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Preparation of zinc oxide nanoparticles using punica granatum and elettaria cardamomum fruit extract and determining its antibacterial activity against lactobacillus

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ABSTRACT

To determine the antibacterial activity of Zinc oxide nanoparticles synthesised using *Punica granatum* and *Elettaria cardamomum* fruit extract against Lactobacillus. *Punica granatum* and *Elettaria cardamomum* have been known for their antimicrobial, antioxidant and anti-inflammatory activity. The phytochemicals present in these fruits have experimented for the preparation of various metal and metal oxide nanoparticles. Zinc oxide is a widely used metal oxide nanoparticle known for its good antimicrobial activity against a host of microbes. The current study was conducted to determine its effect against Lactobacillus, a bacteria known for its role in the progression of dental caries. Preparation of fruit extract mediated zinc oxide nanoparticles. Determining the characteristics of the nanoparticles using UV spectroscopy and SEM. Analysing the activity of these nanoparticles against Lactobacillus using agar well diffusion method. The zone of inhibition increased if the concentration of the fruit mediated zinc oxide nanoparticles increased. But it was incomparable to the standards. Hence, further studies need to be conducted using different concentration of *Punica granatum* and *Elettaria cardamomum* to determine the optimum fruit extract required for the preparation of the nanoparticles. The resultant nanoparticles can be used as an effective antimicrobial agent against Lactobacillus.

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INTRODUCTION

Dental caries is the most common disease in the oral cavity and is one of the foremost public health problem (Heng, 2016). Numerous Microorganisms are associated with the incidence and progression of dental caries and the most common ones being, Streptococcus mutans, Actinomyces and Lactobacillus (Chokshi *et al.*, 2016). Lactobacillus is well known for its role in the progression of dental caries with the dentin being its main ecological site (van Houte *et al.*, 1972). Numerous studies have been undertaken to check the antimicrobial activity of various metal oxide nanoparticles against Lactobacillus Feng *et al.* (2017); Tian *et al.* (2018). It has been proven that Zinc oxide nanoparticles and silver oxide nanoparticles have improved antimicrobial activity when compared to copper oxide and ferric oxide nanoparticles (Feng *et al.*, 2017). The metal and metal oxide nanoparticles are known to possess an intense action against the disease-causing pathogens in the urinary tract and gastrointestinal tract (Vanaja *et al.*, 2014; Lv and B, 2017).

Zinc oxide in the form of nanoparticles has been found to have an increased antimicrobial activity because of the increased specific surface area of the reduced particle size, which enhances the particle surface reactivity (Sirelkhatim *et al.*, 2015). It is believed that the zinc oxide nanoparticles causes the bacterial community shift by reducing the species richness if the EBPR system (Hu *et al.*, 2017). The antimicrobial activity of zinc has been attributed to its ability to inhibit glycolysis, glucosyltransferase production and polysaccharide synthesis, which is required for bacterial survival (Almoudi *et al.*, 2018). Though the chemically prepared nanoparticles possess an excellent antimicrobial activity, they are found to possess toxic effects which are more than the beneficial effects (Gupta and Xie, 2018). Hence, Green synthesis using various plants, fungus, bacteria, and algae have been employed for the synthesis of zinc oxide nanoparticles (Happy *et al.*, 2019; Agarwal *et al.*, 2017).

Punica granatum and *Elettaria cardamomum* are both known for their excellent antimicrobial property (Khalil *et al.*, 2015; Kote *et al.*, 2011). Hence, in the current study, *Punica granatum* and *Elettaria cardamomum* mediated zinc oxide nanoparticles have been formulated to determine its antimicrobial activity against Lactobacillus.

MATERIALS AND METHODS

Preparation of the fruit extract

Elettaria cardamomum and *Punica granatum* were collected from Chennai. The collected fruits were washed 3 or 4 times using distilled water. Fifty gms of *Punica granatum* and 2gms of *Elettaria cardamomum* was weighed separately and individually crushed using mortar and pestle. Then the crushed fruits were individually added to 100ml of distilled water in separate beakers and boiled for 6-8 mins at 60°C. The solutions were then separately cooled down and filtered using No.1 Whatman filter paper. Both the filtered extracts were then mixed in a beaker and boiled for 6-8 mins at 60°C. The fruit extract was then stored at 4°C to be used further for the synthesis of zinc oxide nanoparticles.

