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Green synthesis and characterization of Zinc oxide nanoparticle with polyspice extractantioxidant and antibacterial potential against oral pathogens

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ABSTRACT

Because it is environmentally beneficial, the synthesis of metal oxide nanoparticles utilizing medicinal plants is growing quickly. The polyspice extract made by soxhlet extraction with ethylacetate is used in this research to create zinc oxide nanoparticles. GCMS and qualitative phytochimcal analysis were carried out. ZnONP and extract antioxidant activities were measured and compared to a standard. Using the disc diffusion approach, an antibacterial experiment was conducted against oral pathogens. Polyspice was found to include alkaloids, flavonoids, and phenol. Ten distinct secondary compounds were detected using GCMS. UV-Vis Spectroscopy analysis was used to describe the synthesized NPs, with a peak detected at 396-398 nm. SEM with EDX analysis verified the NPs' spherical form 30-98 nm and the primary constituents are oxygen and zinc. At 50–100 µg levels, ZnO NPs inhibition assay on DPPH, metal chelation, and hydroxyl radical activity demonstrates good inhibition against free radicals. The ZnONPs that were biosynthesized showed higher antibacterial effectiveness than the standard at least 25µg, which is equivalent to standard chlorohexidine. The pattern of the nanoparticles' antibacterial activity against the chosen bacteria was *P. gingivalis, S. mutans*, and Fusobacterium sp. According to the study's findings, synthesizing zinc oxide nanoparticles with a spice extract is an economical and environmentally responsible way to produce green ZnO nanoparticles that have strong anti-oxidant and anti-microbial properties against oral infections.

Introduction

Chronic periodontitis is a significant potential aspect for several systemic health conditions in addition to being one of the primary root causes of decaying teeth. For bacteria in subgingival biofilms to infiltrate the deeper regions of periodontal tissues, they must first penetrate the gingival epithelium1. The primary causative component in the pathophysiology of periodontitis is polymicrobial biofilms found in subgingival fissures2. In the pathophysiology of periodontitis, it was recently shown that the red complex pathogen P. gingivalis, an obligate anaerobe, serves as a keystone pathogen3.The pathogenic potential depends on its primary proteases, gingipain and Lys gingipain4. It has been proposed that F. nucleatum may interact together with other oral infections to cause periodontal disorders. Together with

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other bacteria in plaque biofilms, Fusobacterium species are essential to this phenomena5 and generate According to Porter6, volatile sulfur compounds (VSCs) are thought to be the primary cause of halitosis. primarily the substances that occur in spices not only enhance the flavor and aroma of food but also have antibacterial qualities7. Some of the most often utilized natural antimicrobial agents in food are spices that contain antibacterial components8. Since historic times, spices were used extensively in festivals, as additives, and as colorants. However, a growing number of recent studies have documented the antibacterial activity of spices against common Gram-positive and Gram-negative bacteria that cause respiratory illnesses in humans and issues with food safety9. Ten percent 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 205010. 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 ratio11. Due to 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 frequently12. 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 these13. 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 method

Polyspice extraction

The following spice such as Cinnamomum verum, Piper longum, N. sativa, C.tamala, Piper nigrum, Illicium verum, Elettaria cardamomum, Syzygium aromaticum and dried Zingiber officinale finely powdered and mixed at 1:1 ratio. Phenol, flavonoid and alkaloid of indigenous spices were determined qualitatively. About 100g sample packed in to soxhlet column. The round bottom flask is filled with 200 ml solvent 99% ethyl acetate and kept under 50° C for 6h. Then the mixture was subsequently filtered and concentrated at 40° Celsius. About 1 mg/mL of residues was dissolved in ethylacetate for qualitative GCMS analysis

DPPH antioxidant test [14]

The antioxidant activity of biosynthesized ZnO NPs was determined using the DPPH method. To evaluate the DPPH scavenging activity at various doses, a solution containing 100 µM DPPH was produced in 99.9 % methanol. The analysis was then carried out by combining 3.0 mL of DPPH solution with 1 mL of sample solution at various doses (50 µg/mL, 100 µg/mL, 200 µg/mL, 250 µg/mL, and 500 µg/mL). This combination was then allowed to incubate for 30 min at ambient temperature in the dark. Ascorbic acid was used as the standard and methanol as a blank. The optical density was then determined at 517 nm. To find the synthesized ZnO NPs' DPPH scavenging activity, the following formula

