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Phytochemical screening and antimicrobial activity of *Albizzia lebeck*

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ABSTRACT

Phytochemical screening and antibacterial activity of Albizzia lebeck leaves were assessed. Phytochemical screening of Successive extract Albizzia lebeck leaves shows presence of alkaloids, glycoside, tannins, saponins, flavanoids, and carbohydrates. The Successive ethyl acetate extract Albizzia lebeck leaves are found inhibitory effect against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus. The extract shows sensitivity for both gram positive and gram negative bacteria with maximum against Pseudomonas aeruginosa and minimum against Escherichia coli.

Key Words: Alkaloids, *Bacillus cereus*, antimicrobial activity.

INTRODUCTION

Man has been using herbs and plants products for combating diseases since times immemorial. Indian systems of medicine have a deep root in our culture heritage and cater to the Medicare of large sections of our population. These systems mainly use herbs. If we dwell for a moment on our hoary past, the *Rigveda*, one of the oldest repositories of human knowledge, mentions the use of 67 plants for therapeutic use, the *Yajurveda* enlist 81 plants whereas the *Atharveda* written during 1200 BC describes 290 medicinal plants of medicinal value. *Charak Samhita* written during 990 BC describes 341 medicinal plants. The land mark in Ayurveda was *Sushrut Samhita* written during 600 BC mentioned 395 medicinal plants. *Dhanwantari Nighantu* mentions 750

medicinal plants, 450 are mentioned in the *Bhavaprakash*, 480 in *Madanapala Nighantu* and 450 in the *Kaiyadeva Nighantu*. India unquestionably occupies the top position in the use of herbal drugs. It is one of the foremost countries exporting plant drugs and their derivatives. It also excels in home consumption. It is not at all surprising that herbal drugs are so prevalent in India given the great biodiversity and abundance of flora and the variety of geographical condition which allows the most exotic medicinal plants to be grown here.[1]

Albizzia lebbeck Benth. (Shirish, Family: *Leguminosae*) is a deciduous tree with compound leaves, flat oblong fruits, round cream colored seeds, grows wild. The plant is found throughout India, Bangladesh, tropical and subtropical Asia and Africa [2]. Barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea. Ethanolic extract of pods possesses antiprotozoal, hypoglycemic and anticancer properties. The methanolic extract of the pod was investigated for antifertility activity [3, 4]. The plant extract also evaluated in allergic rhinitis [5] and memory and learning of mice [6]. Phytochemical investigations showed that the pod of the *Albizzia lebbeck* contains 3', 5 Dihydroxy 4', 7 dimethoxy flavone, and N- Benzoyl L phenyl alaninol [7]. The beans of the plant contain albigenic acid-a new triterpenoid sapogenin [8]. The plant also contains saponins [9, 10], macrocyclic alkaloids [11], Tannins [12], and flavonols [13]. The decoction of *Albizzia lebbeck* stem bark was found to be effective against bronchospasm induced by histaminic acid phosphate and shown to exert di-sodium chromoglycate like action on mast cells[14]. *Albizzia lebbeck* bark extract show the antimicrobial activity. The active constitute of bark extract is anthraquinone glycosides. The main constituent from bark is active against aerobes and mechanism of action is that glycosides cause the leakage of the cytoplasmic constituents.[15]

