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RESEARCH ARTICLE

Green Synthesis of Copper Nanoparticles Using Nutmeg Oleoresin and Its Antimicrobial Activity Against Oral Pathogens.

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ABSTRACT

Background: Nanotechnology has a major impact in a variety of applications in the biomedical, engineering, chemical, medicinal and food industries. Nanotechnology is employed to treat microbial infections. Nanotechnology is seen as an emerging scientific field in many aspects, and has a great impact on human daily life with various applications. Nutmeg oleoresin is used as a flavouring agent for foods. Nutmeg oleoresin has strong antimicrobial activity with less cytotoxic activity.

Aim: The aim of the study was to analyse the antimicrobial activity of copper nanoparticles synthesized using nutmeg oleoresin

Materials and Methods: The nutmeg oleoresin mediated copper nanoparticles was synthesized and confirmed by UV visible spectroscopy and antimicrobial activity was tested aganist staphylococcus aureus, Enterococcus faecalis and Streptococcus mutans and Candida albicans, by agar well diffusion method using respective media.

Results: Nutmeg oleoresin mediated copper nanoparticles showed good results for antimicrobial activity. Among the four organisms, Candida albicans (fungal organism) showed more zones of inhibition when compared to Staphylococcus aureus, Streptococcus mutans and Enterococcus faecalis (bacterial organisms).

Conclusion: Antimicrobial activity of copper nanoparticles prepared using nutmeg oleoresin is exhibited with significant antimicrobial activity with minimal side effects. Antimicrobial activity of copper nanoparticles showed a maximum zone of inhibition at 100µl.The sensitivity Candida albicans > Staphylococcus aureus> Streptococcus mutans > Enterococcus faecalis.

KEYWORDS:
Nutmeg oleoresin; Innovative technology; Innovative technique; Antimicrobial activity; Green synthesis; Copper Nanoparticles; Oral pathogens.

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INTRODUCTION

Nanoscience is a modern interdisciplinary science that has recently emerged. Nanoscience is characterised as a complete knowledge of nano size objects basic properties (1). Nanotechnology is employed to treat microbial infections. Nanotechnology has recently been widely accepted in nanoparticles as clusters of atoms in the 1-100nm size range (2). Nanotechnology is described as engineering and science elaborate in the synthesis, design and application of materials and characterisation of the materials (3). Current research of nanoparticles also deals with the bio labelling, cancer therapeutics, biosensors etc… Biological methods of preparation of nanoparticles are considered as the safest one(4). Now-a-days nanoparticles are considered as the greatest commercialised one and also have a lot of applications in catalysis(5).

The use of plant extract phytochemicals has become a unique Nps synthesis technology, as they have a dual nature of reduction and capping agents to the nanoparticles (6). Nutmeg oleoresin is the natural product extracted from the dried seeds of Myristica fragrans Houttyn of family myristicaceae (7) Oleoresin has a wonderful aroma and also a warm taste sensation (7). Oleoresin are natural mixtures of essential oils and resin that are responsible for the complete flavour profile of species that can be extracted from the plants with (or) without an auxiliary solvent such as ethanol, using organic solvents (or) CO2 extraction of supercritical fluids (8). Oleoresin volatile oil produces the aroma. There are some advances over the dry powder and also help for taste as well as having a minimal microbial load (9).

Copper with a nuclear number 29, which is a chemical element. In nature, it is a smooth, soft and bendable material that also has high thermal and electrical conductivity (6). Copper nanoparticles are more affordable. Silver, gold and platinum (11). Copper nanoparticles have physical, chemical and optical catalytic properties(10). Currently most of the methods used to prepare the copper nanoparticles are sono chemical reduction, thermal reduction and capping agent, etc…(11). Antimicrobial activity is used to kill the microorganisms (or) stop their growth. Nutmeg oleoresin has further properties like anti-inflammatory, cytotoxic and antioxidant properties (7). Previously, our team had conducted numerous studies using plant extract, essential oil and oleoresins directly or after preparing different nanoparticles (12)- (13). Our team has extensive knowledge and research experience that has translated into high quality publications(14–18)(19).The aim of this study was to analyse the antimicrobial activity of copper nanoparticles synthesized using nutmeg oleoresin.

