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Research Article

Polyspice Extract Dependent ZnO Nanoparticle Synthesis, Characterization in Respective to Antioxidant and Antibacterial Activity on Oral Pathogens

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ABSTRACT

Background: Green Zinc Oxide nanoparticles (ZnO) have many advantages in biomedical application due to nontoxic, ecofriendly and sustainability. The aim of the research was to synthesis of zinc oxide nanoparticle with phytochemical extracted from polyspice and to evaluate the antibacterial and antioxidant activity. **Materials and Methods:** Polyspice extract was prepared by Soxhlet extraction and ZnO NP were synthesized and characterized. The biopotential of ZnO on antioxidant and antibacterial was performed against oral pathogens. **Results:** The spices extract showed presence of all major phytochemicals and GCMS showed 10 different compounds. The reduction of ZnO NP confirmed by UV-vis absorption maxima at 240 nm. SEM, EDX and XRD confirmed crystalline rod 80-150 nm particles. Polyspice extract and ZnO nanoparticles showed an antibacterial effect that depended on how much of each was present. They act against both bacteria *S. mutans* and *Fusobacterium* sp. ZnO nanoparticles showed more activity, with the largest zones of inhibition (21.3 mm) at 100 µg, which is similar to chlorhexidine. The findings demonstrate that PS-ZnO nanoparticles exhibit considerable antibacterial properties and may function as viable alternatives for the management of oral pathogens. The antioxidant activity of polyspice extract, ZnO nanoparticles, and a standard compound exhibited a distinct concentration-dependent enhancement in DPPH, hydroxyl radical scavenging, and metal chelating assays. The polyspice extract showed very strong antioxidant activity, especially in the metal chelating assay (up to 86.66%), which shows that it has the most antioxidant potential. ZnO nanoparticles more activity than extract (up to about 87%), which is equal to the standard. **Conclusion:** This concludes that the prevention of oxidative damage by extract and ZnO NP. In addition, the green ZnO np were effectively inhibit the oral pathogen may helps to prevent tooth and gingival infection.

Keywords: Antibacterial, *Fusobacterium*, Oral Infection, Red Complex Pathogen, Spice, Zinc Nanoparticle.

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INTRODUCTION

Bacteria in subgingival biofilms must first pierce the gingival epithelium in order to enter the deeper areas of periodontal tissues (Ji *et al.*, 2015) and form polymicrobial biofilms in subgingival fissures is the main cause of periodontitis problem (Anju *et al.*, 2022) Recent research has demonstrated that the red complex pathogen *P. gingivalis*, an obligatory anaerobe, is a keystone pathogen in the pathogenesis of periodontitis. The pathogenic potential depends on its primary proteases, gingipain and Lys gingipain (Curtis *et al.*, 2002) It has been proposed that *F. nucleatum* may interact together with other oral infections to cause periodontal disorders. Together with other bacteria in plaque biofilms, *Fusobacterium* species are essential to these phenomena and generate Volatile Sulfur Compounds (VSCs) are thought to be the primary cause of halitosis (Liu *et al.*, 2009; Porter, 2011) primarily the substances that occur in spices not only enhance the flavor and aroma of food but also have antibacterial qualities (Maharjan *et al.*, 2019). Some of the most often utilized natural antimicrobial agents in food are spices that contain antibacterial components (Indu *et al.*, 2006). Spices have been widely employed in cuisine and aromatherapy since ancient times. However, an increasing number of recent research have shown that spices have antibacterial properties against common human respiratory pathogens and food contamination problems (Irshad *et al.*, 2017). 10% of all consumer antibiotic prescriptions are written by dentists. According to the World Health Organization, resistance to antibiotics is among of the largest dangers to international health and is predicted to cause 10 million deaths annually by 2050. In addition to being one of the

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main underlying causes of tooth decay, chronic periodontitis is a substantial potential component for a number of systemic health disorders. The fundamental components of nanotechnology that give it its practical qualities are called Nanoparticles (NPs). because they have better biological and physico-chemical properties than the bulk material. Because these NPs materials range in size from 1 to 100 nm, they have a higher surface-to-volume ratio (Venkatesan Savunthari *et al.*, 2021) and their unique physical, chemical, and biological properties, a number of metals and MO NPs including zinc, copper, iron, gold, silver, and cerium, among many others, have garnered interest frequently (Rajendran *et al.*, 2017). In addition to their nontoxicity, strong UV-adsorption capacity, broad bandgap, high electron mobility, remarkable visual transparency, and antibacterial properties, Zinc Oxide (Zn-O) Nanoparticles (NPs) are frequently selected among these (Fouda *et al.*, 2018). The goal of the current work was to effectively examine the *in vitro* antioxidant, antibacterial, and anti-inflammatory properties of ZnO nanoparticles made with Polyspice, an environmentally safe sustainable technique for ZnO biosynthesis aided by ultrasonic treatment.

