Assessment of Antimicrobial Efficacy of Zinc Oxide Nanoparticles Synthesized Using Clove and Cinnamon Formulation against Oral Pathogens - An In Vitro Study

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ABSTRACT

BACKGROUND

Zinc Oxide nanoparticle is of particular interest among researchers due to its wide range of applications. Green synthesis of nanoparticles has many benefits like being eco-friendly, less time consuming, cost effective, stable operation, and more importantly the process can be carried out without the involvement of any hazardous chemicals. Clove and cinnamon are known to have antimicrobial activity. Hence, this study was conducted to assess the antimicrobial efficacy of zinc oxide nanoparticles reinforced with clove and cinnamon against oral pathogens.

METHODS

This is an in vitro study. The organisms used were *Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis*. These bacteria were inoculated in their respective medium and incubated overnight. Agar well diffusion method was used to assess the antimicrobial efficacy of the nanoparticles at 25 µL, 50 µL and 100 µL.

RESULTS

Zone of inhibition was found to be highest at 100 µL against *Streptococcus mutans*, *Staphylococcus aureus*, and *Enterococcus faecalis* (15 mm, 13 mm, and 13 mm respectively).

CONCLUSIONS

Findings from this study suggest that zinc oxide nanoparticles reinforced with clove and cinnamon extracts has the potential as an antimicrobial agent against *Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis* and can be used as an alternative to commercially available antimicrobial agents.

KEY WORDS

Zinc Oxide Nanoparticles, Clove, Cinnamon, Anti-Microbial, Oral Pathogens

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BACKGROUND

There has been a rapid evolution of nanoparticle synthesis recently as compared to the early part of the century.[1] Earlier, physio-chemical methods were involved in nanoparticle synthesis. Even though less time is utilized for synthesizing large quantities of nanoparticles using conventional physical and chemical methods, toxic chemicals are required as capping agents to maintain stability, thus leading to toxicity in the environment.[2] Keeping this in consideration, green nanotechnology using plants is emerging as an eco-friendly alternative, as plant extract mediated biosynthesis of nanoparticles is cost-effective.[2] The high surface area to volume ratio of nanoparticle is a notable distinctive feature which enables excellent molecular interaction characteristics with its smaller size, distribution and morphology.[3] Green synthesis of nanoparticles has many benefits like eco-friendly, less time consuming, cost effective, stable operation and more importantly the process can be carried out without the involvement of any hazardous chemicals.[4-6]

Among the various inorganic nanoparticles available, Zinc Oxide (ZnO) has easy processing methods, is inexpensive, has wide range of applications and is a safe material. Due to these properties, Zinc Oxide pulls a particular interest among researchers. Zinc Oxide plays a vital role in the daily life and has the third highest global production volume only after Selenium Oxide (SiO_2) and Titanium Oxide (TiO_2) among the safest metal to use [7,8]. Recent studies have shown that Zinc Oxide nanoparticles are potentially toxic to bacteria but exhibit minimal effects on human cells.[9,10] The bio-compatibility of the Zinc Oxide nanoparticles is due to its particle size and shape, aspect ratio and morphology.[11]

Zinc Oxide nanoparticles are eco-friendly and have biodegradability properties. Zinc Oxide nanoparticles have a large surface volume ratio which facilitates better dissolution and penetration of the bacteria,[12,13] thus having prominent antimicrobial activities. Zinc Oxide nanoparticles antibacterial efficiency is mainly from the nanoparticles as compared to the release of free Zn^{2+} ions which is very low.[14] The antimicrobial property of Zinc Oxide nanoparticles has been proved in many studies.[15-17]

Syzygium aromaticum commonly known as clove, is a median size tree (8-12 m) belonging to the *Mirtaceae* family native of Maluku islands in east Indonesia.[18] For many years the trade of clove has caused the economic development of this region.[18]-Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material.[19] The antimicrobial activities of clove have been proved against several bacterial and fungal strains.[20,21] Sofia et al. tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove.[22] The only sampled that showed complete bactericidal effect against all the pathogens tested Escherichia coli, *Staphylococcus aureus* and Bacillus cereus was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action.

