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Assessment of Antimicrobial activity of novel zinc oxide nanoparticles synthesized through coffee bean and xylitol formulation against oral pathogens

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ABSTRACT

Aim: The aim of this study was to synthesize a novel zinc oxide nanoparticle from a formulation of coffee beans and xylitol and assess its antimicrobial activity against common oral pathogens.

Materials and Method: Antibacterial activity of the respective nanoparticles against the strain S.aureus, S.mutans and E. faecalis and C.albicans was carried out. The nanoparticles with different concentrations were loaded and the plates were incubated for 24 hours at 37 ° C. After the incubation time the zone of inhibition was measured to assess the antibacterial and antifungal activity.

Result: With the present study it can be deduced that with an increase in the testing concentration of the nanoparticle laced solution, it was observed that there was an increase in the zone of inhibition for the above-mentioned oral pathogens. This was not only observed for the gram positive bacterias tested, but also for the fungus- C.albicans.

Discussion: The action potential of ZnO NP could be majorly attributed to the production of ROS (i.e., OH• (hydroxyl radical) and O2−2 (peroxide)), which induces oxidative stress, cell membrane disruption, and DNA damage, resulting in the death of bacterial cells.Also the release of Zn2+ ions, which interact with the bacterial cell, especially the cell membrane, cytoplasm, and the nucleic acid, thereby disintegrating the cellular integrity and eventually resulting in the bacterial cell death. Direct interactions between zinc oxide nanoparticles and bacterial cell membranes through electrostatic forces can also be considered as a potential mode of action of the nanoparticles.

Conclusion: Therefore higher concentration of ZnO NPs can act as a versatile semiconductor photoconductive antimicrobial agent that can be easily employed as an antimicrobial agent in antibacterial creams, lotions, and ointments, mouthwashes, and paints, as well as a biofilm growth inhibitor in surface coatings.

Keywords: *Zinc oxide nanoparticles, Coffee beans, Xylitol, antimicrobial*

INTRODUCTION

Nanotechnology is focused on the research and application of particles ranging in size from 1 to 1000 nm. Nanoparticles have distinctive properties due to their small size in comparison to their bulk counterpart, making them excellent for applications in domains such as electronics, energy, the environment, and health and medical sciences.(Patel and Pathak, 2021) Nanoparticles can generally either be metallic, polymeric, or lipophilic. They can be created with desired characteristics based on their size and shape using various physical, chemical, and biological processes.(Prabhu *et al.*, 2015) However, due to the high cost and toxicity of the chemicals required in synthesis, physical and chemical approaches have received little attention. As a result, substantial research into the biological synthesis or the "green synthesis" of metallic nanoparticles such as silver, gold, titanium dioxide, magnesium oxide, copper oxide, iron oxide, aluminum oxide, and zinc oxide has been conducted. Among these, zinc oxide nanoparticles (ZnONPs) have piqued the interest of experts all over the world due to their extensive therapeutic properties and benefits.(Awasthi, 2021) Zinc oxide nanoparticles have remarkable UV filtering, semiconducting, and catalytic activity, which has piqued the interest of scientists globally. Furthermore, these nanoparticles are non-toxic, biologically safe, and biocompatible. Zinc oxide nanoparticles are also utilized in cosmetics and sunscreen creams because they have the potency to absorb the extremely harmful UV-A and UV-B rays.(Willander, 2014)

Zinc oxide is typically harmless and can also be used as medicine, according to the US Food and Drug Administration. Zinc oxide nanoparticles can also be potentially utilized as an antibacterial agent to kill pathogenic microorganisms.(Abd-Elsalam, 2021) Depending on particle size, shape, concentration, and period of exposure to the bacterial cell, they first break the cell wall, then penetrate, aggregate in the cell membrane, and eventually cause death by interfering with the metabolic operations. For the manufacture of zinc oxide nanoparticles, several methods have been established, including wet chemical, chemical micro emulsion, hydrothermal, vapor phase transparent, and solvothermal.(Pandimurugan *et al.*, 2023) However, these methods are costly and involve the use of toxic and extremely harmful chemicals. As a result, biological synthesis of zinc oxide nanoparticles from plant extracts and microorganisms is an excellent alternative to physical and chemical approaches. (Mazhar *et al.*, 2023) Among these, plants have been extensively explored due to rapid synthesis of zinc oxide nanoparticles and being cost effective.(Thirugnanasambandan and Gopinath, 2023)