MATERIALS AND METHODS

Preparation of Polyspice extract

The spice extraction prepared from the blend of *Cinnamomum verum, Piper longum, N. sativa, C. tamala, Piper nigrum, Illicium verum, Elettaria cardamomum, Syzygium aromaticum* and dried *Zingiber officinale* mixture at 1:1 ratio. About 100g sample packed in to soxhlet column. The round bottom flask is filled with 200 mL solvent 99% ethyl acetate and kept under 50ºC for 6 hr. Then the mixture was subsequently filtered and concentrated at 40ºC. Phenol, flavonoid and alkaloid of indigenous spices were determined qualitatively. About 1 mg/mL of residues was dissolved in ethylacetate for Gas Chromatography-Mass Spectrometry (GCMS) analysis

Synthesis of ZnO Np

The reduction mixture prepared at 1:9 ration of extract and zinc sulphate (50 mM) solution. 90 mL precursor was transferred in a 250 mL flask and kept under magnetic stirrer at 500 rpm, temperature was set 50ºC for 15 min. 10 mL of spice extract was gradually added from a graduated burette. At the end of precipitation UV visible spectrum was taken. The Np collected from reaction mixture by centrifugation (REMI) at 15000 rpm for 5 min. The supernatant disposed of and precipitate underwent two to three washings with deionized water and subsequently with ethanol. The resulted residue was dried at 160ºC for 12 hr to make it moisture free. The sample was then ground with mortar and pestle into fine powders and calcined for 2 hr at 500ºC to get rid of any impurities. The characterization of ZnO NP were characterized by Ultraviolet visible (UV), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray (EDAX) analysis (Naseer *et al.*, 2020).

Antibacterial activity

Three strains of *S. mutans, and Fusobacterium* sp. were tested for the antibacterial activity of synthesized ZnO NPs. The antimicrobial susceptibility test was evaluated using the modified Kirby Bauer disc diffusion method in accordance with the Clinical and Laboratory Institute (CLSI) guidelines. As a positive control, chlorohexidine was used. To obtain 25, 50, 75, and 100 µg, ZNo Np 2 mg/mL in ethanol was produced and placed onto sterile discs at 12.5, 25, 37.5, and 50 µL. The Mueller Hinton (MH) agar plates that had been previously inoculated with test pathogens were covered with all of the discs. Zone size was then measured during an anaerobic incubation at 37ºC. Additionally, polyspice activity was measured from 25 to 100 µg, and the results were compared. Origin 8 is used for statistical analysis of the data, and the test was run in triplicate (Mofid *et al.*, 2020).

$$IC_{50}= C_1+(50\%\max - R_1)/(R_2-R_1)\times (C_2-C_1)$$

Where:

C_1, R_1= lower concentration and response,

C_2, R_2= higher concentration and response,

IC_{50} lies between these two points.

DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant test

The DPPH technique was used to assess the antioxidant activity of biosynthesized ZnO NPs. A solution containing 100 µM DPPH was made in 99.9% methanol in order to assess the DPPH scavenging activity at different dosages (Gülçin and Alwasel, 2023). 3.0 mL of DPPH solution and 1 mL of sample solution at different concentrations (50, 100, 200, 250 and 500 µg/mL) were then combined to perform the analysis. After that, this mixture was left to incubate in the dark at room temperature for 30 min. Methanol was utilized as a blank and ascorbic acid as the standard. Next, the optical density at 517 nm was calculated. The following formula was used to determine the DPPH scavenging activity:

$$\text{DPPH scavenging} = \text{Blank} - \text{test/blank}\ x\ 100$$

Metal chelation assay

As previously mentioned, 0.1 mM Ferric suphate ($FeSO_4$ 0.2 mL) and 0.25 mM ferrozine (0.4 mL) were introduced to 0.2 mL of the test chemical in ethanol at varying concentrations in order to determine the metal chelating activity. The mixture's absorbance at 562 nm was measured following a 10-min incubation period at room temperature. The standard is EDTA (Ethylenediaminetetraacetic acid). The following formula was used to determine chelating activity.

$$\text{Metal chelating activity} = (A\ \text{control} - A\ \text{sample})\ /A\ \text{control}\ x\ 100$$

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The H_2O_2 technique was used to assess each extract's capacity to scavenge radicals. In short, 900 µL of Phosphate Buffer Saline (PBS), 50 µL of 1,10-Phenanthroline (0.75 mM), and 25 µL of $FeSO_4$ (0.75 mM) were added to 1 mL of the reaction mixture in phosphate buffer. The reaction was started by adding 0.1 mL of H_2O_2 (20 mM) solution after the reaction mixture was pre-incubated with 100 µL of test compound at various concentrations for 5 min. The absorbance was measured against the phosphate buffer blank solution at λ_{max} 536 nm after ten min. The formula was used to determine the percentage of H_2O_2 scavenging.

