Antibacterial Efficacy of Acetone Soluble Oil of *Azadirachta indica* on Some Bacterial Strains

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**Abstract**

Medicinal and pesticidal properties of neem (*Azadirachta indica*) have been described for more than 4,000 years. In the present study, antibacterial properties of *Azadirachta indica* seed oil have been investigated against *Escherichia coli*, *Bacillus subtilis*, *Rhizobium mellitii* and *Pseudomonas aeruginosa* by disc diffusion method. At 100% concentration, acetone soluble oil possessed the highest antibacterial activity causing 18 mm zone of inhibition against *E. coli* and 17 mm against *P. aeruginosa* as compared to ciprofloxacin used as a positive control. The 1 inhibition zone showed a significant difference across all the bacteria (p<0.01) and concentrations (p<0.001). This study focuses the use of neem oil as an alternative to ciprofloxacin.

**Keywords**: *Azadirachta indica*; Antibacterial; Acetone soluble oil; Inhibition zone; Antibiotic

**Introduction**

Neem (*Azadirachta indica* A. Juss), belonging to the family Meliaceae, is a tropical evergreen tree plant related to mahogany. In India, neem tree is treated as sacred and is popularly known as the ‘village pharmacy’ because of its healing versatility. As well known that different parts of the neem plant including leaves, flowers, fruits, seeds, roots, and bark have been reported for their valuable. Neem is considered as one of the most promising trees of the 21st century for its great potential in pest management, environment protection, and medicine [1]. Neem oil is the most important product with a great market worldwide. It is rich in bioactive compounds such as steroids, sugars, terpenoids, alkaloids, reducing sugars, tannins, flavonoids, sesquiterpene lactones and phenolic compounds [2,3], which are responsible for the antibacterial properties of almost all parts of this magic tree [4-8]. Moreover, the methanolic, acetonic, and aqueous extracts of neem leaves inhibited the growth of *Escherichia coli* (E. coli), *Micrococcus luteus* (M. luteus), *Enterobacter*, *Bacillus subtilis* (B. subtilis), *Pseudomonas aeruginosa* (P. aeruginosa), *Klebsiella pneumonia* (K. pneumonia), *Streptococcus pyogenes* (S. pyogenes), and *Staphylococcus aureus* (S. aureus) [3,9,10]. Nimbidin, extracted from the oil of seed kernels of *A. indica* oil demonstrated several biological activities. Some tetrnortriterpenes, including nimbin, nimbinin, nimbidinin, nimboide, gedunin, azadirachtin, mahmoodin and nimbidic acid have been isolated from this crude product. Nimbidin and sodium nimbidate possess anti-inflammatory [11], antipyretic activity [12], hypoglycaemic [13] antiagastic ulcer [14,15] antiarthritic [16], Antimalarial, antibacterial [17], antifungal [18], diuretic [19], acetylalicylic acid, indomethacin, stress or serotonin-induced gastric lesions as well as histamine or cytosamine-induced duodenal ulcers [13-16]. Moreover, no much study has been done on *A. indica* oil against bacterial strains. Therefore, the present study was focused on the evaluation of *A. indica* oil against four selected bacterial strains viz., *E. coli*, *B. subtilis*, *Rhizobium mellitii* (R. mellitii) and *P. aeruginosa*.

**Materials and Methods**

**Bacterial strains**

Four bacterial strains viz., *E. coli* - ATCC – 25922, *P. aeruginosa* - ATCC – 27853, *B. subtilis* - ATCC – 23857 and *R. mellitii* - ATCC – 9930 were procured from the Laboratory of the Departmental of Botany and Microbiology, Gurukula Kangri Vishvvidyalaya and stored on nutrient agar slants at 4°C for further study.

**Antibiotics used**

Ciprofloxacin antibiotic was used as standard for positive control. Ciprofloxacin is available in tablet form as Floxip (200 mg) manufactured by Max India Limited.

**Inoculum preparation**

A young colony of each bacterium was inoculated from pre-prepared nutrient agar slant culture into 5 mL nutrient broth and incubated at 37°C for 4 - 6 hour.

**Preparation of disc containing different concentrations on neem oil:** Inhibitory property of neem oil was tested by filter paper (Whatman No.1) disc diffusion method [20]. Whatman filter paper discs (5 mm diameter) were prepared with the help of a punching machine. These discs were dipped in each concentration of neem oil, control and ciprofloxacin.

