of 10 phytochemical compounds. The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula. The table 2 shows the compound name with its molecular formula, Retention time, Peak area, % Peak area and its medicinal importance based on literature survey. The results reveal the presence of (1) 2-Methoxy-4-vinyl phenol;

(2) 3,5-di-tert-Butyl-4-hydroxy benzaldehyde; (3) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; (4) 6-Octadecenoic acid,(Z)-; (5) 9,12-Octadecadienoic acid, ethyl

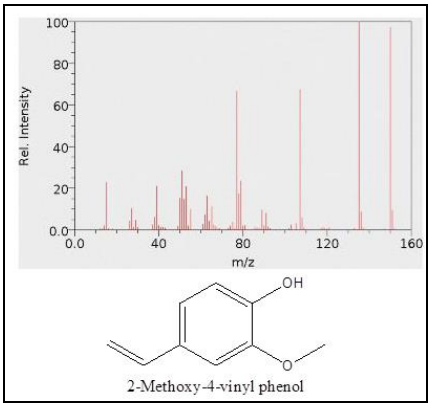
ester; (6) Phenol, 2,6-dimethoxy-; (7) 9-Octadecenoic acid, ethyl ester; (8) Tridecanoic acid, methyl ester; (9) Furfural; (10) Phenol, 2-methoxy-. The individual fragmentation patterns of necessary components were illustrated in Figures 2. The phytochemical

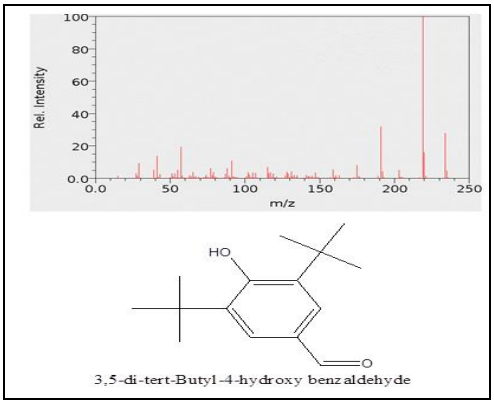
Compounds recognized through GC-MS analysis showed many biological activities are listed in Table 2.

Table 2: Components detected in ethyl acetate extract of *A. lebbeck* L. root bark

S. No.	RT	Compound	% Area	MF	MW	Nature of Compound	Activity≠
1	1.2	2-Methoxy-4-vinyl phenol	1.2	$C_9H_{10}O_2$	151	Phenolic	an aromatic substance used as a flavoring agent.
2	2.9	3,5-di-tert-Butyl-4-hydroxy benzaldehyde	7.68	$C_{15}H_{22}O_2$	234	Cabonyl compound	used as a pharmaceutical intermediate, and can be used for synthesis of antibiotics
3	3.3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	18.27	$C_6H_8O_4$	144	Flavanoid	Beverages and Foodstuffs Resistant to Light Induced Flavour Changes, Processes for Making the Same, and Compositions for Imparting Such Resistance
4	3.9	6-Octadecenoic acid,(Z)-	1.05	C18H 34O2	282	Ester	No Activity reported
5	7.3	9,12-Octadecadienoic acid, ethyl ester	1.01	C20H 36O2	308	Ester	Nematicide, hepatoprotective, antihistaminic, anticoronary
6	13.7	Phenol, 2,6-dimethoxy-	8.21	C8H10O3	154	Phenolic	No Activity reported
7	21.5	9-Octadecenoic acid, ethyl ester	39.2	C20H 38O2	310	Ester	No Activity reported
8	25.5	Tridecanoic acid, methyl ester	2.1	C_5 H_4 O_2	96	Ester	No Activity reported
9	28.6	Furfural	18.64	C14H 28O2	228	carbonyl	No Activity reported
10	29.6	Phenol, 2-methoxy-	3.24	C7H8O2	124	Phenols	Expectorant, antiseptic and local anesthetic

