Phytochemical screening and antimicrobial activity of *Albizzia lebbeck*

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ABSTRACT

Phytochemical screening and antibacterial activity of *Albizzia lebbeck* leaves were assessed. Phytochemical screening of Successive extract *Albizzia lebbeck* leaves shows presence of alkaloids, glycoside, tannins, saponins, flavanoids, and carbohydrates. The Successive ethyl acetate extract *Albizzia lebbeck* 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 landmark 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]

*Albizia 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 *Albizia 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 *Albizia 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]. *Albizia lebbeck* bark extract show the antimicrobial activity. The active constituent 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 *Albizia lebbeck*. [16] *Albiziahexoside* a new hexaglycosylated saponin was isolated from leaves of *Albizia 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 *Albizia lebbeck* leaves protected mice against maximal electroshocks. [19] Ethyl ether and alcoholic extracts of leaves of *Albizia 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 form 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 *Albizia 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 *Albizia 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 dehydrated 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 shaked 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 shaked 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 shaked 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 4°C. 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
37°C 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 Albizzia 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 37°C for 48 hr. The zone
of inhibition is measured in mm for each organism. Controls with DMSO did 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

<table>
<thead>
<tr>
<th>Solvent → Phytochemical</th>
<th>Ethyl Acetate</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Presence of constituent, - Absence of constituent

Table 2- Showing Effect of Extract on Microbial Growth

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>25µl</th>
<th>50 µl</th>
<th>100 µl</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli (ATCC 25922)</td>
<td>11</td>
<td>13</td>
<td>17</td>
<td>37</td>
</tr>
<tr>
<td>Staphylococcus aureus(ATCC 29213)</td>
<td>12</td>
<td>17</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa(ATCC 27853)</td>
<td>26</td>
<td>27</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>Bacillus cereus(ATCC 6633)</td>
<td>23</td>
<td>25</td>
<td>28</td>
<td>33</td>
</tr>
</tbody>
</table>

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
Phytochemical Screening
Phytochemical screening of successive extracts Albizia lebbeck 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 Albizia lebbeck 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 Albizia lebbeck leave. Also, the presence of saponin is support the observation of Ueda M 2003(26) who reported that saponin in Albizia lebbeck 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 Albizia lebbeck 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 Albizia lebbeck leaves had maximum inhibitory effect against Pseudomonas aeruginosa and minimum inhibitory effect against Escherichia coli. This study tends to express that the leaves of Albizia lebbeck have active ingredients against these gram positive and negative bacteria.
REFERENCES