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Synthesis of zinc oxide nanoparticles using fruit peels of bottle guard and their biological potential.

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ABSTRACT

The growing concern of antibiotic resistance and the need for sustainable medicines have fuelled interest in plant-based nanotechnology. In this research, green synthesis of zinc oxide nanoparticles (ZnONPs) from kitchen waste in the form of bottle gourd fruit peels was investigated as a sustainable, eco-friendly, and cost-effective approach compared to traditional processes. The ZnONPs synthesized were tested for antioxidant, antidiabetic, anti-inflammatory, and antimicrobial properties and compared with crude plant extracts. The ZnONPs exhibited much improved bioactivity, with more powerful free radical scavenging, α -amylase inhibitory, and anti-inflammatory activities than crude extracts. The enhanced bioefficacy is due to the surface area and reactivity of nanoparticles and synergistic effects of bioactive compounds in bitter gourd peels. This research is revealing a double advantage: the valorization of kitchen waste and the promotion of sustainable nanotherapeutics. The discovery proposes ZnONPs prepared from fruit peels as potential agents for fighting oxidative stress, diabetes, and microbial infections and promoting green nanomedicine development.

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INTRODUCTION:

Because of the special qualities of materials at the nanoscale, nanotechnology has transformed a number of scientific disciplines. Because of its many uses in electronics, catalysis, cosmetics, and biological domains including antibacterial agents and drug delivery systems, zinc oxide nanoparticles, or ZnONPs, have been highly prized (1). However, traditional ZnONP synthesis techniques use hazardous chemicals that can be harmful to human health and the environment (2).

By avoiding the use of hazardous chemicals and providing a sustainable way for producing nanoparticles, green synthesis techniques—which use natural items like plant extracts—have become an environmentally friendly substitute (3).

The synthesis of ZnONPs using fruit peels and other plant materials has been investigated in a number of research. For instance, ZnONPs with superior antibacterial qualities have been effectively synthesized using extracts from aloe vera and magnolia leaves (4,5). Fruit peels, which are frequently discarded, are excellent candidates for green synthesis because they are high in flavonoids, phenolic compounds, and antioxidants (6). In particular, it is known that the peels of bottle guard (*Lagenaria siceraria*) contain bioactive substances such as flavonoids, carotenoids, and polyphenols that can function as stabilizing and reducing agents during the creation of nanoparticles (7).

The green synthesis of ZnONPs utilizing bottle guard peel extract is the main focus of the current

investigation. This work assesses the biological activity of the generated ZnONPs, including their anti-inflammatory, antibacterial, antifungal, antidiabetic, and antioxidant qualities, in addition to their environmentally friendly manufacture. The findings of this work will aid in evaluating the possible environmental and biomedical uses of ZnONPs produced using environmentally friendly processes.

MATERIALS AND METHODS:

Synthesis of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles were synthesized using fruit peels of bottle guard through a green synthesis approach. The collected fruit peels were washed, dried, and ground into powder. An aqueous extract was prepared by boiling the peel powder in distilled water, which was then filtered. Zinc acetate solution was added to the extract, followed by heating and stirring to induce the formation of ZnONPs. The nanoparticles were then separated by centrifugation, washed, and dried for further analysis.

Characterization:

The synthesized ZnONPs were analyzed using FTIR analysis which was performed using a FTIR spectrometer (PerkinElmer 95163). The dried ZnONPs were mixed with KBr and pressed into pellets. The spectra were recorded in the range of 400-4000 cm^{-1} to identify functional groups associated with the nanoparticles. The morphology of the synthesized ZnONPs was examined using FESEM (Zeiss). A small amount of ZnONPs was mounted on a carbon tape and coated with gold before imaging to observe particle size and shape. EDX analysis was conducted in conjunction with FESEM to determine the elemental composition of the synthesized ZnONPs. The weight percentage of zinc and oxygen was obtained to confirm the successful synthesis of ZnO. The crystalline nature of ZnONPs was evaluated using XRD (Panalytical Xpert Pro) in the 2θ range of 20° to 80° . The diffraction patterns were compared with standard patterns (JCPDS Card No. 36-1451) to confirm the hexagonal wurtzite structure of ZnO. The stability of the synthesized ZnONPs was assessed using a zeta potential analyzer (Zeta sizer Nano ZSP; ZEN 5600). A dilute suspension of ZnONPs in distilled water was prepared, and the zeta potential was measured to determine the stability in aqueous media.

Antioxidant Activity:

The antioxidant activity of the ZnONPs and crude extracts was evaluated using the DPPH free radical scavenging assay. Various concentrations of the samples (200-1000 $\mu\text{g/ml}$) were tested, and the percentage of free radical scavenging activity was

calculated to determine the IC_{50} value(8).

Antidiabetic Activity:

The antidiabetic potential was assessed using an α -amylase inhibition assay. The ability of the ZnONPs and crude extracts to inhibit the enzyme was measured at different concentrations, and the IC_{50} value was determined (9).