MATERIALS AND METHODS

Study setting

The study was conducted in the Nanomedicine laboratory, Saveetha dental college and hospitals, chennai, India. Before the study, ethical approval was obtained from the ethical approval committee with ethical approval number IHEC/SDC/UG-1966/21/124.

Plant material

The Nutmeg oleoresin was collected from synthite industries Pvt Limited, kerala, with a product code: 4010000484. The preparation was done by solvent extraction of the dried seeds. It contains volatile oil in a composition about 40-48 ml/100g. The initial stock solution was 1mg/ml.

Preparation of plant extract

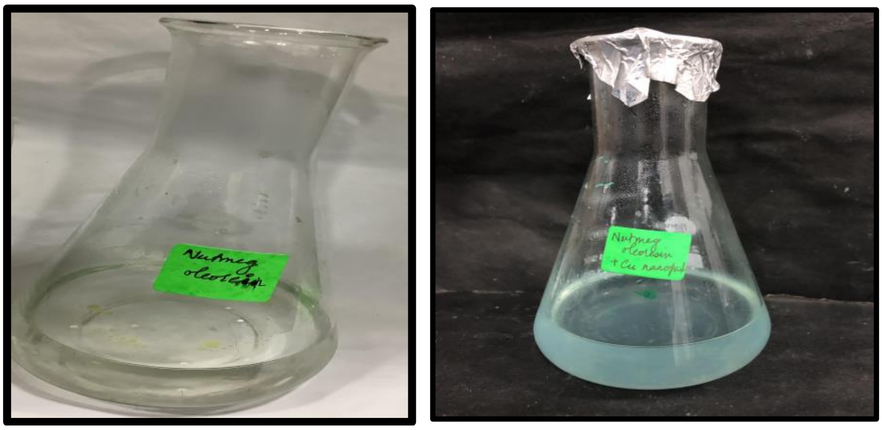
0.2 ml of nutmeg oleoresin was mixed with 100 ml of distilled water and dissolved using a heating mantle with the temperature about 50-60 degree Celsius for 5-10 minutes. The extract was stored in the beaker and covered with foil and used for biosynthesis of nanoparticles.

Synthesis of copper nanoparticles

20 millimolar of anhydrous copper sulphate was initially prepared by dissolving 0.477 in distilled water. 80 ml of this was mixed with 20 ml of prepared nutmeg oleoresin solution. The mixture was kept in an orbital shaker for the formation of copper nanoparticles and colour change was observed visually and confirmed further by using UV- visible spectroscopy . There was a color change from dark blue to light blue. The UV spectroscopy revealed an excitation wavelength of 280 nm of the surface plasmon resonance band.The absorbance was noted at regular intervals and a graph was plotted (Figure 1,Figure 2).

Antimicrobial activity

The nutmeg oleoresin mediated copper nanoparticle was analysed fortis antimicrobial activity against pathogenic bacterial organism like staphylococcus aureus, Candida albicans, Enterococcus faecalis and Streptococcus mutans by agar well diffusion method . Muller Hinton Agar plates were used for the study using standard procedure (6,11). 25µl, 50µl and 100µl of the prepared nanoparticles were placed on the three wells on all plates using sterile micropipette. After incubation at 37 degree Celsius for 24 hours, the zone of inhibition was measured and graph was plotted (Figure 3)



A B

Fig.1: Preparation of Nutmeg oleoresin mediated copper nanoparticles extract. (A) Plant extract. (B) The solution of Nutmeg oleoresin and Copper sulphate.