$$\%\ \text{scavenging}\ H_2O_2 = (A_0 - A_1)/A_0) \times 100$$

RESULTS AND DISCUSSION

Phytochemical profile of spice extract

The qualitative phytochemical of selected spices showed presence of alkaloids, flavonoids, and phenolic compounds were summarized on Table 1. Spice extract of *C. verum* and *Illicium verum* had a lot of alkaloids (++), and *Illicium verum* also had all three phytochemicals. *Zingiber officinale* also showed positive results for all of the phytochemicals that were examined which shows that it has a lot of different phytochemicals (Singh and Ningthoujam, 2025; Jagtap *et al.*, 2004). Flavonoids were identified in various spices, including *Nigella sativa, Elettaria cardamomum, Illicium verum, Syzygium aromaticum, Piper longum* and *Zingiber officinale*, while they were not present in *Cinnamomum* spp and *P. nigrum*. Phenolic compounds were mainly found in *Cinnamomum* spp, *Illicium verum, Syzygium aromaticum, and Zingiber officinale*, which suggests that they may have antioxidant properties. The polyspice extract had a very strong presence (+++) of alkaloids, flavonoids, and phenols. This shows that when spices are mixed together, they become even more bioactive. Figure 1 represents the GCMS of polyspice extract shows ten different peaks with high amount of 13-docosenamide, (z)-(63.3%) and 6.25% 2h-pyridazino(4,5-b) indole-1,4-dicarboxylic acid. bis(2-ethylhexyl) ester of hexanedioic acid was the first eluted compound at 32.866 min and the last eluted one was 1h-indole, 1-acetyl-3-((1,2,3,6-tetrahydro-1-methyl-4-pyridinyl) carbonyl)- with retention time 38.3 min. Further hexanedioic acid, docosenamide, carboxylic acid, prenyloxindole and indole derivatives were additionally identified at less than 3% level. the retention time and corresponding compound were given in Table 2.

Green synthesis of ZnO Nanoparticle

The formation of Zinc Oxide Nanoparticles (ZnO NPs) was confirmed using UV-visible spectroscopy and Energy Dispersive X-ray Analysis (EDAX) (Figure 2). The UV-visible spectrum exhibited a characteristic absorption peak around 240 nm, which is typical for ZnO nanoparticles due to their intrinsic band-gap absorption. This peaks 480 and 503 indicates the successful reduction and agglomerization of zinc ions. The broad nature of the absorption curve suggests a degree of particle size distribution and possible surface defects. Based on the 200 nm scale bar, the particle size is estimated to be in the range of 80-150 nm, suggesting nanoscale ZnO with slight agglomeration. NPs, which informed through the change in color, may be attributed to excitation of surface plasmon vibrations of nanoparticles which results in surface plasmon resonance (Datta *et al.*, 2017). According to previous studies suggested that ZnO NPs exhibit a characteristic broad absorption peak between 330-460 nm with other peaks confirms the synthesis of ZnO NPs with the aid of active biomolecules in the plant extract (Dobrucka *et al.*, 2018).

SEM analysis can be used to understand the crystalline characteristics and size of the synthesized NPs are shown in Figure 3a. SEM images displaying the spherical series of particle size that were between 32 to 96 nm. The EDAX spectrum further confirmed the elemental composition of the synthesized nanoparticles. The EDX (Figure 3b) reveals presence of some impurities, C (15.26%),

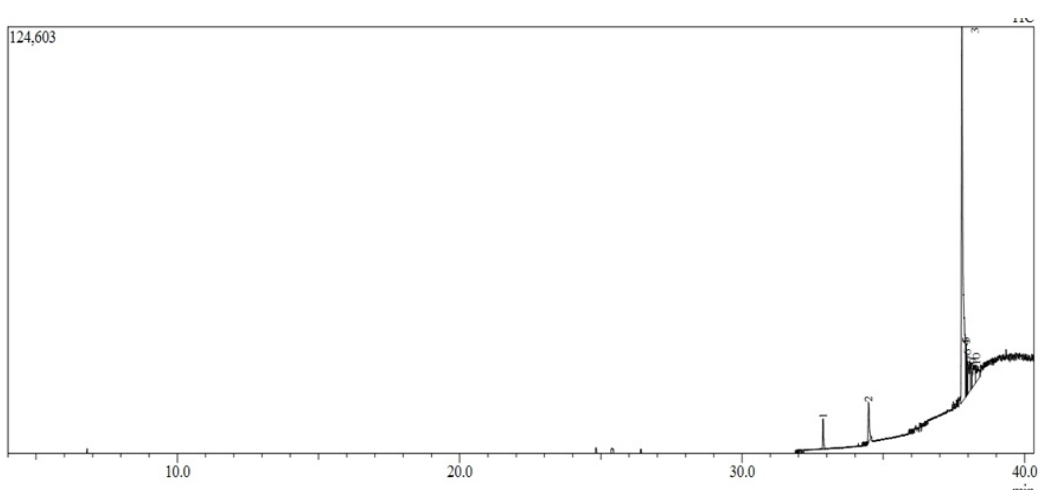


Figure 1: Gas chromatogram of ethylacetate extract of polyspice.