DPPH scavenging =Blank- test/blank x 100

Metal chelation assay

Metal chelating activity was measured as described previously, by adding 0.1 mM FeSO4 (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of test compound in ethanol added at different concentration. After incubating at room temperature for 10 min, absorbance of the mixture was recorded at 562 nm. EDTA used as Standard. Chelating activity was calculated using the following formula:

Metal chelating activity = (A control – A sample) /A control x 100

Hydrogen Peroxide Scavenging Activity

The radical scavenging activity of individual extracts was determined using the H2O2 method. Briefly, 1 mL of reaction mixture in phosphate buffer prepared by adding 900 µl PBS, 50µL 1,10-phenanthroline (0.75 mM) and 25 µL of FeSO4 (0.75 mM). The reaction mixture pre incubated with 100µL of test compound at different concentration for 5 min. the reaction initiated by addition of 0.1 mL of H2O2 (20 mM) solution. After 10 min, the absorbance was measured at λmax 536 nm against the phosphate buffer blank solution. The percentage scavenging of H2O2 was calculated using the equation:

$$\%\text{ scavenging of } H_2O_2 = [(A0 - A1)/A0] \times 100,$$

Synthesis of ZnO np synthesis[15]

The 90 mL aqueous zinc sulphate (50mM) solution was transferred in a 200 mL reagent bottle positioned above ultrasonication bath. The temperature was set 65 °C and 450Hz for 15 min was applied. 10 ml of spice extract was gradually added from a graduated burette. At the end of 15 min 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 160 °C for 12 h to make it moisture free. The sample was then ground with mortar and pestle into fine powders and calcined for 2 h at 500 °C to get rid of any impurities. The characterization of ZnO NP were characterized by UV, XRD, SEM and EDAX analysis.

Antibacterial activity[16]

The research investigation examined the antibacterial activity of synthesized ZnO NPs against three strains of S.mutans, P.gingivalis, and Fusobacterium sp. The modified Kirby Bauer disc diffusion method followed by the Clinical and Laboratory Institute (CLSI) guideline was utilized to assess the antibacterial susceptibility test. Chlorohexidin was considered as a positive control. ZNo Np 2mg/mL in ethanol was prepared and loaded on sterile disc at 12.5, 25, 37.5 and 50µl to get 25, 50,75 and 100µg. All the disc was placed over the MH agar plates pre inoculated with test pathogens. After which incubation at 37 °C was performed under anaerobic condition zone size was recorded. Activity of polyspice also performed at 25 to 100µg and the data was compared. The test was performed in triplicate manner and the data is statistically analyzed using origin 8.

Results and discussion

The qualitative phytochemical test for major phyto chemical was given in table 1. Test on alkaloid showed negative on E cardamomum, S aromaticum, C.tamala and others are positive. Detection of flavonoid showed negative on C.verum, C.tamala and P nigrum whereas N. sativa, E cardamomum, P longum and P nigrum showed negative on phenol test. The presence of all three were confirmed on polyspiece extract. Further figure 1 represent 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. 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. During synthesis, the change in color of the solution and formation of a yellowish-white precipitate was an indication that zinc nitrate had been reduced. The formation of ZnO.NPs was initially confirmed by UV-vis spectroscopy within the range 200–800 nm. The absorption spectrum of green synthesized ZnO.NPs showed a characteristic peak at 374 nm. The reduction of Zn nitrate into ZnO. NPs, which informed through the change in color, may be attributed to excitation of surface plasmon vibrations of nanoparticles which results in Surface Plasmon Resonance17.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 extract18.The crystalline peaks positioned at (2θ) peaks angles of 15.80°, 20.45°, 25.28°, 29.59°, 36.65°, correspond to the reflection from (100), (002), (101), (102), (110), (103), crystal planes, respectively. SEM analysis can be used to understand the crystalline characteristics and size of the synthesized NPs are shown in Fig. 3a. SEM images displaying the spherical series of particle size that were between 32 to 96 nm. The EDX (Fig 3b) reveals presence of some impurities, C (15.26%), Cu (0.47%) along with Zn (57.52%) and O (26.75%).The diffraction peaks of the sample obtained from XRD indicated the purity of ZnO nanocrystalline formation. In accordance with the JCPDS 36–1451car