Cinnamon is a spice obtained from the inner bark of several tree species from the genus Cinnamomum [23]. Cinnamon has many health benefits like-it is loaded with antioxidant, has inti-inflammatory properties, may reduce the risk of heart disease, lowers blood sugar level, protective against cancer, and anti-microbial properties. The anti-microbial activity of cinnamon is due to its constituents-cinnamaldehyde (4.3%) and eugenol (0.32%).[23]

The rationale of this study is that no study has been conducted so far in which the antimicrobial properties of Zinc Oxide nanoparticles reinforced with clove and cinnamon have been assessed. Hence the aim of the study was to assess the antimicrobial efficacy of Zinc Oxide nanoparticles reinforced with clove and cinnamon against oral pathogens.

METHODS

Study Design
In vitro study.

Study Organisms
The test organisms used in this study were obtained from the Culture Collections of the Nanobiomedicine Laboratory, Saveetha Dental College, Chennai. The organisms used in this study were *Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis*.

Preparation of Plant Extract
Clove buds and cinnamon bark sticks were purchased from the market and were powdered using a mixer grinder. 500 mg of clove powder and 500 mg of cinnamon powder were dissolved in 100 mL of distilled water. The solution was boiled in a heating mantle at 60 ^{o}C for 10 minutes until the bubbles appeared. The solution was filtered using a funnel and a 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 Nanoparticles Using Clove and Cinnamon Extract
20 mM of Zinc Sulphate powder was prepared using 60 mL of distilled water and mixed thoroughly. 40 mL of the plant extract was added to this solution and was placed in the orbital shaker. Colour change of the solution was noted every 2 h. Readings were recorded every 2 h in U V Spectrophotometer and after around 36 h, centrifugation was done at 7000 rpm for 10 minutes. Zinc Oxide nanoparticles pellets reinforced with clove and cinnamon were obtained after centrifugation.

Antimicrobial Activity
Agar Well Diffusion Method was used to assess the antimicrobial efficacy.

Media Preparation
100 mL of Mueller Hinton agar for *Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis* and was prepared, sterilized and poured onto the Petri plates. The plates were allowed for solidification.

Swabbing

After solidification, the respective plates was swabbed with the oral pathogens-*Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis*.

Well Formation

After swabbing, three wells on each plates were formed using a gel puncher. To those three wells, Zinc Oxide nanoparticle with clove and cinnamon solution was loaded in the concentration range of 25 µL, 50 µL and 100 µL. The plates were then incubated at 37°C for 24 hrs. and after incubation, the zone of inhibition was measured and calculated.

Measurement of Zone of Inhibition

The test plates were held in front of a desk lamp, and the zones were measured with a ruler held against the back of the petri plate. The diameters of the zones of inhibited growth were measured to the nearest whole millimeter.

RESULTS

Figure 1 depicts the antimicrobial activity of Zinc oxide nanoparticles reinforced with clove and cinnamon extract against *Streptococcus mutans*. Zone of inhibition was found to be highest at concentration of 100 µL (15 mm). Figure 2 depicts the antimicrobial activity of Zinc oxide nanoparticles reinforced with clove and cinnamon extract against *Staphylococcus aureus*. Zone of inhibition was found to be highest at concentration of 100 µL (13 mm). Figure 3 depicts the antimicrobial activity of Zinc oxide nanoparticles reinforced with clove and cinnamon extract against *Enterococcus faecalis*. Zone of inhibition was found to be highest at concentration of 100 µL (13 mm).