Table 1: Major Phytochemicals of ethylacetate spice extract.

Sample	Flavonoid	Alkaloid	Phenol
C. verum	-	++	++
C. tamala	-	-	+
E. cardamomum	+	-	-
I. verum	+	++	+
N. sativa	+	+	-
P. longum	+	+	-
P. nigrum	-	+	-
S. aromaticum	+	-	++
Z. officinale	+	+	+
Polyspice	+++	+++	+++

Table 2: Peak compounds identified through NIST library.

Peak	Retention time	Area %	Height %	Name
1	32.86	2.8	4.4	hexanedioic acid
2	34.47	5.1	5.9	3,5,7-trimethyl-2e,4e,6e,8e-decatetraene
3	37.78	65.3	55.5	N-((4-Hydroxy-3-methoxyphenyl)methyl)-13-docosenamide (Z)-
4	37.92	1.9	7.5	1H-furo(3,4-c)pyrrole-4-carboxylic acid, 6-(2-furanyl) hexahydro-1,3-dioxo-4-phenyl-, methyl ester,
5	37.93	2.8	7.6	ethanone, 1-(4-((1,1-dimethylethoxy)methyl)phenyl)-
6	37.96	2.9	5.7	2h-pyridazino(4,5-b)indole-1,4-dicarboxylic acid
7	38.05	6.2	4.4	2h-pyridazino(4,5-b)indole-1,4-dicarboxylic acid
8	38.13	2.8	2.9	Triflic anhydride
9	38.17	5.5	3.2	3-phenyl-1,3-dihydroindol-2-one
10	38.3	4.1	2.4	1H-indole, 1-acetyl-3-((1,2,3,6-tetrahydro-1-methyl-4-pyridinyl) carbonyl)-

Cu (0.47%) along with Zn (57.52%) and O (26.75%). Additional minor peaks such as Carbon (C) and Copper (Cu) may be attributed to the sample holder or organic residues from the extract used during synthesis. The diffraction peaks of the sample obtained from XRD indicated the purity of ZnO nanocrystalline formation. In accordance with the JCPDS 36-1451card (Somani *et al.*, 2025). The XRD pattern of the synthesized sample exhibits distinct diffraction peaks at 2θ 15°, 21°, 22°, 24.4°, 30.1° and 38.2° indicating its crystalline nature(Ismail *et al.*, 2023). The presence of well-defined peaks confirms successful formation of the material with good structural ordercorrespond to the reflection from (100), (101), (102) crystal planes, respectively(Figure c).

Antibacterial activity of ZnO NP

The antibacterial results on the studied bacterial pathogens showed significant sensitivity pattern by ZnO nanoparticles by concentration dependent manner. Both extract and ZnO NPs have the capability of inhibiting the growth of both Gram-positive and Gram-negative bacteria, at 5 µg and found to be effective at 100 µg. The zone of inhibition by polyspice extract (Table 3) was 12.3, 13.3, 17, 20.3 against *S. mutans* and 12,15.3,16.6, 18.66 mm towards *Fusobacterium* sp. moderately 17-19 mm antibacterial activity was recorded at 50 µg and less significant at 25 µg. Chlorohexidine showed 15.33±0.57 and 16±1 mm on *S. mutans* and *Fusobacterium* sp (Table 4). The inhibitory activity of PSZnO nanoparticle were recorded as 11.3,15.3,18 and 21.3 against *S. mutans* whereas 8,14,17.3 and 21.3 mm against *Fusobacterium* sp (Table 5). It has been demonstrated that the concentration and particle surface area of zinc oxide nanoparticles directly correlate with their antibacterial properties (Zhao *et al.*, 2020). Studies investigated the antibacterial activity of a polymer including bivalent zinc against *oral pathogens*, specially against *S. mutans* found to be sensitive (Vergara-Llanos *et al.*, 2021). Numerous researchers have already documented the antibacterial and other medicinal qualities of spice preparations against a wide range of bacteria (Park, 2008). The antibacterial efficacy of garlic, ginger, clove, and cinnamon (Kubo et al., 2004) towards *S. typhi, B. subtilis, S. aureus, and E. coli* were frequently studied and reported (Ahmed *et al.*, 2015). Spice oils are natural, non-toxic, harmless, and have antibacterial properties due to rich of aromatic compounds (Chan *et al.*, 2018). Spices contain a diverse range of active ingredients, primarily secondary metabolites, that