Table 1. Major phytochemical of ethylacetate spice extract

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

Table 2. peak compounds identified through nist library

Peak	Retention time	Start time	End Time	Area %	Height %	Name
1	32.866	32.83	32.925	2.85	4.46	bis(2-ethylhexyl) ester of hexanedioic acid
2	34.476	34.44	34.56	5.19	5.96	3,5,7-trimethyl-2e,4e,6e,8e-decatetraene

3	37.782	37.74	37.915	65.36	55.57	13-docosenamide, (z)-
4	37.925	37.915	37.93	1.93	7.54	1h-furo[3,4-c]pyrrole-4-carboxylic acid, 6-(2-furanyl) hexahydro-1,3-dioxo-4-phenyl-, methyl ester, (3a. alpha.,4.beta.,6.beta.,6
5	37.935	37.93	37.955	2.83	7.68	ethanone, 1-[4-[(1,1-dimethylethoxy)methyl]phenyl]-
6	37.965	37.955	37.99	2.94	5.78	2h-pyridazino[4,5-b]indole-1,4-dicarboxylic acid, 4a,5-dihydro-5-methyl-4a-(1-propenyl)-, dimethyl ester, (e)-
7	38.058	37.99	38.085	6.25	4.45	2h-pyridazino[4,5-b]indole-1,4-dicarboxylic acid, 4a,5-dihydro-5-methyl-4a-(1-propenyl)-, dimethyl ester, (e)-
8	38.13	38.085	38.135	2.89	2.93	trifluoromethanesulfonic anhydride
9	38.179	38.135	38.265	5.58	3.22	3-prenyloxindole
10	38.3	38.265	38.425	4.19	2.41	1h-indole, 1-acetyl-3-[(1,2,3,6-tetrahydro-1-methyl-4-pyridinyl)carbonyl]-