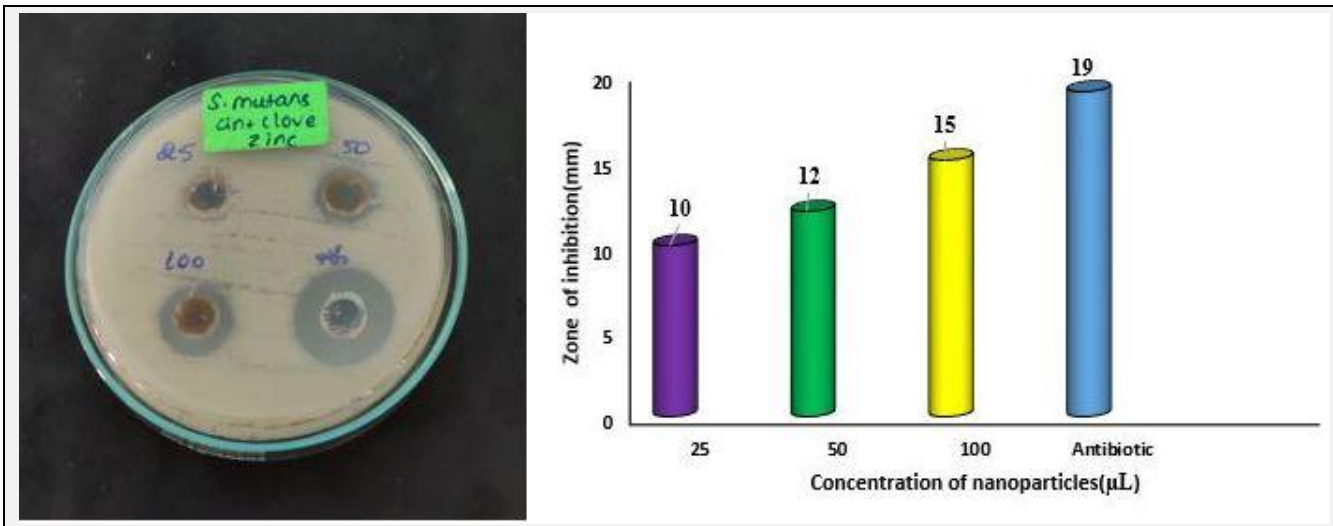

Figure 1. Antimicrobial Activity of Zinc Oxide Nanoparticles Reinforced with Clove and Cinnamon Extract against Streptococcus mutans