Two new tri-O-glycoside flavonols kaempferol and quercetin were identified from the leaves of *Albizzia lebbeck*. [16] *Albizzia* hexoside a new hexaglycosylated saponin was isolated from leaves of *Albizzia lebbeck*. [17] Lignins Present in their cell walls have been oxidized with alkaline nitrobenzene. The phenolic acids were present in the range of 8.8-52.7 mg/g of cell wall. [18] The chloroform fraction of methanolic extract of *Albizzia lebbeck* leaves protected mice against maximal electroshocks. [19] Ethyl ether and alcoholic extracts of leaves of *Albizzia lebbeck* showed positive reaction against bacterial pathogens i.e. [*Staphylococcus aureus*] and [*Escherichia coli*] and fungal pathogen [*Candida albicans*]. Flavonoid contents like Quercetin and Kaempferol were isolated and identified from the leaves and Flavonoid was found contents (2.40 mg/g). [20] Methanolic extract of leaf and methanolic and water extracts of bark have shown in vitro mast cell stabilizing effect against compound 48/80. [21] The effect of saponin containing n-butanolic fraction (BF) extracted from dried leaves of *Albizzia lebbeck* on learning and memory was studied in albino mice and Significant improvement was observed in the retention ability of the normal and amnesic mice as compared to their respective controls. [22]

EXPERIMENTAL SECTION

Plant collection and identification

The leaves of *Albizzia lebbeck* were collected from Jaipur in March 2009. A voucher specimen (Voucher No. RUBL 50033) was kept at the Department of Botany, University of Rajasthan after identification of the plant.

Extraction of the plant material

Plant materials were washed with water and shade dried. The derided leaves were crushed to coarsely powdered by wood-grinder. The powdered material was defatted with petroleum ether (60-80 °C) and then successively extracted in Soxhlet apparatus with solvent. Mark is dried in oven at 40 °C during solvent changing. The extract was concentrated for further studies on water bath at 40 °C. At the time of antibacterial assay 1000 mg of ethyl acetate extract is dissolved in 1 ml of DMSO.

Photochemical screening

The chemical tests were performed for testing different chemical groups present in extracts.

Alkaloids***Dragendorff's Test***

To 2-3 ml of filtrate, few drops of the Dragendorff's reagent were added. Formation of orange brown precipitate indicated the presence of alkaloids.

Carbohydrates***Molisch's Test***

In a test tube containing 2 ml of extract, 2 drops of freshly prepared 10 percent alcoholic solution of α - naphthol was added. Then it was shaken and 2 ml of conc. sulphuric acid was added from sides of the test tube. So the violet ring was formed at the junction of two liquids, indicated the presence of carbohydrates.

Proteins***Biuret's Test***

To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

Amino Acid***Ninhydrin Test***

3 ml of test solution and 3 drops of 5% ninhydrin solution in a test tube were heated in boiling water bath for 10 minutes. Formation of purple or bluish colour indicated the presence of amino acid.

Glycosides***Borntrager Test***

3 ml of extract was treated with dilute Sulfuric acid then boil and filtered. Cold filtrate was treated with chloroform (equal volume) and shaken for some time. The organic layer is separated and treated with dilute ammonia. Pinkish colour of ammonical layer indicated anthraquinone glycoside.[23]

Saponins***Foam Test***

The extract was shaken vigorously with water in a test tube. Formation of persistent foam indicated the presence of saponins.

Steroids*Salkowski Test*

To 2 ml of extract, 2 ml of chloroform and 2 ml of concentrated sulphuric acid were added and shaken, red color at lower layer indicated the presence of steroids.

Tannins*Ferric Chloride Test*

Extract solutions were treated with 5% ferric chloride solution. Formation of blue colour indicated the presence of hydrolysable tannins and formation of green colour indicated the presence of condensed tannins.

Flavanoids

A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavanoids.[24]

Preparation of micro-organism

The organisms used in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus cereus* (ATCC 6633).the strains were maintained on nutrient agar slants at 4⁰C. A loopful of each bacterial strain was inoculated into 50 ml of sterile nutrient broth in 100 ml conical flask. The flask was incubated on a rotary shaker for 24 hr to activate the strain. Mueller Hinton Agar medium was used as bacterial culture medium in the antibacterial assay.

