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## Neem seed extract may be used as topical ointment to treat wound infections caused by *Pseudomonas aeruginosa*

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### Abstract

*Pseudomonas aeruginosa* is an important nosocomial pathogen, usually resistant to many antibiotics. This study was aimed to determine bactericidal effect of the extract of 'Neem Seeds' (*Azadirachta indica*) on *Pseudomonas aeruginosa* isolated from wound infection patients.

In this study, 60 strains of *Pseudomonas aeruginosa* isolated from patients suffering from wound infections.

Undiluted extract of neem seeds obtained after grinding & 106 CFU/ml was performed and the surviving bacteria were detected by culture on PIA (Pseudomonas Isolation Agar).

Minimum Inhibitory Concentration was determined by agar dilution method (10% to 50% concentration of neem seeds extract in 20 ml of molten nutrient agar by inoculation of 104 CFU /spot on MHA.

Out of 60 strains of *Pseudomonas aeruginosa*, 36 (60%) were killed after 1hr exposure to neem seeds extract, 12(20%) were killed after 2 hrs exposure, 8(13.34%) were killed after 4 hrs & remaining 4(6.66%) were killed after 8 hrs exposure respectively. All *Pseudomonas* strains were killed by extract of the neem seeds.

On agar dilution, 51 (85%) of strains were inhibited at 10% concentration of neem seeds extract, 7 (11.67%) at 20% and remaining 2(3.33%) were inhibited at 30% concentration of the extract. The results of the above study clearly indicated that direct extract obtained from grinding the neem seeds, has bactericidal activity on *Pseudomonas aeruginosa*.

**Keywords:** Neem Seeds Extract; Bactericidal activity; Topical Ointment; *Pseudomonas aeruginosa*.

### 1. Introduction

*Pseudomonas aeruginosa* is an important pathogen due to its virulence factors, types of diseases caused, and multi-drug resistance.

Recent increase of multi-drug resistant strains started to pose great difficulty in selecting antimicrobial agents for the management of the infections they cause in large hospitals.

Cephalosporins and other beta-lactam antibiotics have been shown to be clinically ineffective even though certain vitro tests such as 'Kirby-Bauer Disk Diffusion' test would suggest that the strains are susceptible. Heterogeneous resistance to the beta-lactam antibiotics and cephalosporins is also responsible for the problems encountered in performing proper AST. Resistance to cephalosporins, fluoroquinolones and aminoglycosides, has also been reported with these strains<sup>[1]</sup>.

Literature review provided very few studies attempting this aspect of neem seeds extract. Hence, this study was performed to determine the bactericidal effect of neem seeds extract on *Pseudomonas aeruginosa* and the use of agar based dilution method to determine the inhibition of the bacterial inoculum by neem seeds extract<sup>[2]</sup>.

### 2. Materials and Methods

**2.1 Agar Dilution Method<sup>[6]</sup>:** A series of plates were prepared in triplicate starting from 10% to 50% concentration of neem seeds extract in 20 ml of molten Mueller-Hinton Agar [0.2% (w/v)]. To prepare 10% concentration, 2 ml (one part) of the neem seeds extract was added to 18 ml (nine parts) of molten MHA, for 20% concentration, 4 ml of the neem seeds extract was added to 16 ml of molten MHA and so on upto 50% concentration. After homogenization in a water bath having temperature of 45<sup>0</sup> to 50<sup>0</sup> C the agar solution was mixed thoroughly and poured immediately into petri plates maintaining a level of agar depth of about 3-4 mm.

Then, the plates were solidified at room temperature and kept in the refrigerator at 2-8<sup>0</sup> C. One negative control plate (without neem seeds extract) and one positive control plate with 0.5 McFarland turbidity standard of bacterial inoculum (ATCC 27853) were prepared for each dilution<sup>[5]</sup>.

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**2.2** <sup>[4]</sup> **Bacterial Inoculum Preparation:** A standardized inoculum was prepared in nutrient broth for the agar dilution method by growing *Pseudomonas aeruginosa* to the turbidity of the 0.5 McFarland standard. Cultures adjusted to the 0.5 McFarland standard contain approximately  $1.5 \times 10^8$  CFU/ml. This was diluted to achieve a concentration of  $4-5 \times 10^6$  CFU mL (0.75 ml 0.5 Mc Farland added to 25 ml water diluent, 1:33 dilution). An aliquot of diluted inoculum (0.01 mL) was applied to the surface of agar medium with standardized loop with the final inoculum of 104 CFU per spot of 5 to 8 mm in diameter. First growth control plate was inoculated at the beginning and second at the end to ensure there was no contamination or significant antimicrobial carryover during the inoculation. A sample of inoculum was plated on Nutrient agar plate and incubated overnight to detect mixed cultures and to provide freshly isolated colonies in case of retesting.

**2.3 Koch's Plating Technique** <sup>[4]</sup>: The inoculum was streaked on MHA by 'Koch's Plating Technique' i.e. preparation of a well with a sterile plastic disposable loop, then a primary streak from the well, a secondary streak from the primary one and so on up to tertiary streaking ending in a tail end <sup>[4]</sup>.

### 3. Result

Growth of *Pseudomonas aeruginosa* colonies were observed up to tertiary streak in case of 10% concentration, secondary streak in case of 20% concentration, primary streak in case of 30% concentration and on the well in case of 40% concentration of neem seeds extract. There was 'No Growth' observed in case of 50% concentration of neem seeds extract <sup>[2]</sup>.

### 4. Discussion

This study showed 100% susceptibility of *Pseudomonas aeruginosa* to neem seeds extract at varying concentrations.

According to CLSI guidelines two fold serial dilutions of antimicrobial agent (particularly herbal extracts) starting from 2% (v/v) to 0.01% (v/v) is recommended.

So, future studies with lower concentrations of may prove helpful in determining MIC of neem seeds extracts. The initial data appears to prove the antibacterial effect of neem seeds extract. Hence, it may be used as a topical ointment as an alternate to expensive, harmful antibiotics against *Pseudomonas aeruginosa* <sup>[3]</sup>.

However, long term studies with larger number of samples are necessary to study the toxicity in order to set an appropriate formulation for clinical use <sup>[8]</sup>.

### 5. Conclusion

The result of this study clearly indicates that neem seeds extract has antibacterial effect against MDR *Pseudomonas aeruginosa* and thereby reducing the harmful side effects of chemically synthesized antimicrobial agents. Since neem seeds extract exhibited bactericidal effect on *Pseudomonas aeruginosa*, it may find clinical application as topical antibacterial agent <sup>[7]</sup>.

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