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

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Abstract

Medicinal and pesticidal properties of neem (*Aadirachta indica*) have been described for more than 4,000 years. In the present study, antibacterial properties of *Aadirachta indica* seed oil have been investigated against *Escherichia coli, Bacillus subtilus, Rizobium meliloti 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 1inhibition 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, triterpinoids, 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), Streptcoccus pyogens (S. pyogens), 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 tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide, gedunin, azadirachtin, mahmoodin and nimbidic acid have been isolated from this crude principle. Nimbidin and sodium nimbidate possess anti-inflammatory [11] antipyretic activity [12], hypoglycaemic [13] antigastric ulcer [14,15] antiarthritic [16], Antimalarial, antibacterial [17], antifungal [18], diuretic [19], acetylsalicylic acid, indomethacin,stress or serotonin-induced gastric lesions as well as histamine or cysteamine-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 meliloti (R. meliloti) 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. meliloti* - ATCC – 9930 were procured from the Laboratory of the Departmental of Botany and Microbiology, Gurukula Kangri Vishvidyalaya 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 dilution of *A. indica* oil: *A. indica* oil, procured from a village near Muzzaffar Nagar district (Uttar Pradesh), was tested for antibacterial activity against four selected bacterial strains. Four concentrations of *A. indica* oil viz. 25%, 50%, 75% and 100% used along with control were prepared by dispensing the desired amount of oil in culture tubes containing desired amount of acetone under aseptic conditions.

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. subtilus, R. meliloti and P. aeroginosa,* 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

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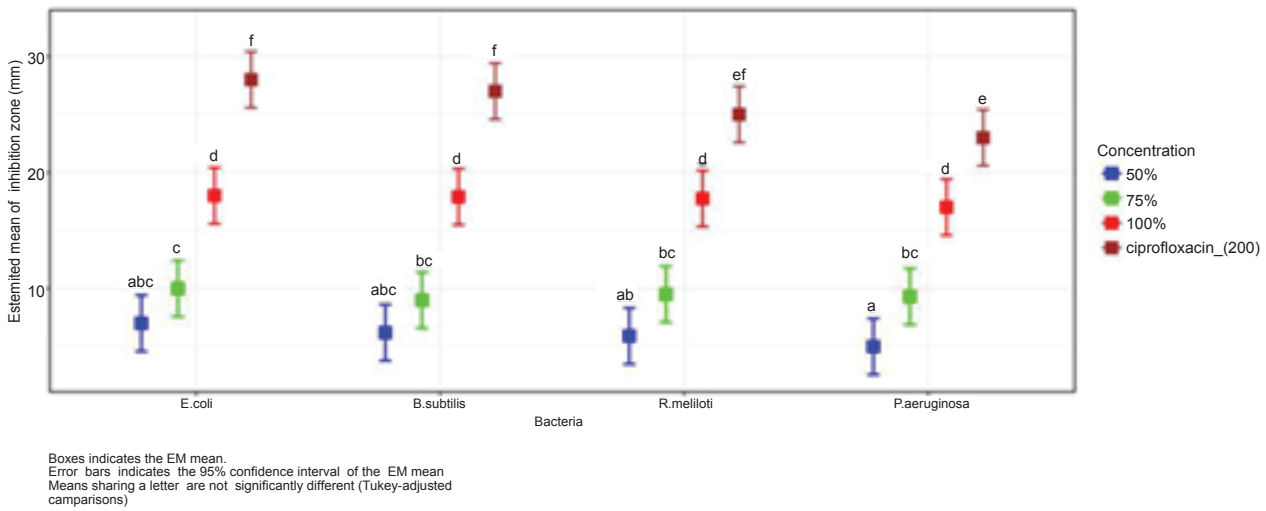

Figure 1: Effect of acetonic concentration of *A. indica* oil and ciprofloxacin on selected bacterial strains.

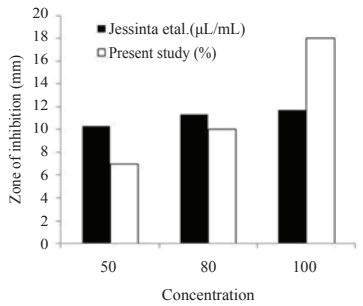

Figure 2: Comparative study of zone of inhibition caused by neem oil against *E. coli.*

Extracts (%)	Zone of inhibition (mm)* on selected bacterial strains			
Neem oil	*E. coli*	*B. subtilis*	*P. aeruginosa*	*R. meliloti*
100	18.0 ± 0.84	17.9 ± 0.86	17.0 ± 0.91	17.75 ± 0.95
75	10.0 ± 0.85	9.0 ± 0.99	9.3 ± 0.84	9.5 ± 0.78
50	7.0 ± 0.87	6.2 ± 0.95	5.0 ± 0.98	5.9 ± 0.92
25	-	-	-	-
Ciprofloxacin	*E. coli*	*B. subtilis*	*P. aeruginosa*	*R. meliloti*
	28.0 ± 0.79	27.0 ± 0.81	23.0 ± 0.94	25.0 ± 0.96

*Mean values ± Standard deviation
- = No zone of inhibition

Table 1: Anti-bacterial activity of neem oil and ciprofloxacin on selected bacterial strains.

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. subtilus*, *R. meliloti* and *P. aeruginosa*. The maximum inhibition of the bacterial growth was recorded at 100% concentration and mimimum at 50% concentration of neem oil. At 100% concentration the oil exhibited the maximum inhibition against *E. coli* (18 mm), followed by *B. subtilus* (17.90 mm), *P. aeroginosa* (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. subtilus* (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. aeroginosa (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 increases 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 µL/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 antifungal, anti-inflammatory,antiarthritic,antipyretic, hypoglycaemic, antigastric 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.

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