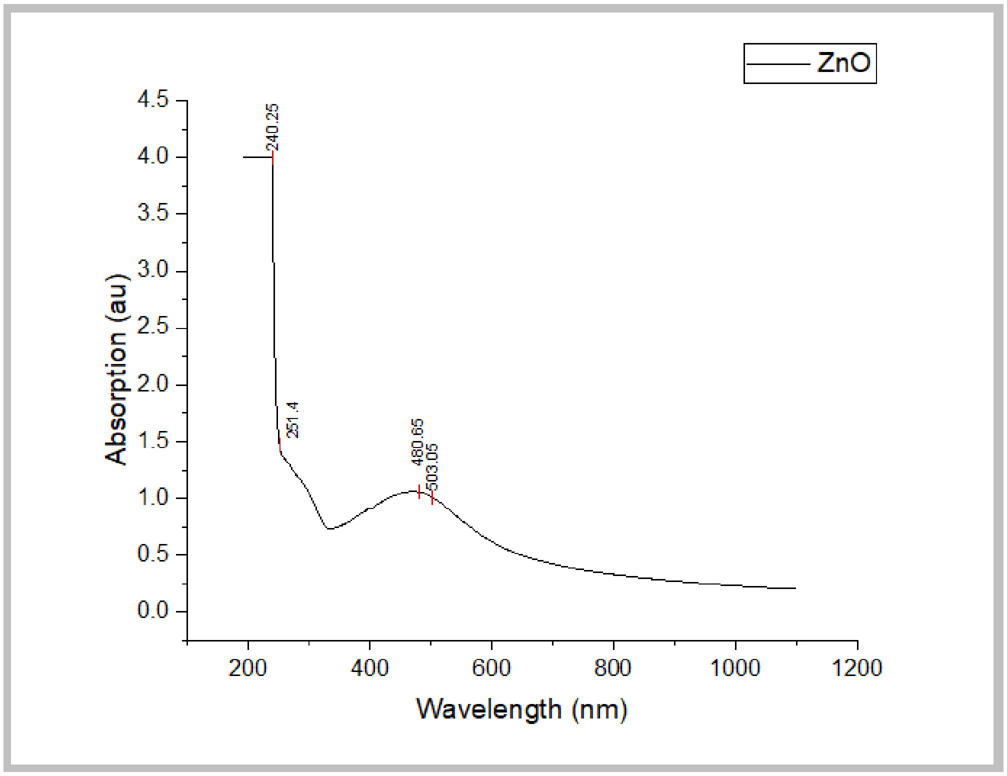


Figure 2: UV-visible spectrum of ZnO reduction.

Table 3: Antibacterial effect of poly spice Extract, inhibitory zone mm in diameter.

S. mutans	μG/mL	Mean	SD	IC_{50}
	25	12.33	0.57	20.6μg/mL
	50	13.33	0.57	
	75	17	1	
	100	20.33	1.527	
Fusobacterium sp	25	12	0	19.4μg/mL
	50	15.33	0.577	
	75	16.66	1.527	
	100	18.66	1.154	

Table 4: Antibacterial effect of chlorohexidine inhibitor zone mm in diameter.

Pathogen	Zone size mm	SD
S. mutans	15.33	0.57735
Fusobacterium sp	16	1

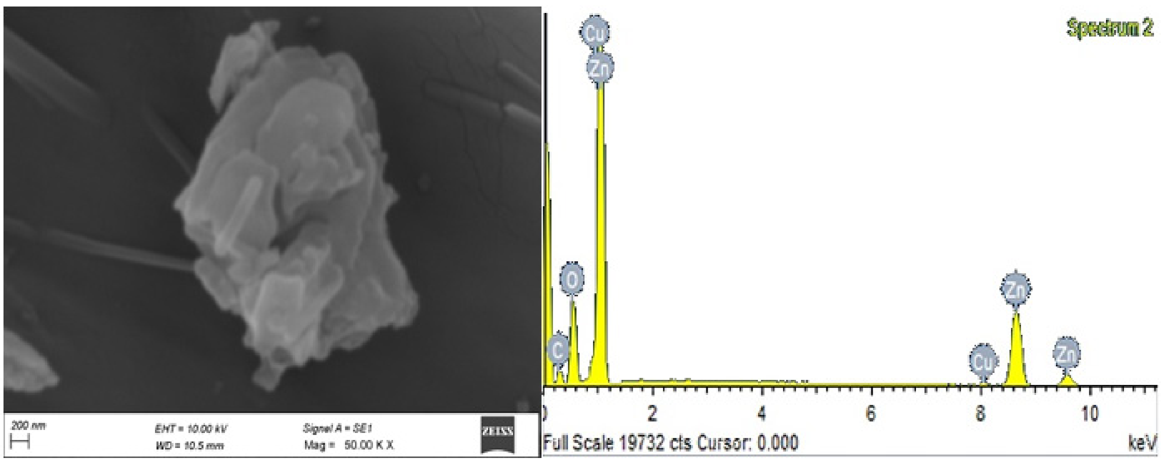
have antibacterial properties. As food additives, spice extracts have shown implicit efficacy against some foodborne infections already investigated for their antibacterial qualities (Gottardi *et al.*, 2016).

