Antimicrobial activity of *Azadirachta Indica* (neem) leaves and stem bark aqueous extracts

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Abstract

**Background:** *Azadirachta indica* is a multipurpose tree called as Neem which is used in Ayurvedic medicine for skin infections and household pesticide.

**Methods:** Aqueous extraction of plant material such as leaf and stem bark were investigated for anti-microbial activity without altering concentration.

**Results:** Results of study revealed that all extracts had inhibitory activity against *Staphylococcus aureus* with the clear zone of 2.6cm, which is higher than streptomycin control. The inhibition zone was found descending order against *Klebsiella*, *Psedumonas* and *Bacillus* species. Antifungal activity of bark extract of *Azadirachta indica* against *Aspergillus* and Yeast showed the inhibitory growth of 64.2% and 64% whereas the leaf extract showed less inhibition than bark extract.

**Conclusion:** The findings reveal that bark and leaf aqueous extracts have wide-ranging activity.

**Keywords:** *Azadirachta Indica*; Leaf and Stem Extracts; Antimicrobial Activity.

1. Introduction

Neem (*Azadirachta indica*) generally called ‘Margosa’, belongs to the family Meliaceae. It is the most adaptable, diverse trees of tropics with immense potential. It is a fast growing tree that extents a height of 15-20 metres but rarely to 35-40m. It is evergreen, but in severe drought it may shed most or nearly all its leaves. The branches are wide and spreading (Kausik, 2002). Various parts of the Neem such as leaves, bark, flowers, fruits, seed, gum and oil (Sonia & Srinivasan, 1999) have been used in Ayurvedic medicine in India and Sri Lanka. Neem oil, the bark and leaf extracts have been medicinally used for controlling leprosy, intestinal helminthiasis, respiratory disorders, skin infections, ulcers, itching and constipation and also as a general health promoter (Nat VD et al., 1987; Mahmood et al., 2010). Facts of herbal plants have been transferred from generation to generation for thousands of years to conserve food and treat health diseases. Treatment of diseases is interrelated with the antimicrobial agents in the plant sources (Mohashine et al., 1997). Neem leaves are used to control airborne bacterial contamination (Zahed Mohammadi, 2008; Saseed &Aslam, 2008; El mahmood et al., 2010) in the environment and seeds are used to control eye and ear infection (Behl et al., 2002). Neem flower actively involved in the oestrous cycle and act as infertility agent (Sinha et al., 1984; Botelho et al., 2008; Ghotolorum et al., 2008). At present medicinal plants have developed for the cure of different disease conditions, such as diabetes, malaria, anaemia and tumors (Chopra et al., 1958; Pillai & Santhirakumari, 1981; Fujiwara et al., 1982; Patil et al., 2013). Periodical screening of them may result in the detection of unique effective compounds (Tomoko et al., 2002). In past few eras, the interest to investigate plants having antimicrobial, antifungal, anti-inflammatory activity for various diseases and used as household pesticide due to the biologically active compounds in plants that are chemically diverse and structurally complex (Khan & Wassilew, 1987; Chattopadhyay , 1993; Ketkar et al., 1995; Chattopadhyay et al., 2005; Ahana, 2005; Kupeli et al., 2007; Bhowmik et al., 2010). The plant extracts were shown the effect of antidermatophytic, anti-inflammatory and hepatic diseases (Venugopal PV &TV, 1994; Majumdar et al., 1998). The therapeutic properties of *Azadirachta indica* were studied and found that can be used as anti-pyretic (Khattak et al., 1985; Okpanyi & Ezeukwk, 1987) and antioxidant activity (Bandyopadhyay et al., 2002). In this study, the antimicrobial activity of Neem plant bark and leaf aqueous extract were examined against bacterial and fungal species by using agar well diffusion method.
2. Materials and methods

2.1. Sample collection and extract preparation

Leaf and bark of A. indica was collected from the premises of University of Jaffna, Sri Lanka. For aqueous extract preparation, 2.5g of plant material was weighed and washed well in tap water. Then they were sterilized by giving a quick dip in alcohol and washed with sterilized water again. The weighed plant material was crushed with 10mL of sterile water and it was filtrated using Whatman’s filter paper No.1. The filtrate was collected in sterile beaker.

2.2. Anti-bacterial activity

The preliminary screening of antibacterial activity was done using well in agar method. Bacillus sp, Staphylococcus aureus, Klebsiella, E.coli and Pseudomonas bacteria were selected for this study. These bacteria were streaked on pure nutrient agar plates separately and stored in refrigerator at 10°C with labelling. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121°C for 15min). Then the petri dishes were used for the experiment. Peptone broth and agar standard solution were prepared and 100ppm streptomycin standard solution was used as positive control. The inoculum was spread in nutrient agar plates with bacterial strain and incubated at 37°C for 24 hours. Wells were prepared with UV sterile and wet heat sterile extracts, streptomycin solution and sterile water in agar plates for each bacterial species. The diameter of the clear inhibitory zone around the well was measured.

2.3. Anti-fungal activity

Candida sp, Aspergillus sp, Pencillium, Rhizopus fungus were selected for this study. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121°C for 15min). The sterilized extracts and Potato dextrose agar (PDA) media were mixed well and poured in petridishes. They were incubated at room temperature for 2 days. After it had grown enough, disc with the diameter of 7mm were cut using the sterile cork borer. The disc of each fungus was placed on the middle of the plate, which contain herbal product with PDA by using sterile loop. Control plates were also maintained without plant extract. These plates were incubated at room temperature for 2 days. Then the diameter of the clear inhibitory zone around the well was measured.