Synthesis of zinc oxide nanoparticles

0.816 grams of ZnSO4 was taken based on the

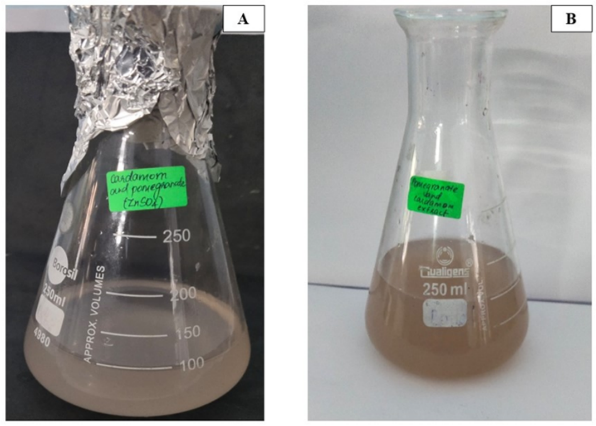

Figure 1: Figure showing A) Pale pink colour solution containing ZnSO4 and fruit extract in 50 ml of distilled water B) Brown colour solution after 72 hours indicating the formation of zinc oxide nanoparticles.

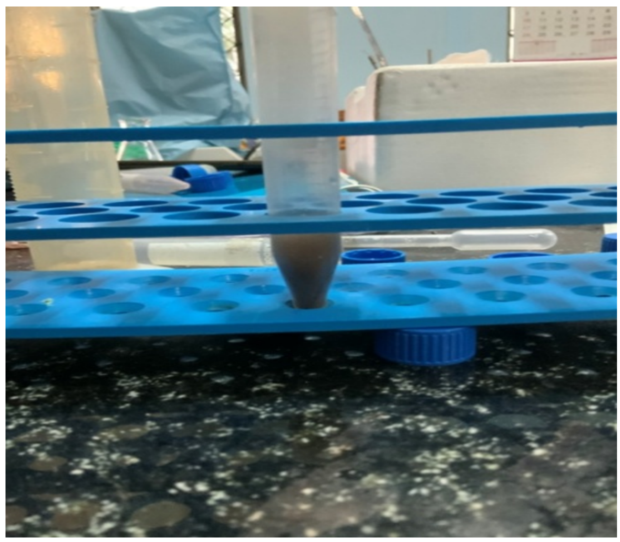

Figure 2: Figure showing the sedimentary pellet obtained after centrifugation.

Table 1: Table indicating the zone of inhibition for various concentrations of the fruit mediated zinc oxide nanoparticles

Concentration (μl)	Zone of inhibition (mm)
50	9
100	9
150	12
Ab	42

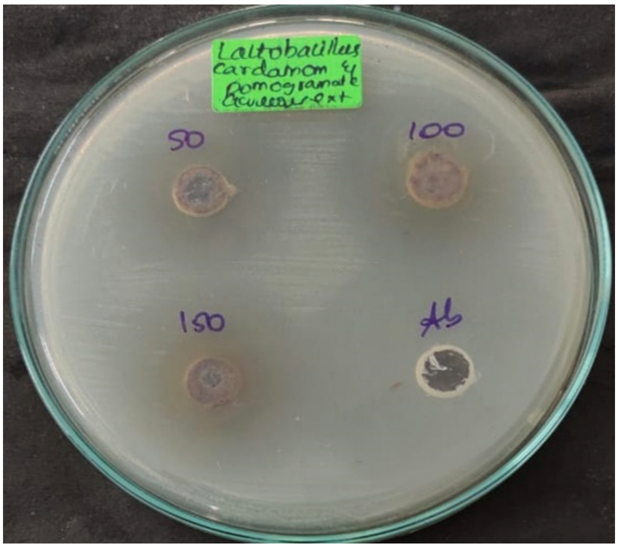

Figure 3: Photograph showing zone of inhibition for different concentrations of ZnO nanoparticles (50,100 and 150 μl) added in the wells against Lactobacillus.

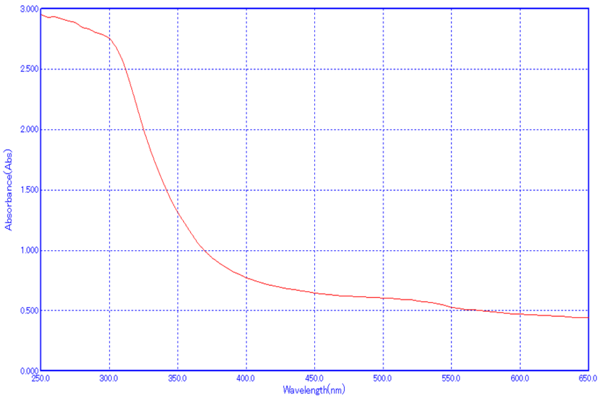

Figure 4: UV spectrometry graph showing a peak in the graph indicating the formation of Zinc oxide nanoparticles.

