Antimicrobial activity of *Azadirachta Indica* (neem) leaf, bark and seed extracts

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ABSTRACT

Screening of medicinal plants for bioactive compounds leads to development of less expensive new antimicrobial agents with improved safety and efficacy. *Azadirachta Indica* (neem) is a multipurpose tree with multiple health benefits. Different parts of the plant are shown to exhibit antimicrobial effects against a wide variety of microorganisms. In the present study we compared the antimicrobial efficacy of aqueous extracts of leaf, bark and seeds of *A. Indica* against human pathogenic bacteria (*Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus fumigatus* and *Candida albicans*). Agar well diffusion method and micro-broth dilution methods were used to determine the minimum inhibitory concentration (MIC). Results showed that leaf extract exhibited strong antimicrobial activity against bacteria and fungi at all the concentrations tested (500, 1000 and 2000µg/ml). Antimicrobial activity of bark extract was found to be moderate on bacteria and fungi (effective at 1000 and 2000µg/ml), whereas seed extract exhibited least antimicrobial activity. Minimum inhibitory concentration (MIC) of leaf and bark extract was found to be in the range of 500 to 2000µg/ml for all the tested microorganisms, where as the seed extract did not inhibit the microorganisms at all the concentrations tested except *Candida albicans* (1000µg/ml). Our results suggest that aqueous extracts of *Azadirachta Indica* leaf and bark exhibit high antimicrobial activity.

Keywords: *Azadirachta Indica*; agar well diffusion method; antimicrobial; MIC; pathogenic microorganisms

INTRODUCTION

Drug resistance is a serious global problem, and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry. Use of herbal medicines in the developed world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs (De N et al 2002 and Ncube N S et al 2008). Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. These compounds after possible chemical manipulation provide new and improved drugs to treat the infectious diseases (Natarajan et al 2003, Shah et al 2006). Plant based products/ extracts are cheaper alternatives to the development of synthetic drugs.

*Azadirachta Indica* (*A. Indica*) belongs to the family Meliaceae, commonly known as neem. It is used in traditional medicine as a source of many therapeutic agents. *A. indica* (leaf, bark and seed) are known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B viruses (Biswas K et al 2002). Different parts of neem (leaf, bark and seed oil) have been shown to exhibit wide pharmacological activities including; antioxidant, antimalarial, antimitagenic, anticarcinogenic, anti-inflammatory, antihyperglycaemic, antiulcer and anti-diabetic properties (Talwar et al 1997). The biological activities are attributed to the presence of many bioactive compounds in different parts.

Antimicrobial activity has been investigated for neem leaves, bark and seed, but there are no studies on the comparative evaluation of aqueous extract of leaves, bark and seed. Hence, the current study was designed to investigate the comparative antimicrobial activity of neem leaves, bark and seed aqueous extract against human pathogenic bacteria and fungi. A number of factors such as, thickness and uniformity of the gel, size of the inoculum, temperature and pH that affect the accuracy and reproducibility of the agar diffusion method were also taken into consideration to obtain reliable results.

MATERIALS AND METHODS

Collection of raw materials and preparation of extracts

The leaves, bark and fruits of *Azadirachta Indica* were collected locally and authenticated by a botanist, Ayurveda pharmacy, Tirupati, Andhra Pradesh. Fruits were manually separated into their seeds and seed hulls (kernels) were milled prior to extraction. Raw materials (leaves, bark and seed) were cleaned, shade dried for one week and pulverized to coarse powder. Approx-
The bacterial strains were grown in nutrient slants were maintained at 4°C for 48 hours and fungal cultures at 25°C for 48 hours. Antimicrobial activity was determined by measuring the zone of inhibition surrounding the well. The assays were carried out under aseptic conditions. Ciprofloxacin (5μg/disc) and Amphotericin B (100μg/disc) were used as positive controls for bacteria and fungi respectively and DMSO as a negative control. Each concentration included duplicates and the results are average of two independent experiments.

**Determination of Minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration (MIC) of the aqueous extracts was determined by micro broth dilution method (Andrews JM 2001). For MIC, two-fold serial dilutions of the extracts were prepared (500, 1000 and 2000μg/ml) in microtitre wells. Incubation of the microtitre plates was carried out at 37°C for 24 hours and fungal cultures at 25°C for 48 hours. Antimicrobial activity was determined by measuring the zone of inhibition surrounding the well. The assays were carried out under aseptic conditions. Ciprofloxacin (5μg/disc) and Amphotericin B (100μg/disc) were used as positive controls for bacteria and fungi respectively and DMSO as a negative control. Each concentration included duplicates and the results are average of two independent experiments.

**RESULTS**

All test strains of bacteria were found to be sensitive to Ciprofloxacin and fungal strains were sensitive to Amphotericin B. DMSO was used as the negative control.
which did not show any zone of inhibition against tested bacteria and fungi.

Results of the agar well diffusion method are shown in table-1. Leaf extract exhibited antibacterial activity against all the tested bacteria at all concentrations, where as antifungal activity was observed only at 2000µg/ml.

The bark extract exhibited significant antimicrobial activity on *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterococcus faecalis* at all the concentrations tested, whereas its antimicrobial activity on *Staphylococcus aureus*, *Aspergillus fumigatus* and *Candida albicans* was observed at higher concentrations (>500µg/ml).

Seed extract did not show any antibacterial activity, but antifungal activity was observed at 1000 and 2000 µg/ml against *Candida albicans*. No activity was observed against *Aspergillus fumigatus* at any of the concentrations tested.

Minimum inhibitory concentration (MIC) was tested for the aqueous extract of leaves, bark and seed. Results are shown in the table-2. MIC for leaf and bark extract against bacteria was found to be at 500µg/ml and for fungi at 1000µg/ml concentration. MIC of seed extract for fungi was found to be at 1000µg/ml concentration, but there was no inhibition of bacteria at the tested concentrations.

**DISCUSSION**

Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new antimicrobial agents with improved safety and efficacy (Srivastava et al 2000). In this study, we have shown that the aqueous extracts of neem leaf exhibited highest antimicrobial activity compared with the bark and seed. The difference in the antimicrobial efficacy could be due to variable distribution of phytochemical compounds in different parts. Margolone, margolonone and isomargolonone are tricyclic diterpenoids isolated from stem bark are shown to exhibit antibacterial activity (Pennington et al 1981). Nimbidin and nimbolide from seed oil show antifungal, antimalarial and antibacterial activity including inhibition of *Mycobacterium tuberculosis* (Rojanpo et al 1985, Khalid et al 1989). However presence of high

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**REFERENCES**


**Table 2: MIC values of the *A. Indica* aqueous extracts of leaf, bark and seed**

<table>
<thead>
<tr>
<th>Name of the microorganism</th>
<th>Minimum inhibitory concentration (MIC) in µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>500</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>500</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>500</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>500</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>2000</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1000</td>
</tr>
</tbody>
</table>

ND- not detected


