
BACTERICIDAL EFFECT OF CRUDE NEEM (*AZADIRACHTA INDICA*) LEAF EXTRACT ON PATHOGENIC BACTERIA

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Abstract: Under the limitations of this study, it was concluded that neem leaf extract has a significant antimicrobial effect against various pathogenic bacteria. Microbial inhibition potential of neem leaf extract observed in this study opens perspective for its use in treating various infections. There is possible potentiating of antibacterial effects of some antibiotics against different bacterial infection when co administered with neem extract. Extensive research and standardization of techniques and controlled predetermined combinations of neem extract and these antibiotics could find clinical applications in the treatment of bacterial infections and in prevention of resistance in bacteria. However, preclinical and clinical trials are needed to evaluate biocompatibility and safety before neem can be conclusively recommended as irrigating solution, but *in vitro* observation of neem effectiveness appears promising.

As the global scenario is now changing towards the use of non toxic plant products that have traditional medicinal use, extensive research and development work therefore should be undertaken on neem and its products for their better economic and therapeutic utilization.

Keywords: Neem (*Azadirachta indica*), antimicrobial, bactericidal.

Introduction: “NO ACTION TODAY NO CURE TOMORROW”

The discovery and development of antimicrobials has been hailed as one of the most important advances in the history of modern medicines reducing the suffering from diseases and saving lives. However these “miracle drugs” are losing their efficacy due to the emergence of antimicrobial resistance and emergence of newer multidrug resistance in pathogenic bacteria. This has forced medical science to search for new antimicrobial substances from various sources like medicinal plants.

According to WHO more than 80% of the world's population relies on traditional medicines for their primary health care needs with therapeutic benefits and relatively cheaper and safer. Therefore scientific investigation of medicinal plant, most commonly on *Azadirachta indica* popularly known as neem has been initiated. Neem has been extensively used in Ayurveda, unani and homeopathic medicines and has become a cynosure of compounds. All parts of neem tree such as leaves, flowers, seeds, fruits, bark and roots are used for treatment of various diseases.

The present study was carried using the crude neem leaf extract for studying its bactericidal effect on various pathogenic bacteria isolated from different clinical specimens. Such study can furnish information on the use of medicinal plants.

Materials and Methods: Crude aqueous and alcoholic neem leaf extract were prepared as per the standard guidelines.

Use as a surface disinfectant: A surface intentionally contaminated with 24 hr broth culture of pathogenic bacteria like Methicillin Resistant

Staphylococcus aureus (MRSA), *Pseudomonas* sp, *Klebsiella* and *E.coli* was covered with neem leaf extract. And the efficacy of neem leaf extract was checked by culturing the swabs taken from the contaminated surface treated with neem leaf extract at different intervals of time with half hour intervals.

To study synergistic effect of neem leaf extract with different antibiotics: Nutrient agar plates with two different concentrations of neem leaf extract i.e 0.1g/ml and 0.5g/ml were prepared and the test organism was layered over it. Antibiotics effective for that particular test strain were placed and the results were noted for increased zone of inhibition to show synergism.

To determine the MIC of neem leaf extract for effective bactericidal activity: Serial dilutions of aqueous and alcoholic neem leaf extract were prepared as per the standard guidelines. 10 μ l broth culture of the test organism was added to each tube and incubated aerobically overnight and the minimum inhibitory concentration was determined by subculturing the stock tubes with test organism on nutrient agar to see visible growth. The concentration of neem leaf extract which did not show any growth was considered as the minimum inhibitory concentration.

To observe the bactericidal effect of neem leaf extract on bacteria inhabiting the hands of health care workers: The hands of health care workers like laboratory technicians were exposed to neem leaf extract and swabs were taken at different time intervals to see the bactericidal activity of neem leaf extract on the flora inhabiting the hands of health care workers.

To observe the anti-inflammatory effect of crude neem leaf extract: Crude neem leaf extract was applied on the acne and crude neem leaf extract gargles were done by a HCW with upper respiratory tract infection. The results were observed and noted.

To compare the efficacy of crude aqueous and crude alcoholic neem leaf extract with surface and hand disinfectants i.e sodium hypochlorite and chlorhexidine respectively: The nutrient agar plate was swabbed with test organism and 4 wells were cut with the help of sterile blade. The first well was filled with alcoholic neem leaf extract, 2nd with aqueous neem leaf extract, 3rd with chlorhexidine disinfectant and 4th with sodium hypochlorite solution. Plates were incubated overnight and the zone diameters were measured.