Antibacterial Activity

Mueller-Hinton Agar plates are prepared by pouring 10-15 ml of the medium into each sterilized Petridis and are allowed to set at room temperature. The cell suspension is inoculated over the surface of agar medium using sterile cotton swab. The four cups are scooped in each plate using a sterile cork borer of 8 mm diameter. Then the solution of test compounds (25µl, 50 µl, 100 µl and control 50 µl) are added in cups by using micropipettes and these plates are incubated at 37⁰C for 48 hr. Standard drug Ciprofloxacin (5mcg) is used. The zone of inhibition is measured in mm for each organism. [25]

RESULTS

Phytochemical screening of ethyl acetate Successive extract *Albizia lebbeck* leaves shows presence of glycoside, tannins, saponins, flavanoids, carbohydrates, proteins and amino acids. Methanolic Successive extract shows presence of alkaloids, tannins, saponins, flavanoids and carbohydrates. Water Successive extract shows presence of tannin, saponins, flavanoids and carbohydrates as per as shows in table1.

Antibacterial Activity

Ethyl Acetate Successive Extract is test for this antibacterial activity. The doses of 1000 mg /ml of extracts were made by dissolving appropriate quantity of extracts in DMSO. Standard drug Ciprofloxacin is used.. the solution of test compounds (25µl, 50 µl, 100 µl and control 50 µl) are added in cups by using micropipettes and these plates are incubated at 37⁰C for 48 hr. The zone

of inhibition is measured in mm for each organism. Controls with DMSO do not show any activity. The crude extract shows positive antimicrobial activity against both gram positive and negative bacteria (table 2, figure 1, 2, 3, 4.)

Table 1- Showing phytochemical screening of AL leaves Successive Extracts Successive Extraction (Soxhlet Apparatus)

| Solvent→ Phytochemical↓ | Ethyl Acetate | Methanol | Water |
|----------------------------|---------------|----------|-------|
| Alkaloids | - | + | - |
| Glycoside | + | - | - |
| Tannin | + | + | + |
| Saponin | + | + | + |
| Steroid | - | - | - |
| Flavanoids | + | + | + |
| Carbohydrates | + | + | + |
| Amino acids | + | - | - |
| Proteins | + | - | - |

+ Presence of constituent, - Absence of constituent

Table 2- Showing Effect of Extract on Microbial Growth

| Dose→ Microorganism↓ | 25µl | 50 µl | 100 µl | Standard |
|------------------------------------|------|-------|--------|----------|
| Escherichia coli (ATCC 25922) | 11 | 13 | 17 | 37 |
| Staphylococcus aureus(ATCC 29213) | 12 | 17 | 19 | 26 |
| Pseudomonas aeruginosa(ATCC 27853) | 26 | 27 | 29 | 34 |
| Bacillus cereus(ATCC 6633) | 23 | 25 | 28 | 33 |

Concentration in (ZOI mm)