Antioxidant activity

The antioxidant activity of the poly spice extract was assessed using DPPH radical scavenging, hydroxyl radical scavenging, and metal chelating assays, which demonstrated a clear concentration-dependent increase in activity (Figure 4). In the DPPH assay, the extract exhibited moderate to strong scavenging activity, ranging from 46.33 ± 0.41% at 25 μg to 73.06 ± 0.94% at 100 μg, indicating its ability to donate hydrogen atoms or electrons to neutralize free radicals. Similarly, hydroxyl radical scavenging activity increased from 43.03 ± 0.55% at 25 μg to 70.66 ± 1.15% at 100 μg, reflecting effective reactive oxygen species quenching potential. Notably, the metal chelating activity was comparatively higher across all concentrations, increasing from 67.63 ± 0.32% at 25 μg to 86.66 ± 0.30% at 100 μg, suggesting a strong ability to bind transition metal ions and inhibit radical generation due to polyphenol content of extract (Yang *et al.*, 2020). Investigated various spices for their phenolic content and antioxidant properties. The elevated levels of DPPH, FRAP, and ABTS in clove and cinnamon are greater than those found in other Indian spices (Patra *et al.*, 2016). The antioxidant potential of ZnO nanoparticles synthesized using spice extract (Figure 5) revealing

Table 5: Antibacterial effect of ZnO nanoparticle inhibitory zone mm in diameter.

pathogen	Concentration µg	Mean	Standard Deviation	IC_{50}
S. mutans	5	11.3	1.15	4.7 µg/mL
	25	15.3	0.57	
	50	18	0	
	100	21.3	1.15	
Fusobacterium sp	5	8	0	13.86
	25	14	0	
	50	17.3	1.15	
	100	21.3	1.15	



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b

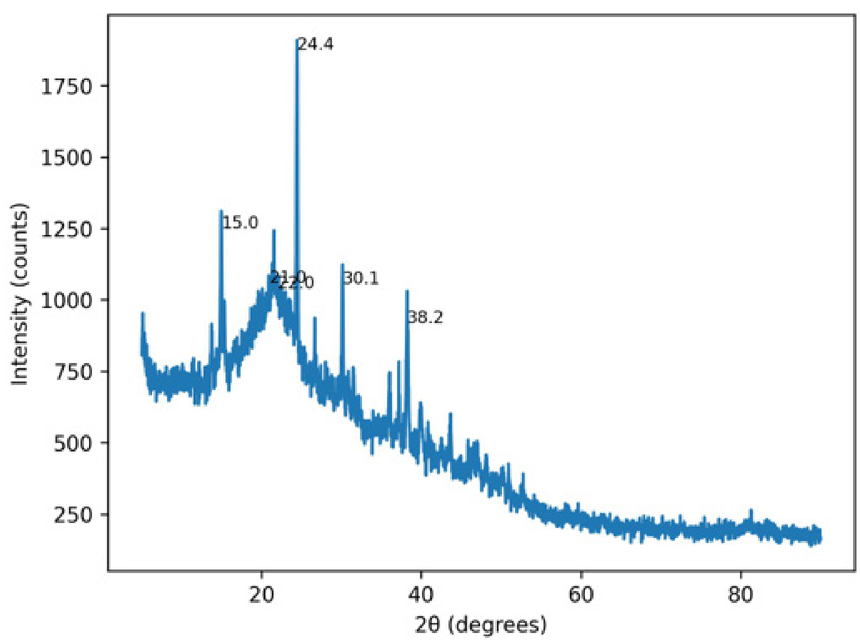

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Figure 3: a) SEM Morphology of ZnO NP b) EDX spectrum of ZnO NP c)XRD spectrum of spice extract dependent green synthesized ZnO Np.