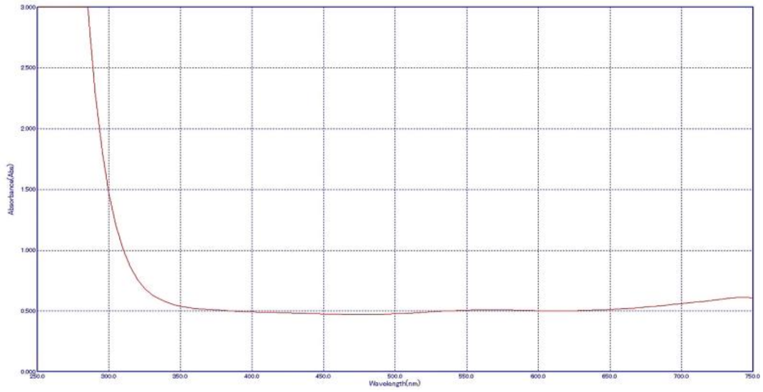


Fig.2: This figure shows a broad and shoulder peak at 280 nm(At 48hours.) X- axis represents wavelength in nm and Y- axis represents absorbance.

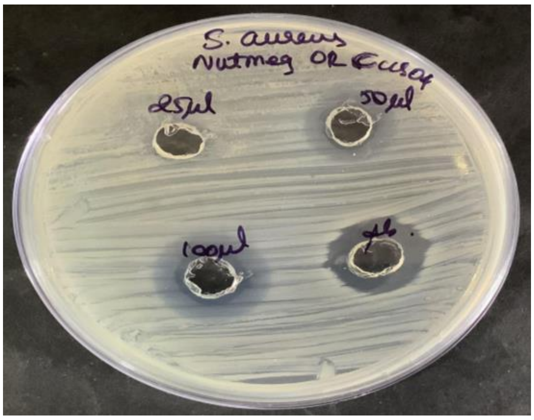
RESULTS

Antimicrobial activity of copper nanoparticles

The antimicrobial activity of copper nanoparticles that was synthesized with the nutmeg oleoresin, by agar well diffusion method against oral pathogens showed a zone of inhibition at different concentrations. The antimicrobial activity of copper nanoparticles was analysed based on their zone of inhibition and the results were compared with standard antimicrobial agents called amoxicillin. Among the four organisms, Candida albicans (fungal organism) showed more zones of inhibition when compared to Staphylococcus aureus, Streptococcus mutans and Enterococcus faecalis (bacterial organisms).

The antimicrobial activity of copper nanoparticles against S. aureus showed a zone of inhibition of 10mm at the concentration of 25µl. The antimicrobial activity of copper nanoparticles against S. aureus showed a zone of inhibition of 17mm at the concentration of 50µl. The antimicrobial activity of copper nanoparticles against S. aureus showed a zone of inhibition of 20mm at the concentration of 100µl. The antimicrobial activity of copper nanoparticles against C. albicans showed a zone of inhibition of 25mm at the concentration of 25µl. The antimicrobial activity of copper nanoparticles against C. albicans showed a zone of inhibition of 30mm at the concentration of 50µl. The antimicrobial activity of copper nanoparticles against C. albicans copper nanoparticles against S. mutans showed a zone of inhibition of 19mm at the showed a zone of inhibition of 35mm at the concentration of 100µl. The antimicrobial activity of concentration of 25µl. The antimicrobial activity of copper nanoparticles against S. mutans showed a zone of inhibition of 20mm at the concentration of 50µl. The antimicrobial activity of copper nanoparticles against C. albicans showed a zone of inhibition of 22mm at the concentration of 100µl. The antimicrobial activity of copper nanoparticles against E. faecalis showed a zone of inhibition of 12mm at the concentration of 25µl. The antimicrobial activity of copper nanoparticles against E. faecalis showed a zone of inhibition of 12mm at the concentration of 50µl. The antimicrobial activity of copper nanoparticles against E. faecalis showed a zone of inhibition of 15mm at the concentration of 100µl (Figure 3).