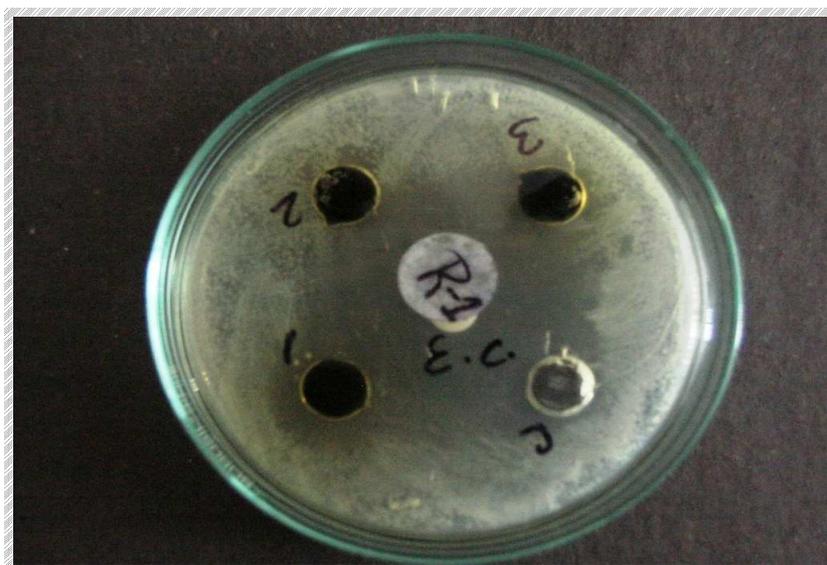


Figure 1- Inhibition Zone against Escherichia Coli

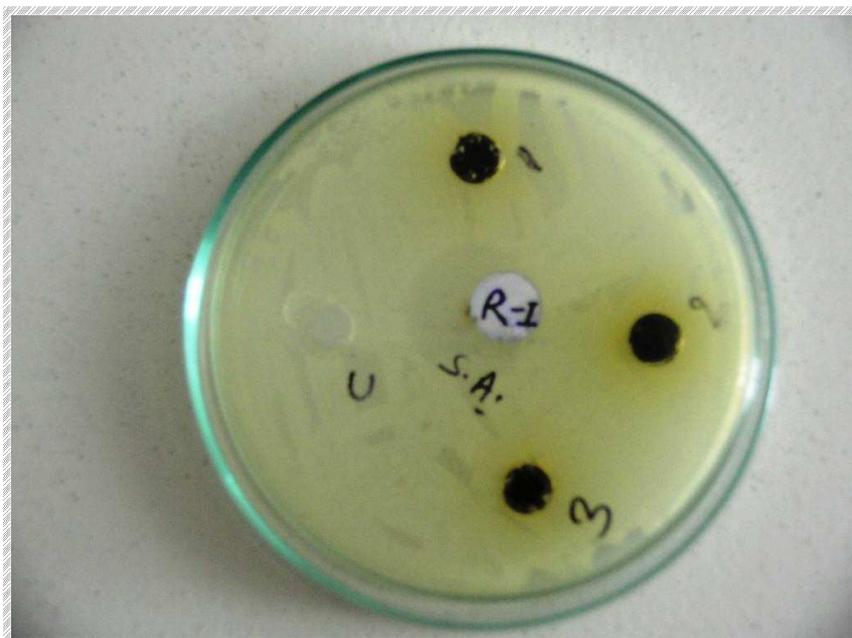


Figure 2- Inhibition Zone against Staphylococcus Aureus

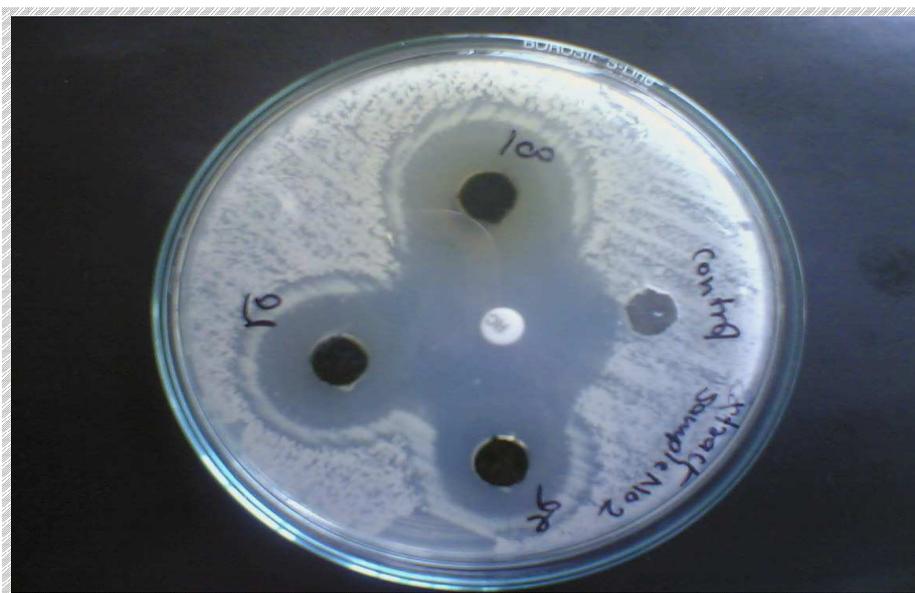


Figure 3- Inhibition Zone against Bacillus cereus

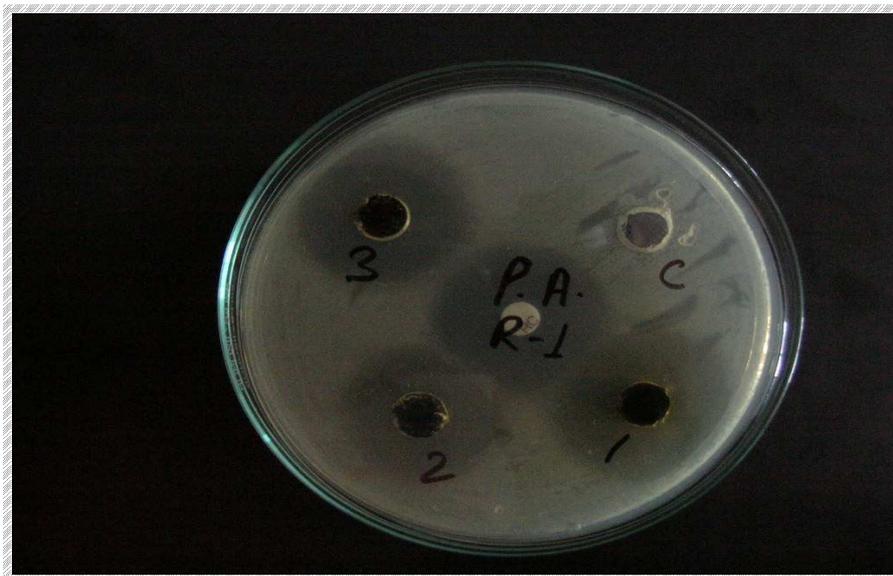


Figure 4- Inhibition Zone against *Pseudomonas aeruginosa*

DISCUSSION

Phytochemical Screening

Phytochemical screening of successive extracts *Albizzia lebbek* leaves was done with ethyl acetate, methanol and water. The study shows presence of carbohydrates, alkaloids, tannin, flavanoids and saponins. Main attraction of phytochemical screening is presence of tannins, saponin and flavanoids in maximum of extracts where absence of proteins and amino acids in successive percolation extract as compare to maceration extract may be indication of denaturation of proteins by heat.

The phytochemical screening of chemical constituents in *Albizzia lebbek* study showed that leaves were rich in flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity. The presence of flavonoids in the present study is support the opinion of Mousallamy AM 1998(17) who noted that flavonoids in *Albizzia lebbek* leave. Also, the presence of saponin is support the observation of Ueda M 2003(26) who reported that saponin in *Albizzia lebbek* leaves. Tannins and saponins were found to be present and steroids are absent in all extracts.

Ethyl Acetate Successive Extract was studied for antibacterial activity against gram positive and gram negative bacteria's. The present investigation clearly demonstrates the significant antibacterial activity of Ethyl Acetate Successive Extract of *Albizzia lebbek* leaves against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* in vitro. These results indicate the potential use of this plant in management of bacterial diseases caused by *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* Since these bacteria's are an important pathogenic bacteria causing a large number of diseases in human being and animals. The anti-bacterial activities of ethyl acetate extract were Compared with standard Ciprofloxacin and the results are showed that the ethyl acetate extract of *Albizzia lebbek* leaves had maximum inhibitory effect against *Pseudomonas aeruginosa* and minimum inhibitory effect against *Escherichia coli*. This study tends to express that the leaves of *Albizzia lebbek* have active ingredients against these gram positive and negative bacteria.

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