Phytochemical Screening and Antibacterial Activity of Ethanol Extract of Leaves and Twigs of Azadirachta indica A. Juss

Ravi K*, Bharavi K, Ravi Kumar P, Vamsi Krishna B

Department of veterinary pharmacology and toxicology NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India – 521102

Abstract

Azadirachta indica A. Juss commonly known as Neem belongs to family Meliaceae. Each part of A. indica has been used extensively for various ailments. In the present study A. indica leaves and twigs were shade dried and made into powder. This powdered material was subjected for extraction using ethanol (10gm powder in 100ml ethanol). Ethanol extracts (EE) of leaves and twigs were qualitatively screened for the presence of phytochemicals viz. alkaloids, flavonoids, saponins, tannins, polyphenols and steroids. The in vitro antibacterial potential of ethanol extract of leaves and twigs against methicillin sensitive Staphylococcus aureus (MSSA) and E. Coli (ATCC 25922) was evaluated by determining minimal inhibitory concentration (MIC). Ethanol extract of A. indica leaves showed favorable MIC values than the ethanol extract of twigs against MSSA whereas against E. coli both the MIC values are comparable. In conclusion, both ethanol extracts of A. indica leaves and twigs were found to have potent antibacterial activity against MSSA and E. Coli.

1 Introduction

Staphylococcus aureus is a gram-positive bacteria, usually found in airway tract and on the surface of the body which is responsible for different types of purulent infections in humans and animals1,2. Escherichia coli are gram-negative bacteria naturally present in large intestine and occasionally in urinary tract. Some strains of E. coli live as harmless commensalism in animals’ intestines while others cause serious diseases. It can cause serious health complications in humans as well as in animals.

Emergence of multiple drug resistant bacterial strains due to indiscriminate use of antibiotics have generated a keen interest in the discovery of effective plant derived drugs3. There is an increasing quest for phytotherapy/searching plant based medicines against resistant bacteria as an effective alternative in different parts of the world4. Furthermore, the antibiotics may be associated with adverse effects, including hypersensitivity and immune suppression5. Antibiotics are sometimes ineffective to bacterial strains, moreover, they are is costly for the poor communities of developing world6,7.

Medicinal plants are reservoir of chemical agents associated with therapeutic properties and gaining importance as an effective alternate for antimicrobials as they are safe and available at low cost8,9. Plant extracts have phytochemical with antimicrobial properties and can be of great significance in therapeutic treatments10.

Azadirachta indica A. Juss, commonly known as Neem is extensively used in Ayurveda, Unani and Homoeopathic medicine. Additionally it has become a cynosure for modern medicine11. A. indica leaves contain a wide range of biologically active and diverse phytochemical constituents12. Many scientific studies are reporting that A. indica leaves have medicinal and pharmacological properties13. In this context present study was conducted to evaluate the phytoconstituents and antibacterial potential of A. indica leaves and twigs against methicillin sensitive Staphylococcus aureus (MSSA) and E. Coli.

2 Materials and Methods

2.1 Phytochemical screening:

The leaves and twigs of A. indica. A. Juss were collected from local source (Gannavaram, Krishna District, Andhra Pradesh, India) and
got authenticated by a qualified botanist, Mr. K. Ramakrishna, Lecturer in Department of Botany, VKR Degree College, Buddavaram, Gannavaram Mandal, Krishna District, Andhra Pradesh. The leaves and twigs were air dried separately in the shade and made into course powder using willey mill with 1 mm sieve size. Twenty grams of powdered leaves and twigs material was taken separately in to two conical flask, and ethanol were added in 1:10 ratio. The flasks were closed properly and subjected to constant stirring using an orbital shaker. Following day, the extracts were filtered using whatman filter paper No.1 and the filtrates were allowed for drying at room temperature until a consistent solid material mass was formed. The ethanol extracts were dissolved in DMSO at concentration of 10 mg/ml. The prepared samples were used for phytochemical screening and in vitro antibacterial activity was investigated.

The leaf and twig extracts was screened for alkaloids, flavonoids, tannins, saponins and steroids using standard methods of analysis described by Sofowora, 1993 and Trease and Evans, 2002.14,15.

2.2 Determination of minimal inhibitory concentration (MIC)16

Minimal inhibitory concentrations (MIC) of the extracts was determined by broth assay against E. coli (ATCC 25922) and methicillin sensitive S. aureus, MSSA (ATCC 25923) using 96-well (round bottom) micro titre plates filled with a 100 µl aliquot of normal saline. To the first column of wells 200 µl of the original extracts was added and mixed with the broth in the wells using a sterile micropipette tip.

After mixing, 100 µl of the mixture was transferred to the next well in each row and this process was continued making two fold dilutions. Each well was inoculated with 50 µl of inoculums (1·2 x 10⁵ – 10⁵ CFU/ml) and incubated overnight at 37°C. As an indicator for bacterial growth, 30 µl of p- iodonitrotetrazolium dye was added to all wells. The plates were then incubated at 37°C for 30 minutes. Positive and negative controls were run parallel with ciprofloxacin (0.25 mg/ml) and normal saline, respectively. Development of pink colour after incubation indicates the presence of bacteria (colourless tetrazolium salt acts as an electron acceptor and is reduced to a red coloured formazan product by biologically active organisms). The concentration at which there was no visually detectable bacterial growth was taken as the MIC.