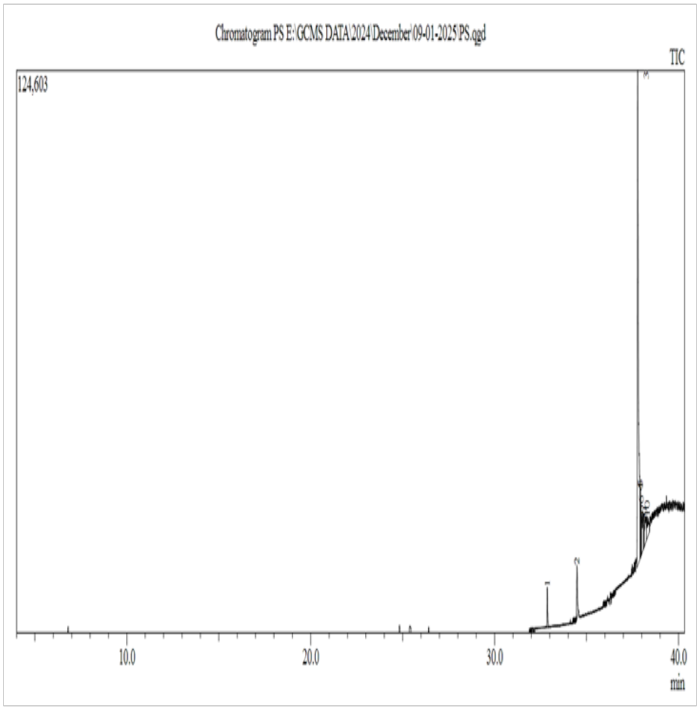

Figure 1. Gas chromatogram of ethyl acetate extract of polyspice

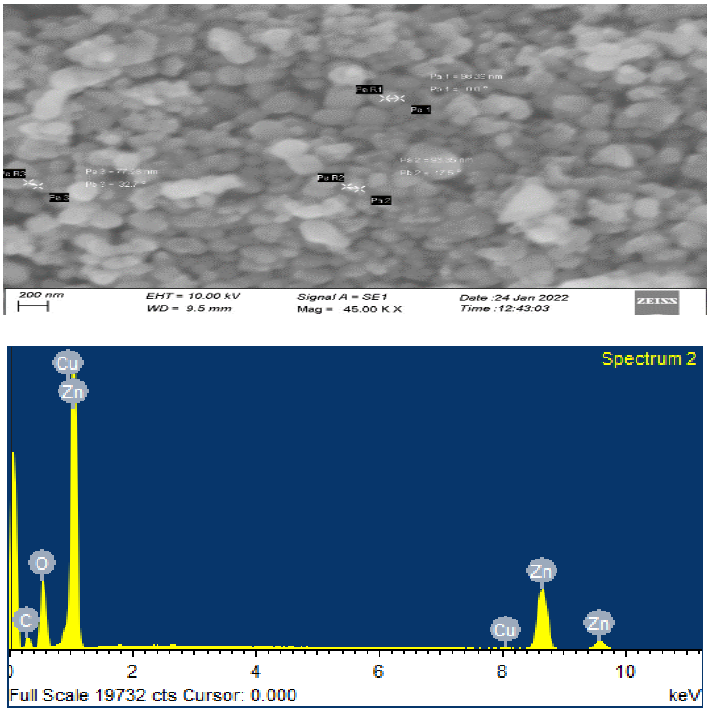

Figure 3. a) SEM Morphology of ZnO NP b) EDAXspectrum of ZnO NP

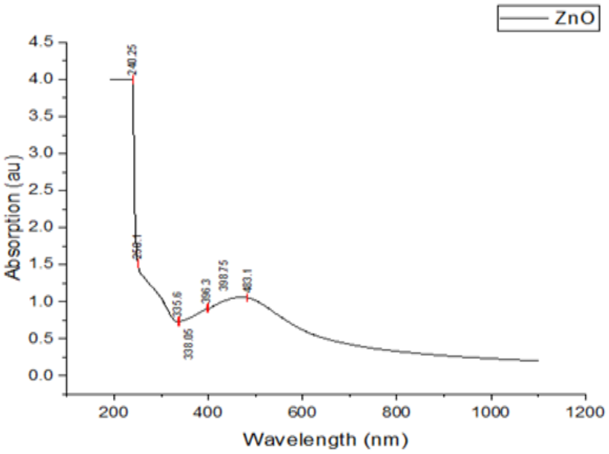

Figure 2. UV Visible spectrum of ZnO reduction

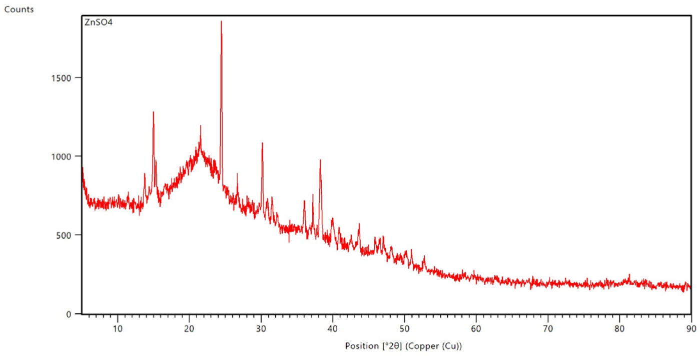

Figure 4. XRD pattern of ZnO NP

Antioxidant activity

The evaluation of antioxidant activity by DPPH-mediated free radical scavenging capability of ZnO NPs was given in table 3. The activity of scavenging was found to be concentration dependent manner. Figure 5a represent the DPPH scavenging of spice extract and ZnO Np. The activity was compared with standard ascorbic acid (Figure 5). The scavenging of polyspice extract was ranged between 46±1 to 72.6 ±1.15 among 25 to 100µg concentration. The activity of ZnO Np was recorded between 36±1 to 83.66±1.15 percentage. In case of standard it was minimum 53±1 to 83±1percentage. Compare to standard polyspice was lower activity and ZnO np have higher activity. At the 0.05 level, the population means are significantly different (<0.0001). 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 antioxidants19.