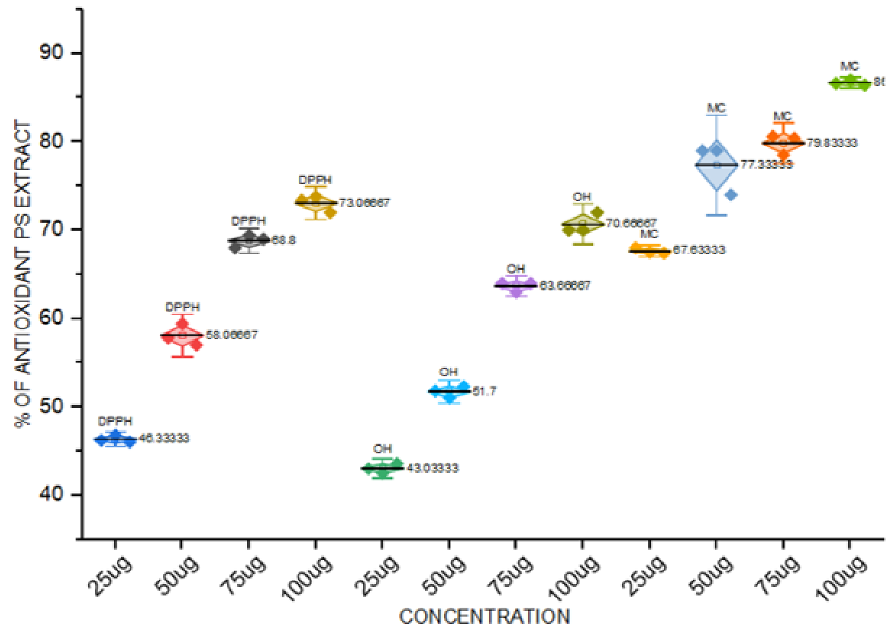

Figure 4: Antioxidant activity of poly spice extract.

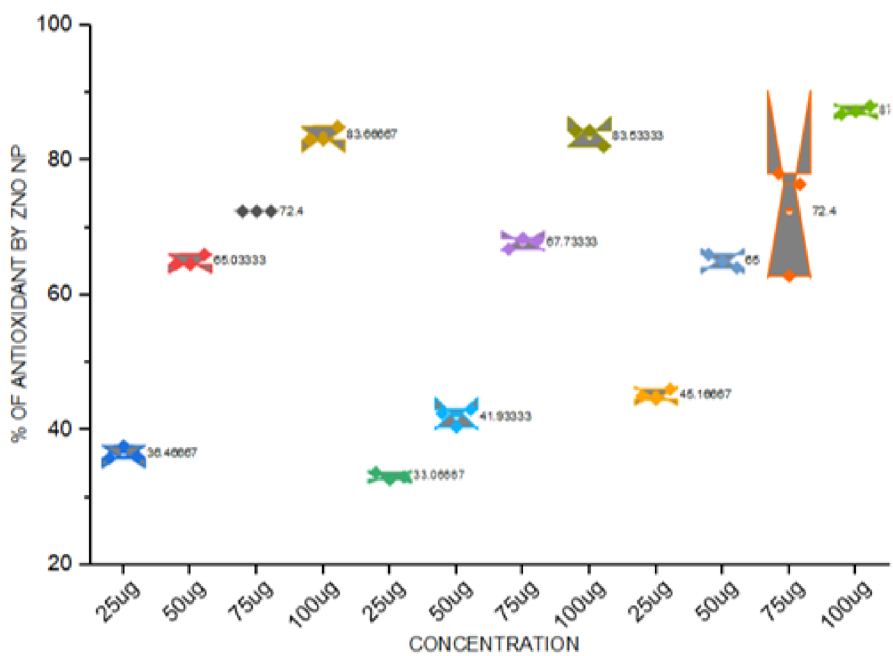

Figure 5: Antioxidant potential of zinc oxide nanoparticle.

a clear concentration-dependent increase in activity. In the DPPH assay, the nanoparticles exhibited scavenging activity ranging from 36.46 ± 0.98% at 25 µg to 83.66 ± 1.15% at 100 µg, indicating strong free radical neutralization capacity. Similarly, hydroxyl radical scavenging activity increased from 33.06 ± 0.50% to 83.53 ± 1.33% across the same concentration range, demonstrating effective reactive oxygen species quenching ability. The metal chelating assay showed activity from 45.16 ± 0.76% at 25 µg to a maximum of 87.33 ± 0.61% at 100 µg, suggesting a pronounced ability to bind transition metal ions and inhibit radical generation. The antioxidant activity of the standard compound (Figure 6) demonstrating a consistent concentration-dependent increase in activity. In the DPPH assay, the standard exhibited scavenging activity from 53.00 ± 0.40% at 25 µg to 81.03 ± 0.83% at 100 µg, indicating strong free radical neutralization efficiency. Similarly, hydroxyl radical scavenging activity increased from 33.33 ± 1.15% at 25 µg to 80.33 ± 0.30% at 100 µg, reflecting effective reactive oxygen species quenching ability. In the metal chelating assay, the activity ranged from 43.93 ± 0.57% at 25 µg to 81.00 ± 0.52% at 100 µg, suggesting a significant capacity to bind transition metal ions and inhibit radical formation. The low standard deviation values across all concentrations indicate good experimental