≠Source: Dr. Duke's : Phytochemical and Ethnobotanical databases





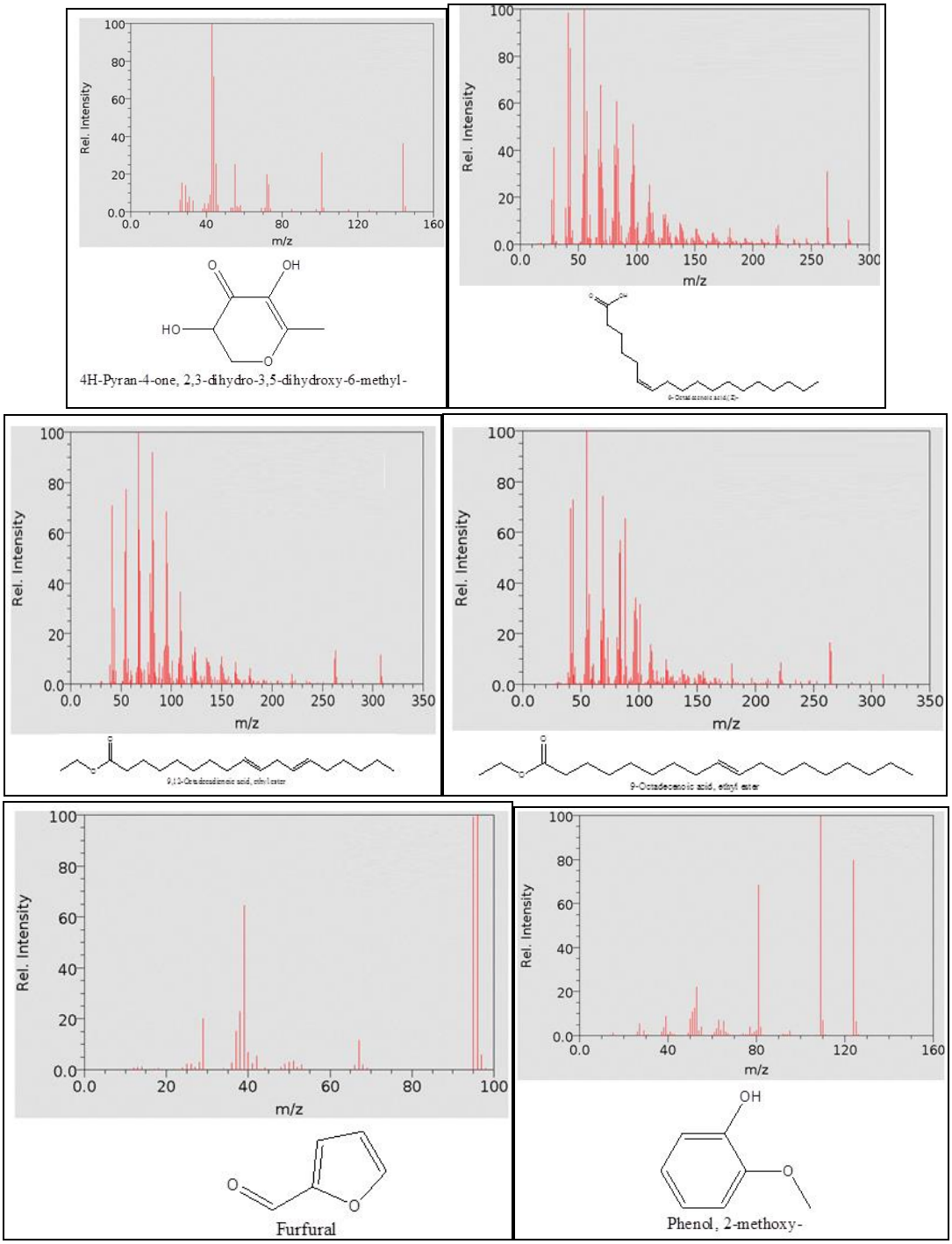


Figure 2: Mass fragmentation spectra and corresponding structures of the phytochemicals isolated from *Albizia lebbeck* root bark extract

Anti-microbial activity showed that the inhibition zones were found increased considerably when the concentration rate increased. Therefore it can be said that quantity of the extract was important for inhibition effect. Among Gram-positive bacterial growths, the maximum zone of inhibition was recorded against *Staphylococcus aureus* i.e. 15.64 mm (table 2). On the other hand Gram negative bacteria strain was tested against microorganism *E. coli* showed maximum zone of inhibition i.e. 22.31 mm. From these it is concluded that root bark extract showed maximum zone of inhibition against *E.Coli* bacterial strains, which indicate that *Albizia lebbeck* root bark extract has capacity to inhibit the growth of both Gram-positive and Gram-negative bacterial strains when used in a higher amount (figure 3).