The metal chelation potential of extract and NP compared with EDTA and the data is given on table 4. Poly spice metal chelation was recorded minimum 68.3±0.57and reached maximum 88±0 Percentage. The standard EDTA43.3±0.5 at 25µg and maximum 80.6±0.5 at 100µg. Where as the ZnO nanoparticle have least metal chelation 46±1.73 AT 25µG and higher activity of 87.3±1.15 percentage. Both the polyspice and ZnO np reached more than 75% activity at 75 µg where as less than 65% activity was noted on standard. Compare to standard higher activity at lower concentration was observed in both samples (figure 6). Further the one way ANNOVA reveals at the 0.05 level, the population means are significantly different (<0.0001).

The hydroxyl scavenging of polyspice between 25 to 100µg was gradually increased from 43±1 percentage to 52±1, 63.6±0.57 and reached maximum 73.1±0.57 percentage respectively at 25, 50, 75 and 100µg level. The standard activity were 33.3±1.1, 43.3±2.1, 58.6±1.1 and 80.6±1.1 at similar 25 to 100µg level. Whereas ZnO NP showed minimum of 32.6±0.5, 41.6±1.5, 67.3±1.1 and 87.3±1.1 percentage of free radical scavenging (table 5). Compare to standard ascorbic acid, polyspice was lesser activity and ZnO np was found to be greater scavenging potential(Fig 7). The probability was <0.0001 found to be the population means are significantly different. A diverse blend of chemicals known as bioactive compounds gives different spices their scavenging activity. The most common and reactive radicals produced naturally through aerobic metabolic processes that cause cell damage in vivo are hydroxyl radicals20. Spice extract's hydroxylscavenging ability is found to be consistent with the Kim et al.21 findings. According to Soren et al.22, zinc nanoparticles' hydroxyl radical scavenging capacity yields comparable outcomes.

