



ANTIMICROBIAL ACTIVITY OF NUTMEG AND FLAX SEED FORMULATION MEDIATED ZINC OXIDE NANOPARTICLES AGAINST PSEUDOMONAS AERUGINOSA

Running Title: Antimicrobial activity of nutmeg and flax seed mediated nanoparticles

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ABSTRACT:

Background: Nanotechnology has provided great possibilities in various fields of science and technology. The aim of this study was to assess the antimicrobial activity of zinc oxide nanoparticles synthesized using nutmeg and flax seed formulation against *Pseudomonas aeruginosa*.

Materials and Methods: Nutmeg and flax seed formulation was prepared and mixed with zinc oxide for zinc oxide nanoparticles formation, and it is centrifuged. It was then placed in the well cultured agar plates against *Pseudomonas aeruginosa*, and incubated for 24 hours. The zone of inhibition was then calculated.

Results: Against *Pseudomonas aeruginosa*, 25 μ L showed 9mm of zone of inhibition, 50 μ L showed 10mm of zone of inhibition, 100 μ L showed 15mm of zone of inhibition and 15mm of zone of inhibition against antibiotic.

Conclusion: The present study suggests that nutmeg and flax seed formulation mediated zinc oxide nanoparticles showed potent antimicrobial effect against *Pseudomonas aeruginosa*. **Keywords:** Antimicrobial, Flax seed, Nanotechnology, Nanoparticles, Nutmeg

INTRODUCTION:

Nanotechnology research has advanced considerably in recent years. It is a multidisciplinary field of science that coordinates the study of physical, natural, and social sciences.(1) By carefully controlling the size and condition of the particles, nanomaterials are created at the nanoscale. Nanostructures of a few metal oxides are being developed because to the unique and significant physical, as well as synthetic, features of nanomaterials.(2) Nanoparticles are useful, multipurpose, eco-friendly, and resistant to extreme environmental conditions. Because of the size, shape, and construction of nanoparticles affect their properties, it is possible to obtain application-based nanoparticles with the desired morphology. Metal nanoparticles have attracted a lot of attention in various branches of innovation and technology.(3)

Zinc oxide (ZnO) is an inorganic compound, which appears as a white powder that is nearly insoluble in water. Zinc oxide nanoparticles have been studied for their various uses in a variety of industries, including medicine.(4) It has been shown that zinc oxide exhibits antibacterial activity, similar to various other metal oxide clusters. However, unlike the others, only a small number of them have been reduced to the nano size and are currently being studied, such as ZnO nanoparticles. The advantage of using inorganic oxides as antibacterial specialists is that they contain mineral components essential for people and demonstrate effective activity in any case, when regulated in small levels. ZnO nanoparticles provide effective antibacterial actions against a wide variety of microbes.(5)

Plant extracts are widely being used in the pharmaceutical industries as they are naturally available and fewer side effects. Some spices are well known for their anti inflammatory and antioxidant activity along with chemo protective activity.(6-13) *Myristica fragrans*, also known as nutmeg, is a member of the *Myristicaceae* family. Nutmeg is reported to have great pharmacological activity. Oil of nutmeg has already being used for medicinal

purposes from ancient time. It is basically used in the form of spice so as to enhance the taste of several dishes such as tea and milk and alcohols. Traditionally, the nutmeg is used as stomach pain relief, stimulant, carminative along with all this they also possesses antipapillomagenic, anticarcinogenic, and anti-inflammatory activities.(14,15)

Flaxseed oil is a vital source of alpha-linolenic acid and some effective phytochemicals such as lignin, phenolic acids, and flavonoids . It offers numerous benefits, including as antioxidant, anti-inflammatory, and anti-atherosclerotic qualities. Due to the antioxidant properties of flaxseed oil, which include phenols and vitamin E, the antioxidant system has been demonstrated to work better when it is used.(16,17)

The rationale of this study was to assess the antimicrobial activity of zinc oxide nanoparticles reinforced with nutmeg and flax seeds against *Pseudomonas aeruginosa*.

MATERIALS AND METHODS:

Preparation of nutmeg and flax seed extract

Nutmeg and flax seeds were purchased and were powdered using a mixer grinder. 5 mg of nutmeg and 5 mg of flax seeds powder was dissolved in 100 mL of distilled water. The solution was boiled in a heating mantle at 60 °C for 10 minutes until the bubbles appeared. The solution was then filtered using a funnel and Whatman filter paper and collected in a conical flask to obtain the plant extract. Then the plant extract was transferred to an airtight container and refrigerated overnight.