Several plant extracts are utilized in the green synthesis of nanoparticles because they include a variety of essential metabolites and biomolecules that act as reducing, stabilizing and capping agents for the synthesis of NPs, including ZnONPs, which have advanced the area of nanoscience.(Lindsay, 2009) Coffee (Coffea arabica) is one of the world's most popular commercially grown beverages. It is thought to be a good source of physiologically active chemicals, especially polyphenols.(Srikhao *et al.*, 2022) The coffee business is responsible for massive volumes of garbage. Solid Coffee Grounds (SCG) is usually made up of (hemi) cellulose, proteins, melanoidins, and polyphenols (tannins, lignins, and chlorogenic acids). Through oxidative coupling, polyphenol-rich aqueous extracts of arabica coffee beans, such as chlorogenic acids and melanoidins, may form various nanostructures.(Galanakis, 2017; Srikhao *et al.*, 2022)

On the other hand, one of the potent antibacterial agents is xylitol, an alcoholic sweetener that does not get fermented by oral bacteria. Xylitol helps to improve oral health by neutralizing oral pH by the process of decreasing the pH.(Söderling *et al.*, 2011) *S. mutans* in plaque and saliva are reduced by consuming a suitable amount of xylitol on a regular basis. *S. mutans* metabolizes xylitol into the cell via the fructose phosphotransferase system. The xylitol is then converted to xylitol-5-phosphate, which is not only incompatible with bacteria but in turn can also be extremely hazardous to them.(Söderling and Pienihäkkinen, 2022) The sweetened sensation caused by the usage of xylitol stimulates the tongue to produce saliva, which acts as a buffer.(Nayak, Nayak and Khandelwal, 2014)

Based on the above mentioned advantages and positive properties of both Coffee and Xylitol this study was carried with a primary aim of synthesizing a novel zinc oxide nanoparticle from a formulation of coffee beans and xylitol and assessing its antimicrobial activity against common oral pathogens.

MATERIALS AND METHOD

Preparation of Coffee bean and Xylitol formulation

Coffee bean extract was prepared by mixing 1 g of freshly grounded coffee bean powder with 100 mL of distilled water. The mixture was boiled for 30 mins at 60 °C on a heating mantle. The mixture was then cooled down to room temperature and double filtered using the Whatman no.1 filter paper. 20m M Zinc nitrate was used as precursor and 5 0 mL of coffee bean extract was used as a reducing agent. The mixture was kept on a magnetic stirrer for uniform dispersion of all the contents at 600-800 rpm for 48hrs. 50mg of Xylitol powder was mixed with 10 mL of distilled water. This mixture was then mixed with the previously prepared coffee bean-zinc nitrate extract. The mixture was stirred for 2 h and UV–Visible readings were recorded, wherein a strong peak was observed at the end of 3 h. The mixture was then centrifuged at 8000 rpm for 10 min.

The sedimented pellet was double washed with distilled water and dried in a hot air oven operating at 80 °C. The brown colored powder that was obtained was then used for characterization.

Characterisation of Zinc Oxide nanoparticle

Crystalline nature and particle morphology of zinc oxide nanoparticles was analyzed through the Scanning Electron microscope.