**Preparation of seeded agar plates:** Distilled water (5 mL) was added to inoculation tube and shaken thoroughly into get better bacterial suspension. Suspensions (0.2 mL) of *E. coli*, *B. subtilis*, *R. mellitii* and *P. aeruginosa*, were separately added in the flasks containing 200 mL of (NAM) and maintained at 45°C to 50°C.

**Transfer of seeded nutrient agar in sterilized Petri plates:** The inoculated medium (20 mL) was poured in Petri plates (six for each
bacterium) with the help of sterilized pipette. Discs having different concentrations of oil were placed on the surface of NAM towards the periphery of the Petri plates along with the control disc in centre. The discs were placed in such a manner that none of the inhibition zone appearing may coalesce. The Petri plates were left open aseptically for 40-45 minutes to evaporate the organic solvents. The plates were incubated at 37°C for 24 hours and the diameter of inhibition zone was measured. The activity of respective solvents was subtracted from the total zone of inhibition. Growth inhibition (I) of bacteria was calculated by using the formula:

\[ I = T - C \]

Where \( T \) = Diameter of total inhibition zone in treatment.

\( C \) = Diameter of inhibition zone in control.

**Statistical Analysis**

Statistical analysis was performed using the computing environment R (R Development Core Team) [21].

**Results**

Neem oil showed antibacterial activity against *E. coli*, *B. subtilis*, *R. meliloti* and *P. aeruginosa*. The maximum inhibition of the bacterial growth was recorded at 100% concentration and minimum at 50% concentration of neem oil. At 100% concentration the oil exhibited the maximum inhibition against *E. coli* (18 mm), followed by *B. subtilis* (17.9 mm), *P. aeruginosa* (17 mm) and *R. meliloti* (17.75 mm) (Table 1 and Figure 1).

However, 10.0 mm (*E. coli*), 9.0 mm (*B. subtilis*), 9.3 mm (*P. aeruginosa*) and 9.5 mm (*R. meliloti*) zone of inhibition were recorded at 75%, while 7.0 mm, 6.2 mm, 5.0 mm and 5.9 mm at 50% concentration. At 25% concentration of neem oil showed no zone of inhibition against all four bacterial strains. There was no inhibition zone was observed in control discs. The inhibition zone exhibited by ciprofloxacin and neem oil showed negligible variations against all the four est bacterial strains. The maximum zone of inhibition was recorded against *E. coli* (28 mm), followed by *B. subtilis* (27 mm), *R. meliloti* (25 mm) and *P. aeruginosa* (23 mm) (Table 1 and Figure 1).

**Discussion**

Uses of herbal drugs have been in practice since ancient times. These drugs are very effective in curing diseases caused by human pathogens. Neem oil is one of the herbal medicinal plants which possess antimicrobial properties. The results obtained from the experiments reveal the presence of potential antibacterial property of neem oil against *E. coli*, *B. subtilis*, *R. meliloti* and *P. aeruginosa* causing
the maximum inhibition zone against *E. coli* (18 mm) and minimum against *P. aeruginosa* (17 mm) at 100% concentration. All the bacteria exhibited more or less similar zone of inhibition at 100% concentration. However, the inhibitory effect of the oil increased with an increase in the concentration for each test organisms ($R^2=0.929$, $p<0.01$), the effective inhibition zone produced by ciprofloxacin was always found more than that of neem oil at 100% concentration. Jessinta et al. [22] reported that the maximum zone of inhibition shown by the *A. indica* oil towards *E. coli* was 11.7 mm at the 100 $\mu$L/mL while, 18 mm zone of inhibition was observed at 100% concentration in our investigation (Figure 2). The zone of inhibition is also increased s with the increase in oil concentration. The antimicrobial activity of neem oil may be explained due to the presence of certain bioactive compound such as nimbidin, nimbin, azadirachtin and mahmoodin which also shows anti fungal, anti inflammatory, antiarthritic, antipyretic, hypoglycaemic, anti gastric ulcer, spermicidal, diuretic, antimalarial and antibacterial properties [23].

**Conclusion**

It may be concluded that acetone soluble neem oil exhibits the growth inhibition of all bacterial strains because of presence of bioactive compound containing. Hence, plant based compounds could be useful in meeting the demand of drug development due to less side effects. Therefore, it can be used for the treatment of several harmful infectious diseases caused by resistant microorganisms.

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**Conflict of Interest**

The authors confirm that this article content has no conflicts of interest.

**References**