3. Results and discussion

3.1. Anti-bacterial activity

The extract of Azadirachta indica found to be effective against the pathogenic bacteria. The inhibition zone is shown in the following graphs has exposed the power against pathogenic bacteria.
Uwimbabazi Francine et al., (2015) stated that aqueous and ethanol extracts of fresh and dried *Azadirachta indica* has an antibacterial effect on *Staphylococcus aureus*. The similar study was done by Gajendrasinh et al., (2012) exposed that *E.coli* was the most susceptible bacterium to aqueous and ethanol extracts of Neem and fresh and dry bark ethanol extracts were effective against *S.aureus* compared to water extracts. Ramadass and Subramaniam (2018) stated that chloroform extract of Neem showed maximum inhibition zone against *Staphylococcus aureus* (2.6 cm) in 75µg/mL and 2.4cm zone of inhibition was recorded against *E.coli*. The aqueous bark extract of Neem in this study showed high inhibition zone against *S. aureus* (2.5cm) and generally bark extract showed higher antibacterial activity than leaf extract of *Azadirachta indica*.

Antimicrobial study done by Ranjit et al., 2014 leaf and bark extract of *Azadirachta indica* showed more inhibition zone against *Vibrio cholerae* (1.7cm) and *Bacillus subtilis* (1.7cm), while *E. coli* (1.1cm) and *S. typhi* (1.0cm) are less susceptible to Neem extract. Methanol and ethanol Extract shows maximum inhibition on *Bacillus pumillus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an ascending order (Maragathavalli et al., 2012). In case of *A. indica* the leaf extract failed to show a wide range of inhibitory action. It produced clear zone against *Bacillus sp 1* and *Staphylococcus aureus* only, *E.coli*, *Bacillus sp 2*, *Pseudomonas* and *Klebsiella* showed resistance to the leaf extract. But the bark extract showed inhibitory action against all bacteria. Among them the diameter of clear zone produced by *Bacillus sp 1* is smaller than that of streptomycin. In all other cases, the area of inhibition is larger than that of streptomycin.

### 3.2. Anti-fungal activity

#### Table 1: The Diameter of the Mycelial Disc in cm and % of growth reduction of samples obtained from *A. Indica* under UV Sterilization

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Diameter of inhibitory zone (cm)</th>
<th>Control (without plant extract)</th>
<th>% of growth reduction in leaf extract</th>
<th>% of growth reduction in bark extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>2.3</td>
<td>2.9</td>
<td>20.68</td>
<td>41</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>8</td>
<td>4.5</td>
<td>15.78</td>
<td>50</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>2.6</td>
<td>4</td>
<td>35</td>
<td>55</td>
</tr>
<tr>
<td>Pencillium</td>
<td>1.4</td>
<td>1.7</td>
<td>17.6</td>
<td>29.41</td>
</tr>
</tbody>
</table>

#### Table 2: The Diameter of the Mycelial Disc in cm and % of growth reduction of samples obtained from *A. Indica* under Wet Heat Sterilization

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Diameter of inhibitory zone (cm)</th>
<th>Control (without plant extract)</th>
<th>% of growth reduction in leaf extract</th>
<th>% of growth reduction in bark extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>2.2</td>
<td>2.9</td>
<td>24.13</td>
<td>64</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>7.3</td>
<td>9.5</td>
<td>23.15</td>
<td>22</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>2.3</td>
<td>4</td>
<td>42.5</td>
<td>64.2</td>
</tr>
<tr>
<td>Pencillium</td>
<td>1.2</td>
<td>1.9</td>
<td>29.41</td>
<td>36.8</td>
</tr>
</tbody>
</table>

Leaf and stem bark extract of *Azadirachta indica* showed anti – fungal activity against all selected fungi. Yeast was inhibited by the UV sterilized leaf extract by 20.68% while by the stem bark extract by 41%. Rhizopus was inhibited 15.78% by the leaf extract and 50% by the stem bark extract. In the case of Aspergillus stem bark proved more efficient than leaf extract. Penicillium showed lowest inhibition as 17.6% in leaf extract and 29.41% in stem bark extract. The effect of stem bark is approximately same on both UV sterilized and wet.
heat sterilized extracts. But the wet heat sterilized leaf extract of A.indica showed a considerable difference from UV sterilized leaf extract. The wet heat sterilized leaf extract inhibits the growth of yeast by 24.13%. Rhizopus by 23.15%. Aspergillus by 42.5% and Penicilium by 29.41%. The phyto constituents alkaloids, glycosides, flavonoids and saponins have antibiotic properties which has defensive mechanism against different pathogens (Faiza et al., 2009; Hassan Amer et al., 2010).

4. Conclusion

The present study indicated that the aqueous extract of Azadirachta indica may have potential use in medicine. The current investigation concluded that, aqueous extract of Neem plant have antimicrobial properties where bark extract has higher inhibition rate than leaf extracts. Neem extracts have higher anti-fungal properties compared to anti-bacterial activity among selected pathogens. The synergistic effect of plants extracts against pathogens will lead for the treatment of diseases in folk medicine.

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References