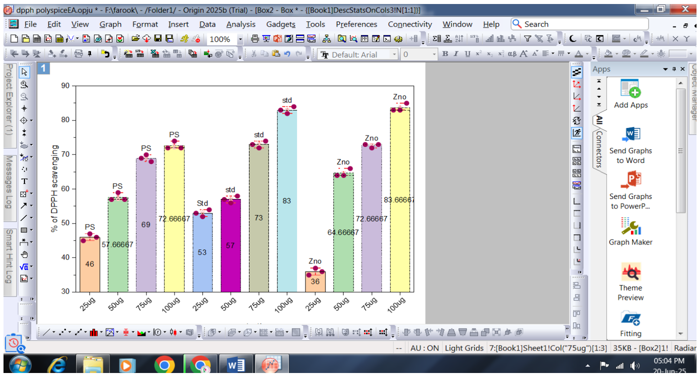

Figure 5. percentage of DPPH scavenging

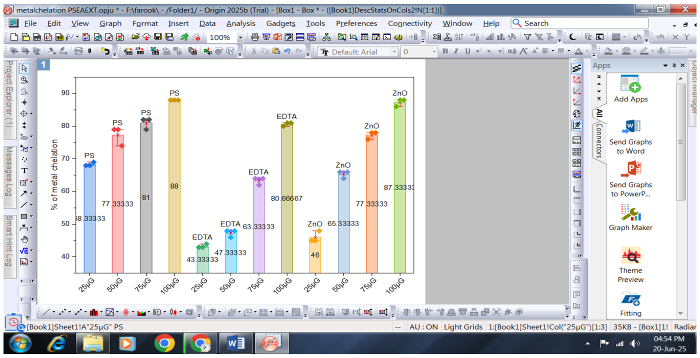

Figure 6. Percentage of metal chelation

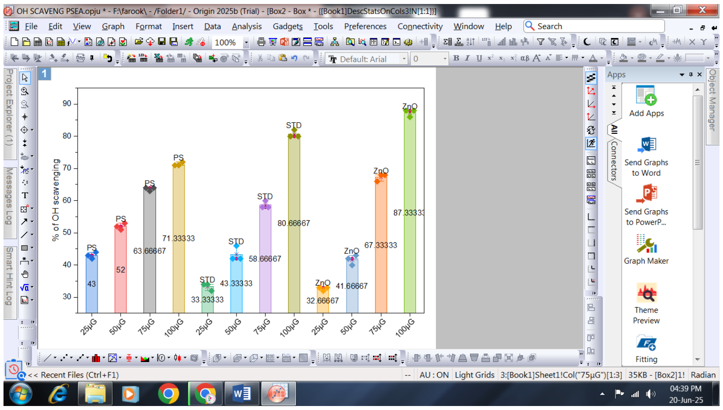

Figure 7 percentage of hydroxyl scavenging

Table 3 percentage of DPPH scavenging by ZnO Nanoparticle

Sample	Concentration µg	Mean	Standard Deviation	SE of Mean
	25	46	1	0.57735
Poly spice	50	57.66667	1.1547	0.66667
	75	69	1	0.57735
	100	72.66667	1.1547	0.66667
	25	53	1	0.57735
standard	50	57	1	0.57735
	75	73	1	0.57735
	100	83	1	0.57735
	25	36	1	0.57735
	50	64.66667	1.1547	0.66667
ZnO Np	75	72.66667	0.57735	0.33333
	100	83.66667	1.1547	0.66667

TABLE 4 percentage of METAL chelation by ZnO Nanoparticle

Sample	Concentration µg	Mean	Standard Deviation	SE of Mean
Poly spice	25	68.33333	0.57735	0.33333
	50	77.33333	2.88675	1.66667
	75	81	1.73205	1
	100	88	0	0
standard	25	43.33333	0.57735	0.33333
	50	47.33333	1.1547	0.66667
	75	63.33333	1.1547	0.66667
	100	80.66667	0.57735	0.33333
ZnO Np	25	46	1.73205	1
	50	65.33333	1.1547	0.66667
	75	77.33333	1.1547	0.66667
	100	87.33333	1.1547	0.66667

Table 5 percentage of hydroxyl Scavenging by ZnO nanoparticle

Sample	Concentration µg	Mean	Standard Deviation	SE of mean
Poly spice	25	43	1	0.57735
	50	52	1	0.57735
	75	63.66667	0.57735	0.33333
	100	71.33333	0.57735	0.33333
standard	25	33.33333	1.1547	0.66667
	50	43.33333	2.3094	1.33333
	75	58.66667	1.1547	0.66667
	100	80.66667	1.1547	0.66667
ZnO Np	25	32.66667	0.57735	0.33333
	50	41.66667	1.52753	0.88192
	75	67.33333	1.1547	0.66667
	100	87.33333	1.1547	0.66667

Antibacterial activity of extract

The bactericidal activities of spice extract and ZnO NPs against tooth infecting pathogens were evaluated at different concentration. Table 6 displays the zone of inhibition of polyspice on test pathogens. The polyspice alone showed maximum activity at 100µg and the zone of inhibition against S.mutans was 20.33±1.5 mm, and 20.67±1.15 mm on P.gingivalis whereas 18.66667±1.15 mm against Fusobacterium sp. the most important finding was that the extract showed activity at all concentration against bacterial pathogens but only active at 75 and 100µg against yeast C.albicans. The standard chlorohexidine showed 15.3±0.57, 16.66±0.57, 16±1 mm respectively over S.mutans, P.gingivalis and Fusobacterium sp. Numerous researchers have already documented the antibacterial and other medicinal qualities of spice preparations against a wide range of bacteria23-24. Anees et al.25 recently showed the antibacterial efficacy of garlic, ginger, clove, and cinnamon towards Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. Spice oils are natural, non-toxic, harmless, and have antibacterial properties, according to Chan et al.26. They are also good for your health. Spices contain a diverse range of active ingredients, primarily secondary metabolites, that have antibacterial properties. As food additives, spice extracts have shown implicit efficacy against some foodborne infections27 thoroughly investigated their antibacterial qualities.