Antimicrobial activity of Zinc Oxide nanoparticle

Antibacterial Activity

Antibacterial activity of the respective nanoparticles against the strain *S.aureus, S.mutans and E. faecalis* was carried out. MHA agar was utilized to assess the antibacterial activity by determining the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media was then poured into the sterilized plates and left undisturbed for solidification. The wells were cut using the well cutter and the test organisms were swabbed individually. The nanoparticles with different concentrations were loaded and the plates were incubated for 24 hours at 37 ° C. After the incubation time the zone of inhibition was measured to assess the antibacterial activity.

Antifungal Activity

Antifungal activity of *C.albicnas* was assessed by agar well diffusion assay. Rose Bengal Agar was used to prepare the medium. The prepared and sterilized medium was swabbed with test organisms and nanoparticles with different concentrations were added to the wells. The plates were incubated at 28° C for 48-72 hours. After the incubation time, the subsequent zone of inhibitions were measured to assess the antifungal activity.

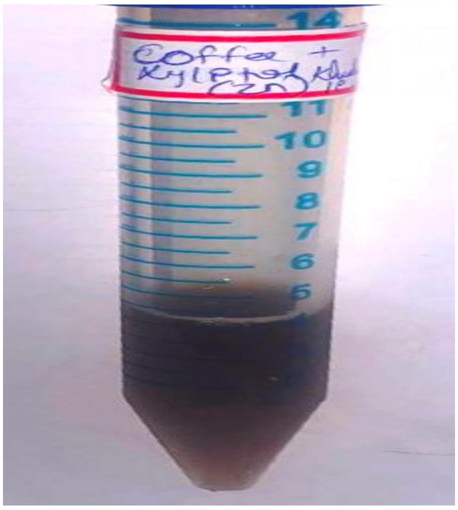

FIGURE 1: Coffee bean-Zinc nitrate-Xylitol formulation

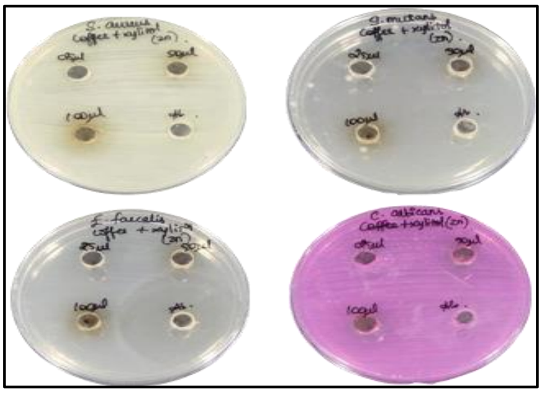

FIGURE 2: Antimicrobial assay for *S.aureus, S.mutans, E.faecalis and C.albicans*.

RESULTS

TABLE 1: Antimicrobial activity of Zinc oxide nanoparticle synthesised through coffee bean and xylitol formulation among all groups of different concentration (25, 50, 100 micro lit)

Groups	Sample size	Mean difference±S.D	Std. error	95% confidence interval		df	F value	P value
				Lower	Upper			
25 µL	*S.mutans*	9.00 ± .00	.00	9.00	9.0	3	10.00	0.00*
	S.aureus	9.00 ± .00	.00	9.00	9.0			
	E.faecalis	9.00 ± .00	.00	9.00	9.0			
	C.albicans	9.00 ± .00	.00	9.00	9.0			
50 µL	*S.mutans*	9.33 ± .81	.33	8.47	10.19	3	22.88	0.00*
	S.aureus	9.83 ± 1.16	.47	8.60	11.06			
	E.faecalis	10.66 ± 1.50	.61	9.08	12.24			
	C.albicans	14.33 ± 1.03	.42	13.24	15.41			
100 µL	*S.mutans*	9.50 ± .54	.22	8.92	10.07	3	36.76	0.00*
	S.aureus	11.66 ± .51	.21	11.12	12.20			
	E.faecalis	12.50 ± .83	.34	11.62	13.37			
	C.albicans	16.16 ± 1.94	.79	14.12	18.20			
Control	*S.mutans*	12.00 ± 1.26	.51	10.67	13.32	3	104.33	0.00*
	S.aureus	14.50 ± 2.16	.88	12.22	16.77			
	E.faecalis	28.33 ± 1.96	.80	26.26	30.39			
	C.albicans	21.50 v 1.51	.61	19.90	23.09			