Synthesis of zinc oxide (ZnO) nanoparticles using nutmeg and flax seed extract

0.1 g of zinc oxide was added to 100 ml of the filtered extract and was placed in the orbital shaker. Colour change of the solution was noted every 2 hours. Readings were recorded every 2 hours in UV Spectrophotometer and after around 36 hours, centrifugation was done at 7000 rpm for 10 minutes. The synthesis of nanoparticles was done in a darker niche. The synthesized nanoparticle solution was preliminarily characterized using UV Spectroscopy; 3 ml of the solution was taken in cuvette and scanned in double beam in UV Spectrophotometer from 200 nm to 600 nm wavelength. The results were recorded. The final reaction mixture was centrifuged using Lark refrigerated centrifuge at 800 rpm for 10 minutes. The final mixture of nutmeg and flax seeds extract mediated ZnO nanoparticle was concentrated to 100 ml. Then collected and stored in an air-tight Eppendorf tube.

Antimicrobial activity

Agar well diffusion method was used to determine the antimicrobial activity of nutmeg and flax seed extract mediated zinc oxide nanoparticles. Different concentrations of the extract mediated zinc oxide nanoparticles were tested against *Pseudomonas aeruginosa*. Different concentrations of nutmeg and flax seed extract mediated zinc oxide nanoparticles (25 µL, 50 µL, 100 µL) were incorporated into the prepared wells and the plates were incubated

at 37 °C for 24 hours to study its effect. Antibiotics (Amoxicillin) was used as positive control against *Pseudomonas aeruginosa* and their zone of inhibition was recorded.

RESULTS:

Zone of inhibition using different concentration of nutmeg and flax seed extract mediated zinc oxide nanoparticles shows antimicrobial activity against *Pseudomonas aeruginosa*. Against *Pseudomonas aeruginosa*, 25µL showed 9mm of zone of inhibition, 50µL showed 10mm of zone of inhibition, 100µL showed 15mm of zone of inhibition and 15mm of zone of inhibition against antibiotic. (Table 1)

Concentration (µL)	Zone of inhibition (mm)
	<i>Pseudomonas aeruginosa</i>
25	9
50	10
100	15
Antibiotic	15

Table 1: Zone of inhibition using different concentrations of nutmeg and flaxseed formulation mediated zinc oxide nanoparticles against *Pseudomonas aeruginosa*

DISCUSSION:

The present study demonstrated that nutmeg and flax seed extract mediated zinc oxide nanoparticles showed antimicrobial activity similar to the tested conventional drug against *Pseudomonas aeruginosa*.

The alkaloid from the fruit of the nutmeg showed antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) while the alkaloid compound was showed antifungal effect against *Candida albicans* and *Cryptococcus neoformans*. The isolated alkaloid compound was not showed any cytotoxicity of human blood solution. From this result, it was concluded that the alkaloid isolated from *Myristica fragrans* can be used in treatment of various disorders because of the effectiveness of the compound against bacteria and fungi.(18)

Also, ethanol and acetone extract of *M. fragrans* was assessed for its antibacterial efficacy against *Escherichia coli*, *Bacillus subtilis*, *Bacillus megaterium*, and *Proteus mirabilis*. The study results demonstrated that *M. fragrans* seed extract exhibited significant antibacterial activity against selected microorganisms.(19) In addition, antibacterial effectiveness test of nutmeg showed potent effect against *Shigella sp.* and *Clostridium sp.*(20)

In another study, flaxseed freeze dry extract was tested against *Streptococcus mutans*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* using agar-well diffusion method and compared their antibacterial activities with the antibiotics (Streptomycin) as a positive control and dimethyl sulfoxide as a negative control. Flaxseed extract effectiveness was tested using zone of inhibition. This study has shown that flaxseed extract has antibacterial effect towards selective oral pathogens comparable to streptomycin.(21) Based on the findings of the study, it can be concluded that nutmeg and flax seed formulation mediated zinc oxide nanoparticles with has a synergistic effect and can be used as an alternative to commercially available antimicrobial agents.

CONCLUSION:

The present study suggests that nutmeg and flax seed formulation mediated zinc oxide nanoparticles showed potent antimicrobial effect against *Pseudomonas aeruginosa*.

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