Antibacterial activity of ZnO NP

According to our results (Table 7), the studied bacteria pathogen had significantly varied interactions toward the synthesized ZnO nanoparticles as antimicrobial agents by concentration dependent manner. ZnO NPs have the capability of inhibiting the growth of both Gram-positive and Gram-negative bacteria, at 5µg and found to be effective at 100µg estimated as 21.33±1.15 mm against S.mutans, 22.66±1.15 Over P.gingivalis and 21.33±1.15mm towards Fusobacterium sp. moderate antibacterial activity was recorded at 50 µg recorded between 17-19 mm and less significant antibacterial effect was noted at 2 to 25µg. figure 7 represents the zone of inhibition among the pathogens was increased by increasing concentration. The data among three strains sensitivity over Zno Np reveals the P-Value is not significant at0.05(p=0.90053) and test Static F:0.106. It has been demonstrated that the concentration and particle surface area of zinc oxide nanoparticles directly correlate with their antibacterial properties28. Zhao et al.29 investigated the antibacterial activity of a polymer including bivalent zinc against P. gingivalis, while Vergara-Llanos et al.30 demonstrated action against S. mutans.

Table 6 Antibacterial effect of poly spice extracts inhibitor zone mm in diameter

	µG/mL	T1	T2	T3	Mean	SD
S.mutans	25	12	13	12	12.33333	0.57735
	50	14	13	13	13.33333	0.57735
	75	18	16	17	17	1
	100	22	19	20	20.33333	1.527525
P.gingivalis	25	14	12	14	13.33333	1.154701
	50	16	15	18	16.33333	1.527525
	75	18	16	18	17.33333	1.154701
	100	20	22	20	20.66667	1.154701

	25	12	12	12	12	0
	50	15	15	16	15.33333	0.57735
	75	18	15	17	16.66667	1.527525
Fusobacterium sp	100	20	18	18	18.66667	1.154701
	25	0	0	0		
	50	0	0	0		
	75	14	16	16	15.333	0.9428
CA	100	18	20	18	18.666	0.942
SM		15	15	16	15.33333	0.57735
PG	CH	16	17	17	16.66667	0.57735
FB		15	17	16	16	1
CA		12	14	14	13.333	0.94

Table 7. Antibacterial effect of ZnO nanoparticle inhibitory zone mm in diameter

		Mean	Standard Deviation	SE of mean	Minimum	Median	Maximum
S.mutans	5	11.33333	1.1547	0.66667	10	12	12
	25	15.33333	0.57735	0.33333	15	15	16
	50	18	0	0	18	18	18
	100	21.33333	1.1547	0.66667	20	22	22
P.gingivalis	5	14.66667	1.1547	0.66667	14	14	16
	25	16.66667	1.1547	0.66667	16	16	18
	50	19.33333	1.1547	0.66667	18	20	20
	100	22.66667	1.1547	0.66667	22	22	24
Fusobacterium sp	5	8	0	0	8	8	8
	25	14	0	0	14	14	14
	50	17.33333	1.1547	0.66667	16	18	18
	100	21.33333	1.1547	0.66667	20	22	22