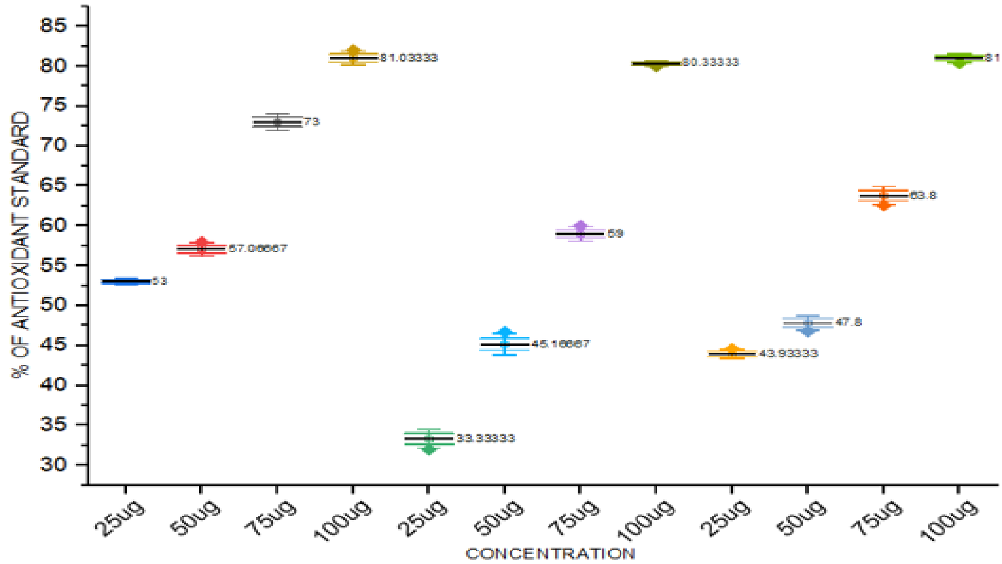

Figure 6: Antioxidant activity of standard ascorbic acid and EDTA.

reproducibility and reliability. In addition to providing additional health benefits in the prevention of numerous serious illnesses like inflammatory conditions, cancer, and heart disease, spices are abundant sources of polyphenolic compounds with potent antioxidant abilities that may eventually replace chemical antioxidants (Hossaina *et al.*, 2018). The most common and reactive radicals produced naturally through aerobic metabolic processes that cause cell damage *in vivo* are hydroxyl radicals. Spice extract hydroxyl scavenging ability is found to be consistent with the Kim *et al.* (2011) findings. According to Soren *et al.* (2018). Zinc nanoparticles hydroxyl radical scavenging capacity yields comparable outcomes of our findings was observed.

CONCLUSION

Zinc oxide nanoparticles mediated by polyspice extract and its phytochemicals have been shown to be beneficial against oral infections. According to our research, ZnO NPs made with spice extract have stronger antibacterial activity at lower concentrations than usual. These results provide credence to ZnO NPs as a potentially effective treatment approach for oral infections.

ACKNOWLEDGEMENT

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ABBREVIATIONS

ZnO: Zinc Oxide; **NP:** Nanoparticles; **NPs:** Nanoparticles; **GCMS:** Gas Chromatography-Mass Spectrometry; SEM: Scanning Electron Microscope; **EDX:** Energy Dispersive X-ray; **EDAX:** Energy Dispersive X-ray Analysis; **XRD:** X-Ray Diffraction; **UV:** Ultraviolet; **VSCs:** Volatile Sulfur Compounds; **MO:** Metal Oxide; **CLSI:** Clinical and Laboratory Standards Institute; **MH:** Muller Hinton; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EDTA:** Ethylenediaminetetraacetic acid; **PBS:** Phosphate-buffered saline; **JCPDS:** Joint Committee on Powder Diffraction Standards.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Dr. Nithyadharani R. conceptualized the study and supervised the project. Abdul Farook Samath performed the experiments and drafted the manuscript. Both authors reviewed and approved the final manuscript.

SUMMARY

This study successfully synthesized ZnO nanoparticles using a green polyspice extract. The polyspice extract and nanoparticles demonstrated significant, concentration-dependent antioxidant and antibacterial activity against oral pathogens *S. mutans* and *Fusobacterium* sp., presenting a sustainable alternative for managing oral infections.

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