Results: The present study revealed that all the organisms except *Pseudomonas aeruginosa* which took approximately 60 minutes to be killed by crude neem leaf extract. *Pseudomonas aeruginosa* was killed after approximately 90 minutes of exposure to crude neem leaf extract (Figure -1). Present study proved a good synergistic activity of neem leaf extract with 0.5g/ml concentration with aminoglycosides like gentamycin, amikacin and flouroquinolones like levofloxacin and ciprofloxacin which commonly prescribed drugs against all the test organisms. Also the neem leaf extract showed synergism with chloramphenicol, ceftazidime and cefoparazone+sulbactam. Best synergistic activity was found against *E. coli*. (Table-1). This experiment proved that the minimum inhibitory concentration for mostly all the organisms ranged from 1:4 to 1:8 dilutions per ml (10^{-2} to 10^{-3} dilution). (Table-2).

An exposure of approximately 30mins of neem leaf extract to hands of HCWs was found to be effective for disinfecting hands of HCWs. This experiment proves the potential use of neem leaf extract as disinfecting agent which can be used in form of soap solutions.

This experiment showed a very good anti-inflammatory activity of neem leaf extract when applied on face wherein the inflammation settled within a day of exposure to neem leaf extract also gargling with the aqueous neem leaf extract help in decreasing the inflammation in the upper respiratory tract due to infection. This property of neem extract is being used in cosmetic industry. This experiment proved that alcoholic neem leaf extract had a better efficacy as compared to aqueous neem leaf extract. Also the zone sizes were compared with chlorhexidine and sodium hypochloride solution, wherein the alcoholic neem leaf extract showed nearly equal zones of inhibition against *Acinetobacter*

and MRSA.

Discussion: The indiscriminate use of synthetic drugs has led to emergence of multiple drug resistance among pathogenic bacteria particularly responsible for causing hospital acquired infection. This drives the need to screen medicinal plants for their novel bioactive compound as plant based drugs are safe and have fewer side effects and are comparatively cheaper (Kaushik et al 2002). This study was a sincere effort to prove the bactericidal activity of neem leaf extract. As the results indicate experiment no 1, 2 & 4 showed significant antibacterial activity against all the test organisms in the form of log reduction (Maragathavalli et al 2012). *Pseudomonas* being highly resistant bacteria was resistant to neem leaf extract also as the time taken to kill *pseudomonas* sp was the maximum. Similar results were seen in other studies (Margathavalli et al 2012). The use of neem extract as surface disinfectant has been studied by many researchers and even our studied proved the potent activity of neem extract against MRSA (Saba Irshad et al. 2011). MIC results tested also were correlating with other studies (Raja Ratna Reddy et al., 2013).

Although sodium hypochlorite and chlorhexidine showed comparatively more antimicrobial effect than both aqueous and alcoholic neem leaf extract but still had an observable effectiveness against test organisms (Vibha Hegde et al., 2013). Good synergistic activity was found with many antibiotics maximum being found with aminoglycosides and flouroquinolones which are commonly used drugs for treating infections caused by pathogenic bacteria (Hemraj et al., 2012).

Conclusion: Under the limitations of this study, it was concluded that neem leaf extract has a significant antimicrobial effect against various pathogenic bacteria. Microbial inhibition potential of neem leaf extract observed in this study opens perspective for its use in treating various infections.

There is possible potentiating of antibacterial effects of some antibiotics against different bacterial infection when co administered with neem extract. Extensive research and standardization of techniques and controlled predetermined combinations of neem extract and these antibiotics could find clinical applications in the treatment of bacterial infections and in prevention of resistance in bacterias.

However, preclinical and clinical trials are needed to evaluate biocompatibility and safety before neem can be conclusively recommended as irrigating solution, but invitro observation of neem effectiveness appears promising.

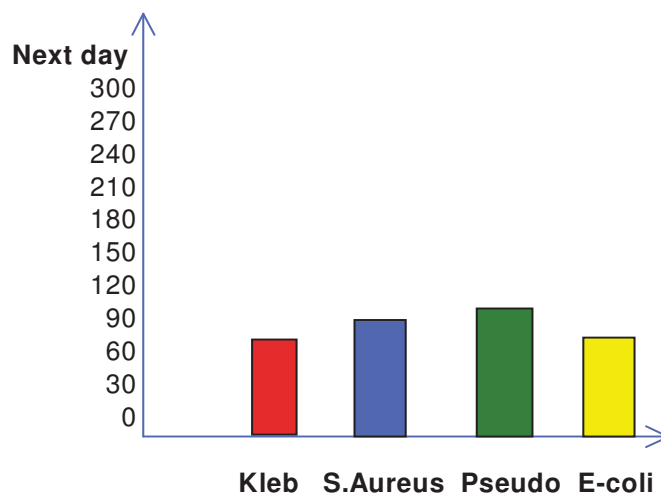
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use of non toxic plant products that have traditional medicinal use, extensive research and development work therefore should be undertaken on neem and its

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Graph1:Show time verses growth of microorganism
 Graph 1 indicates the minimum time taken by different pathogenic bacterias to be killed by the crude neem leaf extract.

TABLE-1: Zone of inhibition with neem powder and antibiotic to show synergism for *E. coli*.

