Evaluation of Antibacterial Activity and Phytochemical Screening of Azadirachta indica Leaves Extracts Against Staphylococcus aureus

Bhuwanendra Singh*, Arshad Ahamad, Vipin Pal

Department of Pharmacognosy, Rajiv Academy for Pharmacy, NH-2, Delhi-Mathura Highway, Mathura (U.P.), India

Abstract

Azadirachta indica is an Indian tree well known for its several pharmacological activities, including antimicrobial activity. A. indica leaves extract was used to screen antibacterial activity against S. aureus. The methanol extract of leaves of A. indica was prepared and its phytochemical screening was performed. The phytochemical analysis exhibited the presence of alkaloids, polyphenol, tannins, flavonoids and glycoside in extract. The methanol extract of A. indica leaves tested against S. aureus using disc diffusion method. Gentamicin was used as standard and compared the effect of antibacterial activity of methanol extract. The methanol extract of A. indica leaves demonstrated potent antibacterial activity against tested bacteria.

1 Introduction

Azadirachta indica is a tree belongs to the botanical family Meliaceae (also called the mahogany family), originated from India. A. indica is the most useful traditional medicine and it is used in the treatment of various disease. It grows well in the tropical and semi-tropical countries. Its twigs are used as tooth brush and are widely used in the Indian sub-continent. Earlier studies on A. indica showed that it contains active substances in almost every part of the seeds, leaves, roots, bark, trunk and branches with multiple medicinal properties. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.

A. indica known for its pharmacological activities, such as anti-inflammatory, anti-malaria, anti-fertility, antimicrobial, anti-acne, acaricidal and nematicidal. It is used for the treatment of diabetes and shows the potential role of anti-diabetic activity. Aqueous extract of A. indica leaf extract has a good therapeutic potential as anti hyperglycemic agent. A. indica leaves have antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise. Neem seeds are used in traditional medicine to treat infections conditions especially those involving the eye and ear. Administration of alcoholic extract of A. indica flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and has the potential of an ideal antifertility agent. Neem aqueous extract has powerful chemotherapeutic and viral agent.

A. indica are rich in phytoconstituents such as glycoside, alkaloids, triterpenoids, tannin, flavonoids etc. Azadirachtin, the main active component of this plant, is a tetranortriterpenoid abundant in the seeds and present in a smaller concentration in the leaves (Fig-1). Other active substances are salalan, 14-epoxiazadiradione, meliintrol, melianone, gedunin, nimbinolane, nimbin, deacetyl salalanin, azadiractol, azadirone, vilosinin, meliacarpine, over 300 isolated and characterized components. The purpose of the present study was to investigate the antibacterial activity of A. indica leaves against disease causing bacteria, such as Escherichia coli, Staphylococcus aureus.

2 Materials and Methods

2.1 Plant material

Azadirachta indica (neem) leaves were collected from College Garden, in Rajiv Academy for Pharmacy, Mathura U.P.

2.2 Preparation of extracts

The fresh leaves of A. indica were collected and dried in shade (Fig-2). About 100 grams of coarsely leaves powdered was exhaustively extracted for 2 h with methanol in soxhlet apparatus. The extracts
obtained were filtered and evaporated under reduced pressure using rotary evaporator. The extracts were dissolved in dimethyl sulfoxide (DMSO) to make the final concentrations which were kept in refrigerator till used.

Fig 1: Chemical structure of Azadirachtin

2.3 Preliminary Phytochemical studies

The extracts were analyzed by the following procedures. To test for the presence of the alkaloids, saponins, tannins, Terpenoids, flavonoids, glycosides, volatile oils and reducing sugars.

2.3.1 Test for alkaloids

(a) Dragendorff’s test: To 1 ml of the extract, add 1 ml of dragendorff’s reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer’s test: To 1 ml of the extract, add 1 ml of mayer’s reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) Hager’s test: To 1 ml of the extract, add 3 ml of Hager’s reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

(d) Wagner’s test: To 1 ml of the extract, add 2 ml of wagner’s reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

2.3.3 Test for Glycosides

(a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager’s test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

2.3.4 Test for carbohydrates and sugars

(a) Molisch’s test: To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(b) Fehling’s test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.
Benedict’s test: To 5ml of Benedict’s reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

### 2.3.5 Test for tannins and phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

### 2.3.6 Test for flavonoids

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) Shinoda’s test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonoids.