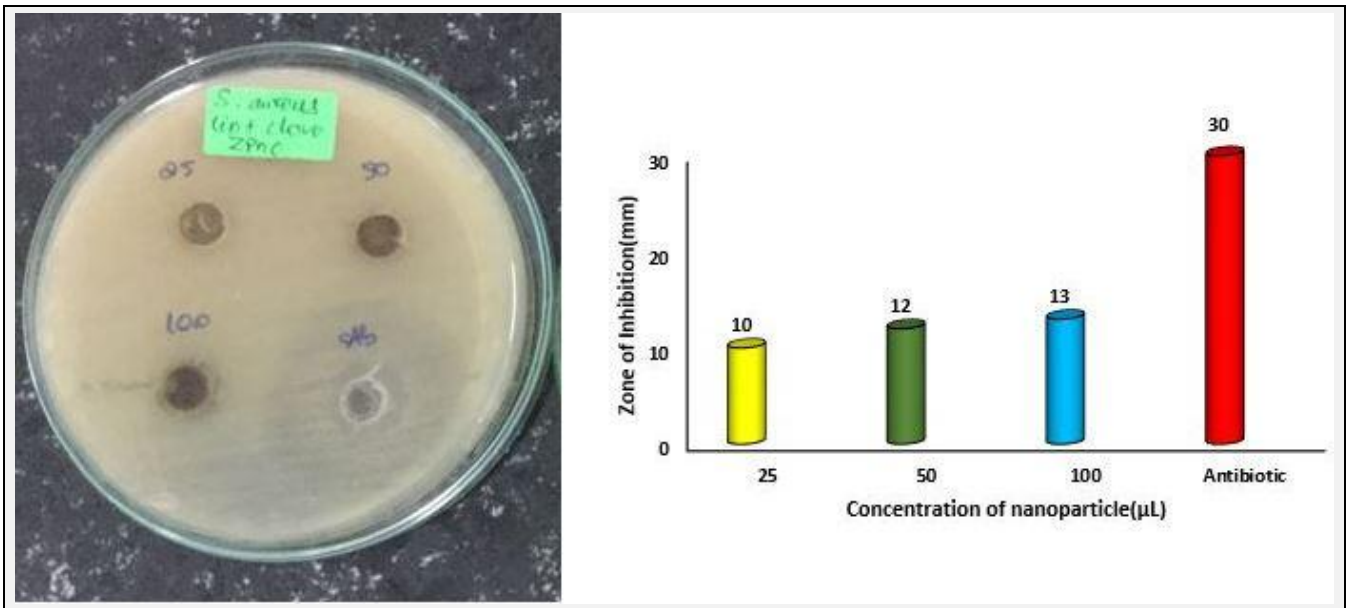

Figure 2. Antimicrobial Activity of Zinc Oxide Nanoparticles Reinforced with Clove and Cinnamon Extract against Staphylococcus aureus

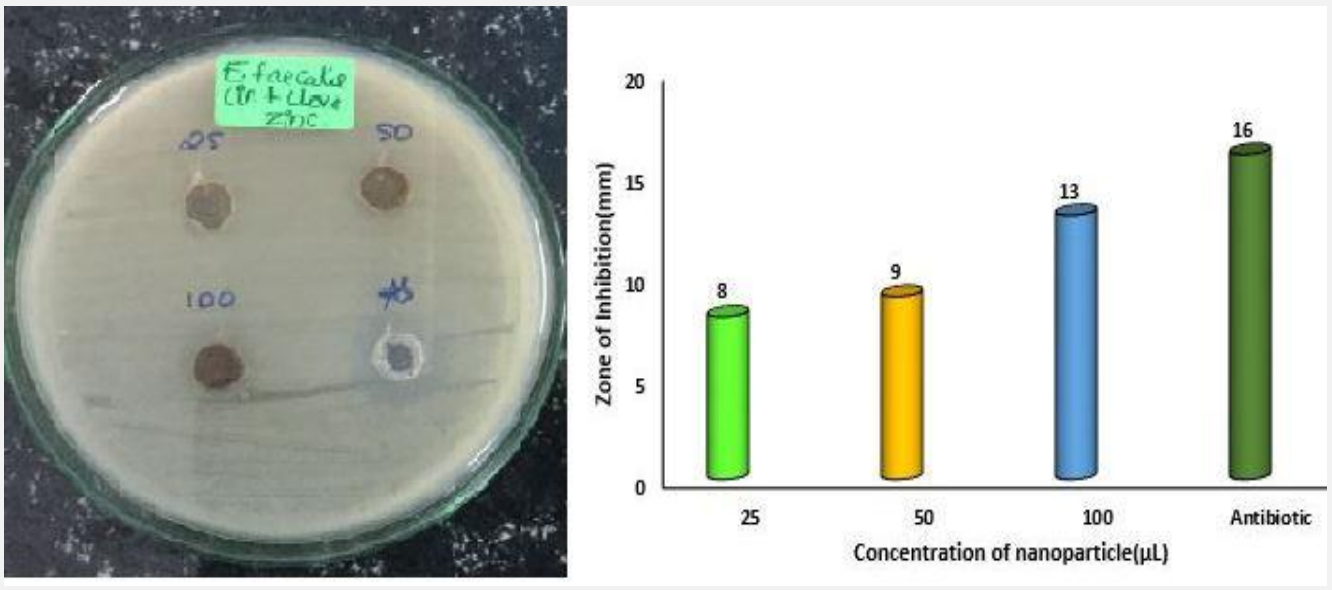

Figure 3. Antimicrobial Activity of Zinc Oxide Nanoparticles Reinforced with Clove and Cinnamon Extract against Enterococcus faecalis