P-value(*) derived using one- way ANOVA

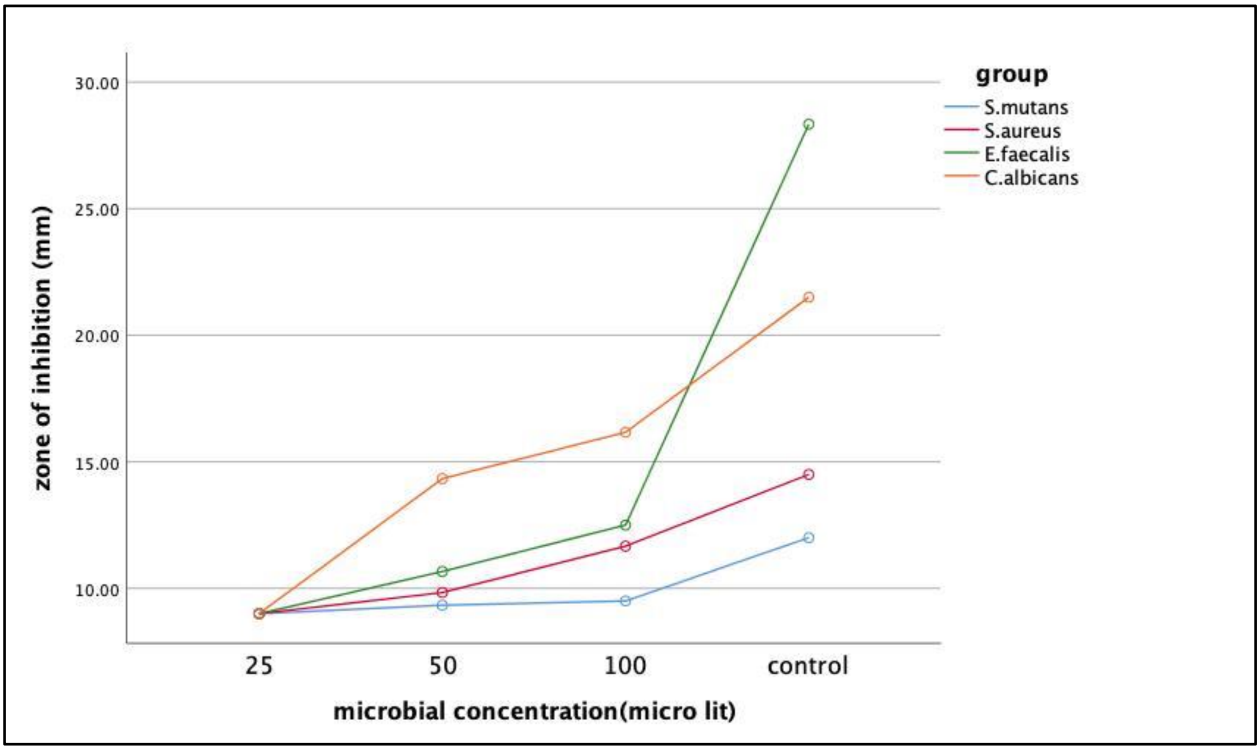

FIGURE 2: line graph depicting the antimicrobial assay for *S.aureus, S.mutans, E.faecalis and C.albicans*.