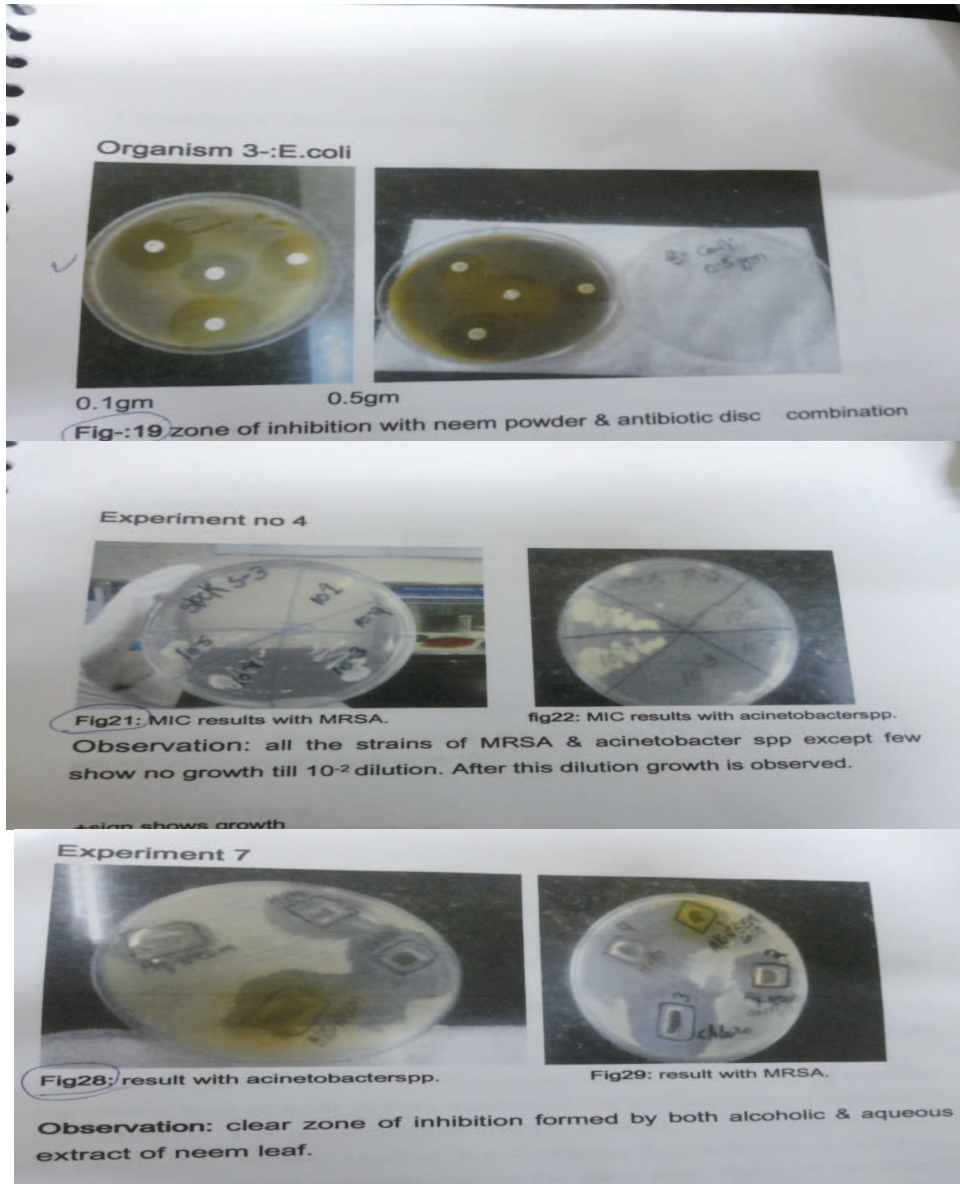
Name of Antibiotic	Diameter of Inhibition zone (in mm)	Neem extract + antibiotic	
		0.1gm/ml	0.5mg/ml
Amikacin	15-16	20	17-18
Levofloxacin	14-16	22	23
Cefoparazone + sulbactum	15-19	20	23
chloramphenicol	18	21	23

Stock = original concentrated solution without dilution.

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TABLE-2: MIC of neem extract for MRSA strains.										
Dilution	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀
Stock	-	-	-	-	-	-	-	-	-	-
10 ⁻¹	-	-	-	-	-	-	-	-	-	-
10 ⁻²	-	-	-	-	-	-	-	-	-	-
10 ⁻³	+	+	+	+	-	+	+	-	+	+
10 ⁻⁴	+	+	+	+	+	+	+	+	+	+
10 ⁻⁵	+	+	+	+	+	+	+	+	+	+

+ = growth, - =no growth
 S = Methicillin-resistant *Staphylococcus aureus* (MRSA)



Figures showing synergistic effect with different antibiotics, MIC and comparative zone sizes of neem extract with sodium hypochlorite and chlorhexidine disinfectants.

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