DISCUSSION

Now microorganisms have become resistance to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So, it has become necessary to find out new antimicrobial agents. Nanotechnology is an emerging technology and has led to a new revolution in every field of science.[24] Among the various inorganic Nanoparticles available, Zinc Oxide has easy processing methods, is inexpensive, has wide range of applications in dentistry and is a safe material. Due to these properties, Zinc Oxide pulls a particular interest among researchers.

Green synthesis offers numerous benefits of eco friendliness and compatibility for biomedical applications, where toxic chemicals are not used for the synthesis protocol. The active ingredients of plants against microorganisms are mostly some of the secondary metabolites (i.e. alkaloids, glycosides etc.) that are present in abundance in herbs.

In this study the zone of inhibition against *Streptococcus mutans* at 25 µL concentration was 10 mm, at 50 µL was 12 mm and at 100 µL was 15 mm. Zone of inhibition against *Staphylococcus aureus* at 25 µL was 10 mm, at 50 µL was 12 mm and at 100 µL was 13 mm. Zone of inhibition against *Enterococcus faecalis* at 25 µL was 8 mm, 50 µL was 9 mm and at 100 µL was 13 mm. So, it was seen that as the concentration of the Zinc Oxide nanoparticles reinforced with clove and cinnamon extract increased, the antimicrobial activity increased. The anti-microbial activity against *Enterococcus faecalis* was found to less as to the other two bacteriae. The reason could be that *Enterococcus faecalis* is highly resistant to antimicrobial agents.

In Ayurveda clove has been used for its medicinal properties.[25] It has an anaesthetic effect which is effective numbing small regions like teeth. Thus, it has been used actively in dentistry. Also, since this is natural and so does not have any side effects.[26] Clove also contains in small quantities active anti-microbial tannins like gallotannic acid and methyl salicylate.

There are many studies done which have proved the antimicrobial activity of clove.[27-29] The main component of clove is eugenol which is responsible for the antimicrobial activity. It is well known that eugenol is a phenolic compound that can denature proteins and react with cell membrane phospholipids. This changes their permeability and inhibit Gram-negative and Gram-positive bacteria as well as different types of yeast [30]. It has also been found that addition of clove in combination with metal nanoparticles have enhanced antimicrobial activity.[31-33]

Antimicrobial activity of zinc nanoparticles by green synthesis using clove [34] and cinnamon have shown promising results.[35] The reason could be that zinc oxide nanoparticles disrupt bacterial cell membrane integrity, reduce cell surface hydrophobicity, and downregulate the transcription of oxidative stress-resistance genes in bacteria which leads to its lysis.[36]

Various biologically active compounds identified in cinnamon such as cinnamaldehyde, cinnamic acid, cinnamyl acetate, eugenol and others are responsible for its antimicrobial, antiulcer, antidiabetic, anti-inflammatory and antioxidant properties. Antimicrobial activity of cinnamon against oral pathogens has been proved in many studies.[37-40] Cinnamaldehyde is one of the main components of cinnamon that is responsible for the antimicrobial activity.

Based on the findings of the study we can say that zinc oxide nanoparticles reinforced with clove and cinnamon extract can be used as an alternative to commercially available antimicrobial agents.

Limitation

The study was conducted in vitro, so it cannot be assumed that the results of antimicrobial efficacy could be translated into clinical effectiveness.

Recommendations

- This product can be given to the patients in the form of a mouthwash.
- In further studies, in vivo studies are recommended with people' acceptance values as well.

CONCLUSIONS

Findings from this study suggest that zinc oxide nanoparticles reinforced with clove and cinnamon extracts has the potential as an antimicrobial agent against *Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis* and can be used as an alternative to commercially available antimicrobial agents.

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