Table 2: Zone of Inhibition of selected microbial cultures

Bacteria	Extracts	Zone of inhibition (mm)
Escherichia coli (Gram-ve)	n-hexane/ethyl acetate (60:40 v/v)	22.31± 0.21
Staphylococcus aureus(Gram+ve)	n-hexane/ethyl acetate (60:40 v/v)	15.64± 0.71

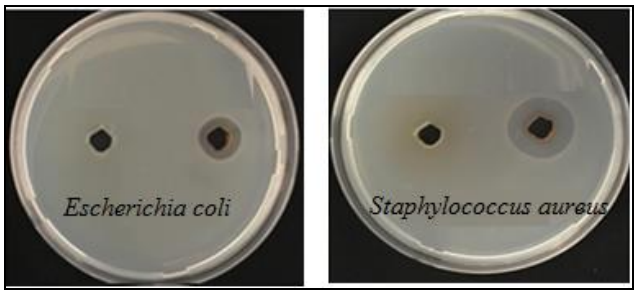


Figure 3: Showing antibacterial activity of n-hexane/ethyl acetate (60:40 v/v) root bark extract of *A. lebbeck* against *Escherichia coli* and *Staphylococcus aureus* microbes

Conclusion

Medicinal plants have curing property of various diseases due to the presence of biologically active compounds which make the plant medicinally important. *A. lebbeck* root bark phytochemical study revealed the presence of phytochemical compounds such as anthraquinones, flavonoids, steroids, phenols, etc., which have unique medicinal properties and used in the treatment for microbial diseases. The n-hexane/ethyl acetate extract showed higher antimicrobial activities than the ethyl acetate extract. This is a preliminary work on screening of bioactive compounds in *A. lebbeck* which needs further investigation

References

[1]. Sharma PC, Yolne MB and Dennis TJ: Database on medicinal plants used in Ayurveda, Volume I. Central council for Research in Ayurveda, and Siddha. Yuganter Prakashan (P) Ltd., New Delhi 2000; PP: 418-430.

[2]. http://easyayurveda.com/2012/12/26/shirisha-albizia-lebbeck-uses-medicinal-qualities-ayurveda-details/

[3]. M Farag; A El Gamal; A Kalil; A Al-Rehaily; O El Mirghany; K El Tahir, Pharmacology & Pharmacy, 2013,4, 473-477.

[4]. NP Babu; P Pandikumar; S Ignacimuthu, Journal of ethnopharmacology, 2009, 125, 356-360.

[5]. CR Resmi; MR Venukumar; MS Latha, Indian J Physiol Pharmacol., 2006, 50 (3), 297-302.

[6]. ZH Muhammad; A Shakeel; Q Mughal; E Sezai, Turkish Journal of Biology., 2013, 37, 25-32.

[7]. K Kokila; PS Deepika; V Sujatha, International Journal of Pharmacy & Pharmaceutical Sciences, 2013, 5 (3),70-73.

[8]. SM Seyydnejad; M Niknejad; I Darabpoor; H Motamedi, American J. of App. Sci., 2010, 7 (1), 13-16.

[9]. M Faisal; PP Singh; R Irchhariya, International Res Jour of Pharmacy, 2012, 3(5), 63-68.

[10]. S Verma, Research Journal of Pharmacy and Technology, 2013, 6, 1307-1313.

[11]. M Pandey; R Pandey; V Singh; V Pandey; U Singh, Mycobiology, 2002, 30, 55-56.

[12]. AK Dixit; LN Misra, Journal of natural products, 1997, 60, 1036-1037.

[13]. A Perwez; M Ali; A Vidhu. Der Pharmacia Lettre, 2011, 3 (6), 74-81.

[14]. ADP Barbosa, Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(11), 32-36.

[15]. A El-Mousallamy, Phytochemistry, 1998, 48, 759-761.

[16]. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7 (12): 1797-1806