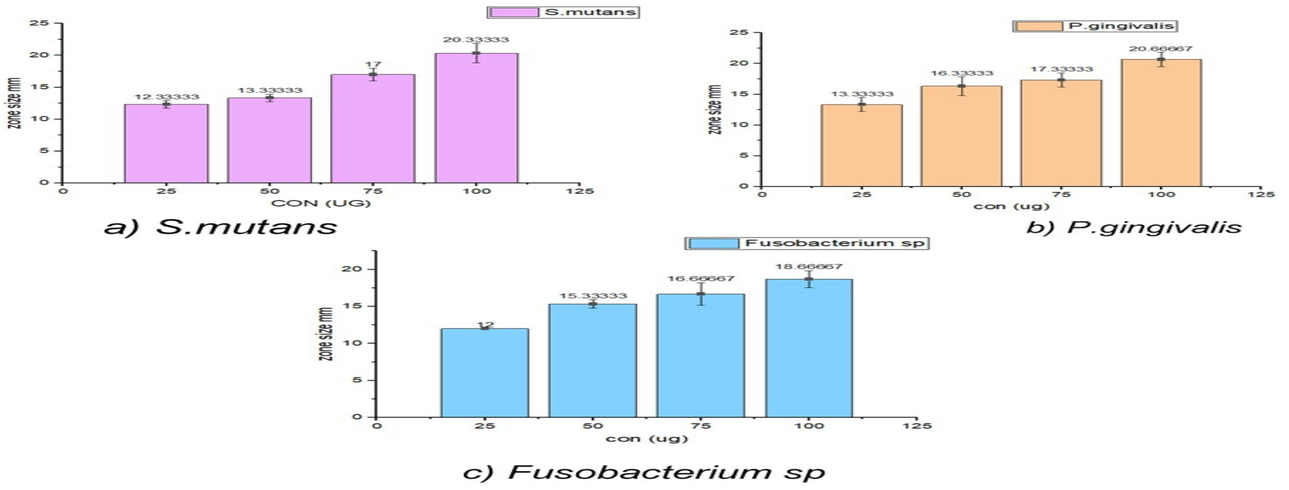

Figure 7. Zone of inhibition of polyspice extract at different concentration

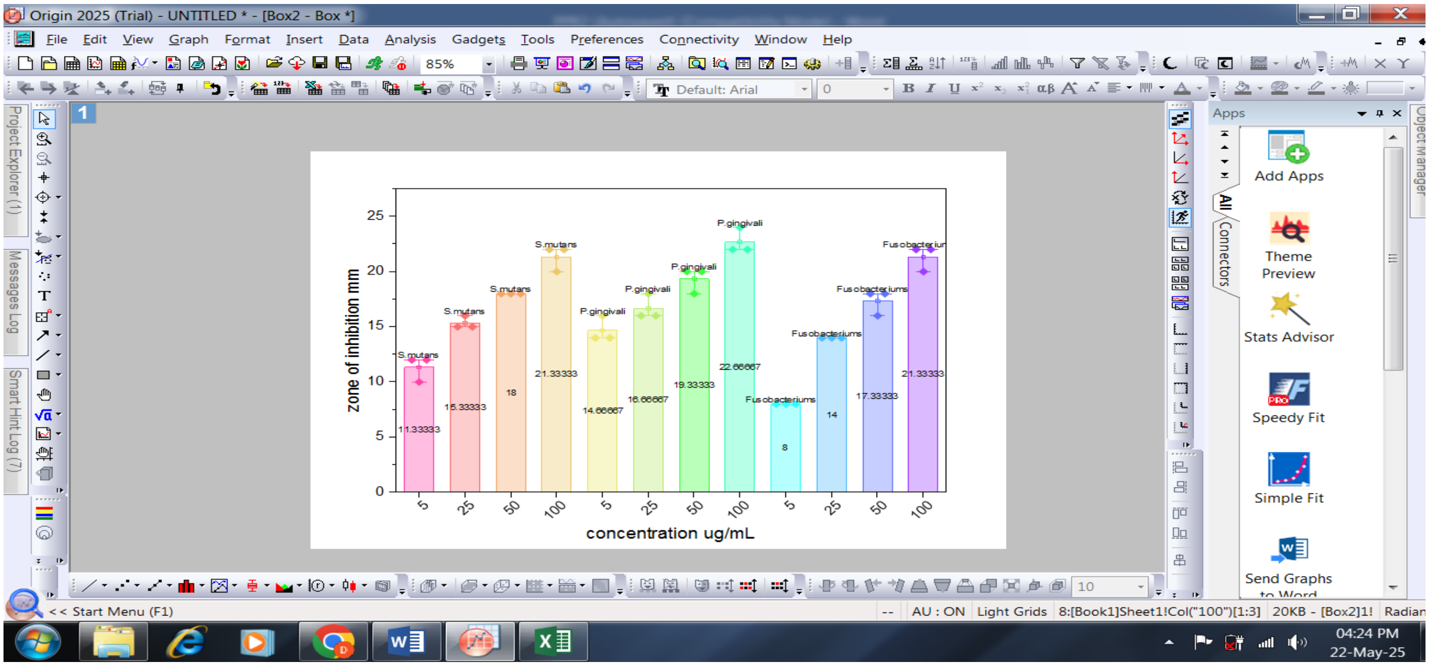

Figure 8Antibacterial activity of ZnO NP

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.

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