After incubation, a small volume of the culture from each well was streaked on Muller-Hinton agar plates. The plates were then incubated at 37°C overnight and the lowest dilution that yielded complete inhibition of growth was taken as the minimal bactericidal concentration (MBC).

Each of the extracts was tested in triplicate, and the average values were obtained from two repeated experiments.

3 Results

3.1 Phytochemical screening

The phytochemical screening of ethanol extract of A. indica leaves and twigs revealed the presence of alkaloids, flavonoids, saponins, tannins, polyphenols and steroids (Table 1).

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Leaf extract</th>
<th>Twig extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falvonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Presence of constituents; - = Absent of constituents

3.2 Minimum inhibitory concentration and minimum bactericidal concentration

MIC and MBC of the EE of A. Indica. leaves and twigs were determined by broth dilution method against methicillin sensitive S. aureus (ATCC 25923) and E. coli (ATCC 25922). The MICs of A. indica leaves against S. aureus and E. coli were 156.25 ± 0.02 µg/ml and 625 ± 0.11 µg/ml and the MBCs were 625 ± 0.15 µg/ml and 2500 ±0.29 µg/ml respectively, while the MICs of twigs against them were 1250 ± 0.31 µg/ml and 625 ± 0.18 µg/ml and MBCs were 2500 ± 0.05 µg/ml and 1250 ± 0.52 µg/ml, respectively (Table 2; Fig 1 and 2).

4 Discussions

Ethanol extracts of leaves and twigs of A. indica was subjected to phytochemical screening. The analysis revealed the presence of flavonoids, alkaloids, tannins, saponins, polyphenols and steroids. Different phytochemical constituents like alkaloids, flavonoids, phenolic compounds, steroids and ketones that are present in various parts of A. indica possess biologically active properties.17 Ethanol extracts of A. indica leaves and twigs were evaluated for their MIC and MBC against MSSA and E. coli organisms. The study revealed that the MIC and MBC levels of A. indica leaves were much lower than the corresponding values of A. indica twigs against MSSA. Against E. coli, the MIC levels were same for both the...
extracts of leaves and twigs. However, the MBC level of twigs was lower than the MBC level of leaves against \( E. \ coli \). Overall, the study revealed the presence of antibacterial activity in the leaves and twigs of \( A. \ indica \) against MSSA and \( E. \ coli \). Numerous studies conducted earlier with different parts of \( A. \ indica \) also revealed the presence of antimicrobial and antiprotozoal effects. Saradhajyothi and Subbarao (2011)\(^{18} \) opined that the antibacterial activity of \( A. \ indica \) might be due to presence of wide array of biologically active chemical constituents. A study by Sarmento et al. (2011)\(^{19} \) also revealed that the ethanol extract of \( A. \ indica \) leaves had antibacterial effect against both methicillin sensitive and methicillin resistant \( S. \ aureus \). \( A. \ indica \) stick extracts possessed a wide spectrum of antibacterial action against gram negative and gram positive microorganism\(^{20} \).

**Table 2: Minimum inhibitory concentration of the ethanol extract of \( A. \ indica \) leaves and twigs against methicillin sensitive \( S. \ aureus \) (MSSA) and \( E. \ coli \).**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Methods</th>
<th>MSSA</th>
<th>( E. \ coli )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf extract</td>
<td>MIC</td>
<td>156.25±0.02 µg/ml</td>
<td>625±0.11 µg/ml</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>625±0.15 µg/ml</td>
<td>2500±0.29 µg/ml</td>
</tr>
<tr>
<td>Twig extract</td>
<td>MIC</td>
<td>1250±0.31 µg/ml</td>
<td>625±0.18 µg/ml</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>2500±0.05 µg/ml</td>
<td>1250±0.52 µg/ml</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>MIC</td>
<td>0.12±0.29 µg/ml</td>
<td>0.24±0.09 µg/ml</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>0.12±0.35 µg/ml</td>
<td>0.48±0.17 µg/ml</td>
</tr>
</tbody>
</table>

*Values indicate the measurement of mean of three time*

**Fig 1:** Minimum inhibitory concentration (µg/ml) of the ethanol extract of \( A. \ indica \) leaves and twigs against \( S. \ aureus \) (pink colour indicates growth). A – ethanol extract of twigs; B – ethanol extract of leaves; C – negative control (Nacl + \( S. \ aureus \) tryptic soya broth); D – positive control (Ciprofloxacin + \( S. \ aureus \) tryptic soya broth).

**Fig 2:** Minimum inhibitory concentration (µg/ml) of the ethanolic extract of \( A. \ indica \) leaves and twigs against \( E. \ coli \) (pink colour indicates growth). A, B - ethanol extract of twigs; C, D - ethanol extract of leaves; E - negative control (Nacl + \( E. \ coli \) tryptic soya broth); F - positive control (Ciprofloxacin + \( E. \ coli \) tryptic soya broth).

**5 Conclusions**

The study revealed the presence of antibacterial activity in the leaves and twigs of neem against MSSA and \( E. \ coli \).

**6 Acknowledgements**

The funds and facilities provided by NTR College of Veterinary Science and Sri Venkateswara Veterinary University, Gannavaram for the postgraduate research are acknowledged.

**7 Competing interests**

There are no competing interests

**8 Author’s contributions**

BK, RKP designed and monitored the experimental protocol. RK and VKB collected the material and performed the whole experimental procedure.

**9 References**