DISCUSSION

Due to the widespread usage of nanoscale metals in industries such as food, medicine,and the environment, synthesizing these materials is currently a hot research topic.(Rai and Posten, 2013) The vast majority of metal nanoparticles are created chemically, which has unanticipated effects such as energy and environmental waste, as well as potential health problems.(Sirelkhatim *et al.*, 2015) It is critical to develop a method of producing ZnO NPs that is environmentally friendly and uses gentle procedures and nontoxic components. In response to these challenges, green synthesis, which decreases metal ions using plant extracts rather than manufactured chemicals, was created.(Dizaj *et al.*, 2014) Green synthesis has several benefits: it is affordable and non hazardous to human health and the environment. Recently, researchers have become interested in the utilization of biomaterials for the manufacture of ZnO NPs. Plant, fungal, bacterial, algal, arthropod,enzyme, animal, and agricultural waste products are all examples of biomaterials.(Acharya, 2021) Because of some characteristics, such as the use of easily available plants, simplicity, and the wide range of ZnO-NP morphologies, the manufacture of plant-based ZnO nanoparticles is preferable to alternative biological methods. (de Mello Donegá, 2014)

With the above mentioned results of our study, it can be deduced that with an increase in the testing concentration of the nanoparticle laced solution, it was observed that there was an increase in the zone of inhibition for the above mentioned oral pathogens. This could be majorly attributed to the production of ROS (i.e., OH• (hydroxyl radical) and O2−2 (peroxide)), which induces oxidative stress, cell membrane disruption, and DNA damage, resulting in the death of bacterial cells.(Sirelkhatim *et al.*, 2015) Also the release of Zn2+ ions, which interact with the bacterial cell, especially the cell membrane, cytoplasm, and the nucleic acid, thereby disintegrating the cellular integrity and eventually resulting in the bacterial cell death. Direct interactions between zinc oxide nanoparticles and bacterial cell membranes through electrostatic forces can also be considered as a potential mode of action of the nanoparticles. (Sirelkhatim *et al.*, 2015; Król *et al.*, 2017; Czyżowska and Barbasz, 2022)

Bhuyan et al.(Khanuja, Uma and Varma, 2017) synthesised ZnO-NPs using Azadiracha indica (neem) leaf extract and zinc acetate dihydrate. The nanoparticles were spherical in form and ranged in size from 9.6 to 25.5 nm. The antibacterial activity of biosynthesized ZnO-NPs against Staphylococcus aureus, Streptococcus pyogenes, and Escherichia coli was tested. Bacterial growth was found to decrease as the concentration of green ZnO-NPs increased, quite similar to the results of our study.

Therefore higher concentration of ZnO NPs can act as a versatile semiconductor photoconductive antimicrobial agent that can be easily employed as an antimicrobial agent in antibacterial creams, lotions, and ointments, mouthwashes, and paints, as well as a biofilm growth inhibitor in surface coatings. (Król *et al.*, 2017)Despite the fact that ZnO NPs have antibacterial activity, aquatic organisms might be extremely susceptible to dissolved zinc. Furthermore, through enhancing permeability via confusion inside the cell membrane, ZnO NPs can in-turn be harmful to methicillin-resistant bacteria such as Streptococcus agalactiae (Gram-positive) and S. aureus. Bacterial oxidative stress is also induced by polyvinyl alcohol-coated ZnO NPs. The antibacterial activity of ZnO NPs can also vary according to their size and shape.(Hassan *et al.*, 2021)

CONCLUSION

While there is very scarce and limited literature in terms of the antimicrobial activity exhibited by nanoparticles extracted from either coffee extract or xylitol, there are no evidences focusing light on zinc oxide nanoparticle extracted and showing antimicrobial activity from a formulation of coffee bean extract and xylitol. With the current study, coffee bean and xylitol formulation not only show the presence of zinc oxide nanoparticles, but also show that with an increase in testing concentration of the formulation, there was a direct increase in the zone of inhibition. This was not only observed for the gram positive bacterias tested, but also for the fungus- *C.albicans*. This antimicrobial potential that was observed with an increase in concentration can further aid in developing several oral hygiene product that not only carry the antimicrobial activity of the novel zinc oxide nanoparticle but also the positive characteristics of the combined formulation of coffee bean and xylitol.

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