(A)



(B)

(C)

(D)

Fig.3: Antimicrobial activity of Nutmeg oleoresin mediated copper nanoparticles on (A) Candida albicans (B) Staphylococcus aureus (C) Streptococcus mutans. (D) Enterococcus Faecalis.

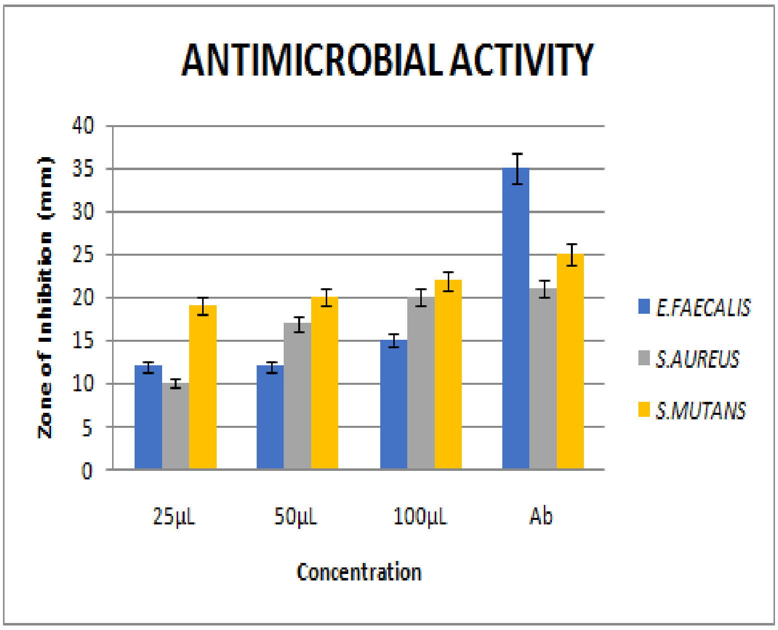


Fig.4: This figure represents the antimicrobial activity of copper nanoparticles mediated nutmeg oleoresin. X axis refers to concentration in µl and Y axis refers to the zone of inhibition of bacterias in mm. Blue colour denotes zone of inhibition of E. faecalis, grey colour denotes zone of inhibition of S. aureus and yellow denotes zone of inhibition of S. mutans. The Zone of inhibition of S. aureus was comparatively closer to the antibiotic(ab) than E.faecalis and S. mutans. (n=3) mean+SD.

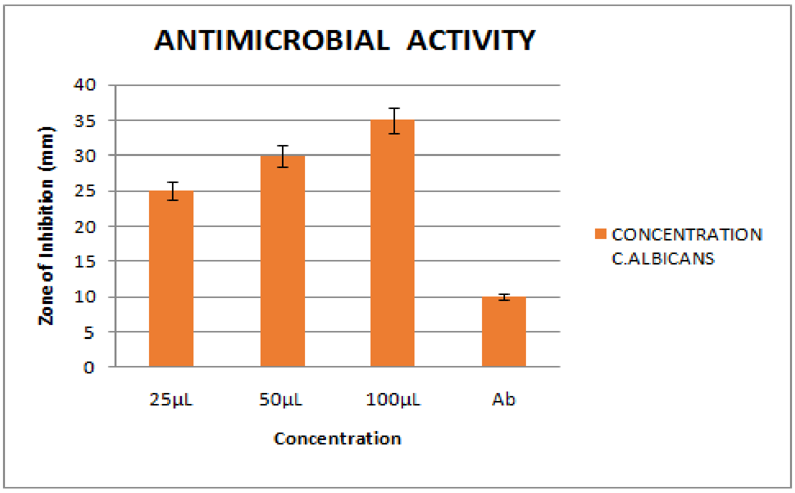


Fig.5: This figure represents the antimicrobial activity of the copper nanoparticles mediated nutmeg oleoresin. X -axis refers to concentration in µl and Y- axis refers to the zone of inhibition of bacterias in mm. Orange denotes the zone of inhibition of C. albicans. Candida albicans shows a higher zone of inhibition when compared to the antibiotic (ab). (n=1) mean+SD.