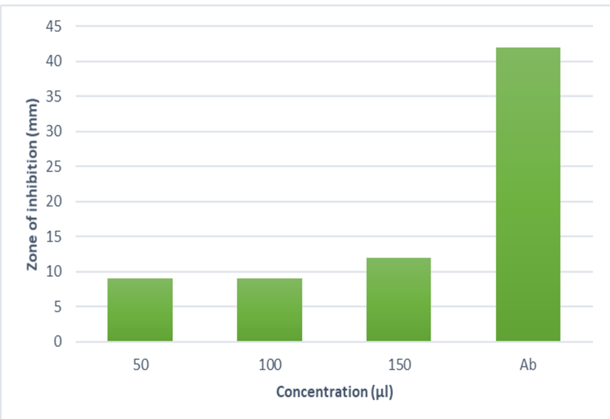

Figure 5: Graph indicating the increase in the zone of inhibition for the increasing concentration of zinc oxide nanoparticles and its comparison with the standard antibiotic

molecular weight, and it was mixed with 50ml of distilled water. To this solution, 50ml of the prepared fruit extract was added. The solution was then placed in a magnetic stirrer, and UV spectrometry readings were taken after every 2hours. After 72 hours, a peak in the graph was noticed along with a visible colour change of the solution from pale pink to brown, indicating the formation of the nanoparticles (Figure 1).

The solution was then transferred to 6 centrifuge tubes containing 13ml of the solution in each. Centrifugation was carried out for 10 minutes at 8000rpm using Lark refrigerated centrifuge. After centrifugation, the supernatant was removed, and the substrate was collected in a single tube and pellet was obtained (Figure 2).

The sedimentary pellet was double washed with distilled water and dried overnight in a hot air oven at 80 °C. A brown coloured powder was collected and stored in an airtight tube for further characterisation.

Characterization of zinc oxide nanoparticles

The synthesis of nanoparticles solution was preliminarily characterised using UV spectrometry. About 3ml of the solution is taken in curettes and scanned in double beam UV spectrophotometer from 300nm wavelength. The results were recorded for graphical analysis. The Shape and size of the nanoparticles were then determined using a Scanning electron microscope (SEM) using the powder obtained after drying in the hot air oven.

Antibacterial activity of nanoparticles against lactobacillus

The agar well diffusion method was the method that was used to determine the antibacterial activity of zinc oxide nanoparticles. Different concentrations of zinc oxide were tested against Lactobacillus. The fresh bacterial suspension was dispersed on the surface of the Muller-Hinton agar plates. Different concentrations of nanoparticles (50, 100, 150μL) {Citation}were incorporated into wells, and the plates were incubated at 37○C for 24 hours. The antibiotics were used as positive control and zone of inhibition was recorded in each plate using a scale (Figure 3).

RESULTS AND DISCUSSION

Visual observation

After the addition of Zinc sulfate solution with *Punica granatum* and *Elettaria cardamomum* fruit extract, the colour was pale pink. After 72 hours of being in the magnetic stirrer, the colour changed into light brown which indicated the formation of zinc oxide nanoparticles (Figure 1).

UV visible spectrometry

The peak found in the spectroscopy indicates the formation of Zinc Oxide nanoparticles (Figure 4).

Antibacterial activity

The present study has revealed that the zone of inhibition for 50 μl of zinc oxide nanoparticles was 9mm, for 100 μl was 9mm and for 150 μl was 12mm. This study has shown that there is a marginal increase in the zone of inhibition with the rise in the concentration of the nanoparticles (Table 1, Figure 5). On comparing with the standard antibiotic, it was found the standard showed a much higher zone of inhibition of 42mm (Figure 5).

Discussion

The present study has shown a marginal increase in the zone of inhibition with growth in the concentration of the nanoparticles; this can be owed to the definitive antimicrobial activity of the fruit mediated nanoparticles against Lactobacilli. Zinc oxide nanoparticles are known to have a broad spectrum of antibacterial activity (Xie *et al.*, 2011; Rajeshkumar, 2018).

The mechanism by which they render this activity is by producing certain specific reactive oxygen species which elevates the membrane lipid peroxidation and causes membrane leakage of reducing sugars, DNA, proteins, and reduces cell viability of the bacteria (Tiwari *et al.*, 2018).

This antibacterial action of zinc oxide is further enhanced by the presence of *Punica granatum* and *Elettaria cardamomum.* Sowmya Kote et al. in their study found *Punica granatum* to cause a 46% decrease in the lactobacillus count in the dental plaque, and this was attributed to the polyphenols present in the fruit (Kote *et al.*, 2011).

On the other hand, Sara.L.Kahil et al. in their study on *Elettaria cardamomum* extract found good antibacterial activity against Lactobacillus, and this was hypothesised because of the essential oil oleoresin present in it (Khalil *et al.*, 2015).

CONCLUSION

Within the limits of the study, we can say that *Punica granatum* and *Elettaria cardamomum* mediated zinc oxide nanoparticles have antibacterial activity against Lactobacillus. But further research is required to enhance the potency of the fruit mediated nanoparticles by altering the ratio of *Punica granatum* and *Elettaria cardamomum*.

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Conflict of Interest

The authors declare no potential conflicts of interest concerning the authorship and publication of this article.

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