(d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

(e) The extract is treated with concentrated H₂SO₄, formation of yellow or orange colour indicates flavones.

### 2.3.7 Test for steroids

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H₂SO₄. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

### 2.3.8 Test for triterpenoids

Noller’s test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids.

### 2.4 Test microgranism

The disease causing strains of *Streptococcus aureus* used as a test organisms and disc diffusion method were used to determine the antibacterial activity of *A. indica*.

### 2.5 Preparation of Discs

From the plant extracts, 50 mg and 100 mg of crude extracts were dissolved in 1 ml of 4 % DMSO and 0.2 ml of the prepared extracts were loaded on to the filter paper discs (Sterilized Whatmann No. 1 filter paper discs of 6 mm diameter) to get 20 mg / disc concentration and allowed to dry at room temperature in laminar air flow chamber.

### 2.6 Antibacterial activity

The antimicrobial activity of the extracts was evaluated by disc diffusion method. The paper discs containing different extracts were placed individually on the surface of the petri plates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions individually (10 CFU/ml). Standard antibiotic Gentamicin (20 μg/disc) obtained from Hi-media, Mumbai, was used as positive controls.

The discs containing methanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded with the help of antibiotic zone reader.

### 3 Results

The qualitative chemical test of *A. indica* leaves powder of methanol extracts exhibited the presence of alkaloids, polyphenol, tannins, flavonoids and glycoside (Table 1). The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract. Imaran khan et al., 2010 studied that phytochemical analysis of *Azadiracta indica* leaves by using different solvent such as Petroleum ether, chloroform, methanol show the presence of triterpenes, glycosides and fatty acids.

The antibacterial activity of methanol extracts was investigated using disc diffusion method, against the *Staphylococcus aureus*. The antibacterial activity of *A. indica* compared with the zone of inhibition of Gentamicin which exhibited greater antibacterial activity. Zone of inhibition of methanol leaf extract of *A. indica* was observed 16 mm
Singh et al. Evaluation of Antibacterial Activity and Phytochemical Screening of Azadirachta indica

diameter and the zone of inhibition of Gentamicin were not observed because gentamicin (Table 2; Fig 3 & Fig 4). It implies that Gentamycin completely inhibit the growth of Staphylococcus aureus in medium, and it indicates 100% inhibition.

**Table 1:** Phytochemical analysis of methanol extracts of Azadirachta indica leaves

<table>
<thead>
<tr>
<th>Plant constituents</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
</tbody>
</table>

-ive - absence of plant constituents  
+ive - presence of plant constituents

**Table 2:** Inhibitory activity of A. indica and Gentamicin

<table>
<thead>
<tr>
<th>Drug</th>
<th>Microorganism</th>
<th>Inhibition activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>S. aureus</td>
<td>0%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>100%</td>
</tr>
<tr>
<td>Methanol A. indica extract</td>
<td>S. aureus</td>
<td>16%</td>
</tr>
</tbody>
</table>

4 Conclusion

It may be concluded from this study that A. indica leaves extract has antibacterial activity against S. aureus. Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects. A. indica leaves possessed good antibacterial activity confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care. The extract of A. indica when used as medicinal plant, could be useful for the growth inhibition of the carcinogenic bacterium, S. sobrinus. The alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanisms of the plants against pathogens.

**Figure 3:** Graphical representation of inhibitory activity of Gentamicin and methanol extract of A. indica

**Figure 4:** Inhibitory activity of methanol extract of A. indica against S. aureus (A) Represent the gentamicin activity on the MHA media; (B) Represent the A. indica extract antibacterial activity

5 Competing interest

None

6 Author's contributions

BS, AA and VK performed experimental work. All authors read and approved the final manuscript.
7 References