DISCUSSION

The antimicrobial activity of Nutmeg oleoresin mediated copper nanoparticles against Candida albicans, Staphylococcus aureus, Streptococcus mutans and Enterococcus Faecalis showed in the graph(Figure 4). The results revealed that nutmeg oleoresin showed the best antimicrobial activity in Candida albicans in Figure 5. The antimicrobial activity of nutmeg oleoresin mediated copper nanoparticles for other bacterial organisms is summarized in (Figure 3). Zone of inhibition of Candida albicans, Staphylococcus aureus, Streptococcus mutans and Enterococcus faecalis was summarized in (Table 1). As the concentration increases the zone of inhibition also increases. From this, study proved that bacterial and fungal species are sensitive to Nutmeg oleoresin.The sensitivity of bacterial and fungal species of Candida albicans > Staphylococcus aureus> Streptococcus mutans > Enterococcus faecalis.

The present study showed good antimicrobial activity for Candida albicans at 100µl concentrations coinciding with Vignesh et al., previously the author concluded that cumin oil mediated silver nanoparticles showed a good zone of inhibition(20). Present showed 35 mm of zone of inhibition at 100µl supported by the Previous study done by garmaseva et al. found that 15 mm of the inhibition zone with higher concentration induced silver nanoparticles from cinnamon extract have shown strong antimicrobial activity(21). Previously the study done by Shivani et al., showed the highest zone of inhibition at 100µl for Streptococcus mutans proved that excellent antimicrobial activity for zinc nanoparticles (8). The study done by Sathvika et al., concluded that silver nanoparticles synthesized using neem and aloevera showed an effective antibacterial activity against streptococcus mutans supports the present study(22).

Zangeneh et al. published a study which aimed to synthesize copper nanoparticles from Falcaria vulgaris' aquatic extract and evaluate their cytotoxicity, antioxidant, antifungal, antibacterial and dermal cures(23). Neha etal., concluded that a remarkable number of antioxidant, antifungal, antibacterial, and cutaneous wound healing potentials with a lower cytotoxicity have been recorded for Aspergillus niger (24). Malaka, et al. found that the silver nanoparticle synthesis with a Cosmos sulphureus aqueous extract displayed an improved activity of antimicrobial agents(25). Dhar, et al, findings showed that silver nanoparticles extracted from Phyllanthus emblica proved to be an effective reducing agent(26). Paramasivam et al, findings have shown that silver nanoparticles synthesized from Azima tetracantha leaf extracts have shown good bacterial activities against the five human disease species Escherichia coli, Seratia sp., Klebsiella sp., Pseudomonas sp., Staphylococcus sp (27). Rajeshkumar et al. have been shown to be an important antimicrobial agent for Staphylococcus aureus, Streptococcus mutans and Pseudomonas species by silver nanoparticles synthesized from oleoresin of the rosemary (28). Previously our team has conducted numerous studies based on nanotechnology(29), (30), (31), (32), (33), (34), (35), (36), (37), (38), (39), (40), (40,41), (42), (42,43), (44), (45)

The limitations of the study was that it included only a few microorganisms. In the future , the study can be extended with more microorganisms, excluding those that have been studied in this research(46) (47) (48) (49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) (60). The future scope for this study can lead to the development of commercial products of various nanoformulations: mouthwash, toothpaste, oral gels, etc that are safe, effective, and are economical.

CONCLUSION

According to the results of our study, we concluded that Nutmeg oleoresin mediated copper nanoparticles showed a good range of zones of inhibition and possessed excellent antimicrobial activity, especially against the C. albicans. It is eco-friendly, effective, simple and powerful against multi-drug resistant bacteria (61)-(62)).Copper nanoparticles can thus be used for traditional antibiotics as a non-toxic substitute.

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CONFLICT OF INTEREST

All authors declare no conflict of interest in the study.

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