Anti-inflammatory Activity:

The anti-inflammatory activity was evaluated using an albumin denaturation assay. The inhibition of protein denaturation by the ZnONPs and crude extracts was measured at various concentrations, and the IC_{50} value was calculated (10).

Antimicrobial Activity:

The antimicrobial activity was tested against various pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*, *Penicilliumchrysogenum*, *Alternaria alternata* and *Fusarium solani*. The inhibition zone (IZ) and activity index (AI) were measured at different concentrations (25-100 $\mu\text{g/ml}$). standard antibiotic streptomycin (for bacteria) and ketoconazole (For fungi) were used to determine activity index(11).

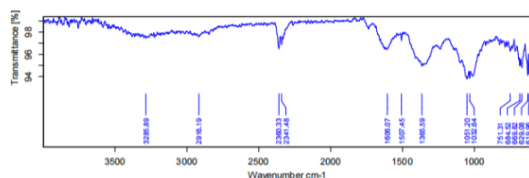
RESULTS:

Synthesis and Characterization:

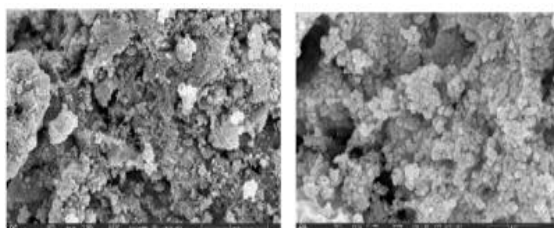
ZnONPs were successfully produced utilizing bottle guard peel extract in a green synthesis, and the resultant nanoparticles demonstrated impressive biological activity. Each result is explained in detail in this discussion, which also contrasts the results with those of earlier research and suggests potential mechanisms of action.

FTIR:

The bioactive substances from the peel extracts were found to be involved in the stability and reduction of the ZnONPs by the FTIR analysis. The presence of polyphenols, flavonoids, and other reducing agents is indicated by peaks corresponding to hydroxyl (-OH) and carbonyl (C=O) groups. These substances are essential for the reduction of zinc ions to ZnO nanoparticles. It is known that polyphenolic chemicals served as capping and reducing agents. These functional groups' interaction with ZnONPs stabilizes the nanoparticles and stops them from aggregating. FTIR analysis revealed the presence of functional groups that participated in the capping and stabilization of ZnONPs. Peaks corresponding to hydroxyl (-OH) and carbonyl (C=O) groups were observed around 3400 cm^{-1} and 1600 cm^{-1} , indicating the role of phenolic compounds in the reduction and stabilization process.

**FESEM and EDS:**

The Field Emission Scanning Electron Microscopy (FESEM) images revealed the spherical morphology of ZnONPs, with particle sizes ranging between 20–40 nm. The biological activities of nanoparticles are significantly influenced by their size and shape; smaller particles often show higher reactivity because of their bigger surface area.

**XRD:**

The X-ray diffraction (XRD) analysis of zinc oxide nanoparticles (ZnONPs) synthesized using different fruit peels confirms the crystalline nature of the synthesized materials. Bottle gourd ZnONPs showed peak positions at 24.7437° and 43.3136° , with the primary peak also corresponding to a high d-spacing value of $3.59822 \text{ \AA}$, although a broader FWHM indicates comparatively lower crystallinity. Overall, it was confirmed the hexagonal wurtzite structure of ZnO.

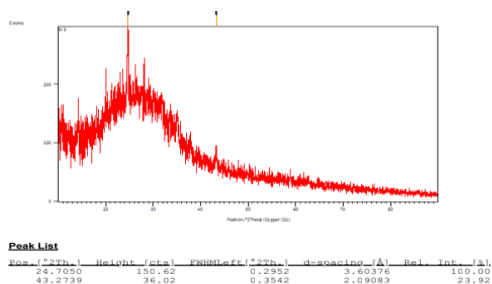
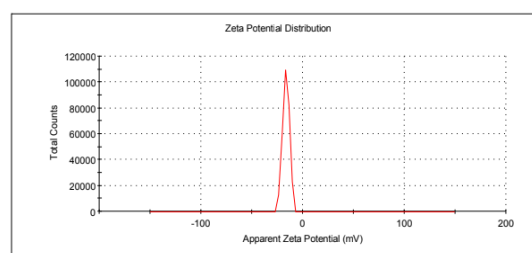


Table: Antioxidant potential of different fruit peel extracts and ZnONPs synthesized.

Name of samples	Anti-oxidant activity at different concentrations (µg/ml)					IC50 value (µg/ml)
	200	400	600	800	1000	
Extract	20.28±1.29	22.34±0.78	24.24±1.07	26.52±1.47	29.50±1.36	2875.80±274.40
ZnONPs	29.22±1.07	32.29±1.29	33.98±0.78	35.66±1.32	37.75±1.29	2187.06±156.09

Zeta Potential:

The zeta potential distribution spectra of zinc oxide nanoparticles (ZnONPs) synthesized using bottle guard peels indicated the colloidal stability and surface charge characteristics of the nanoparticles. Bottle gourd-ZnONPs presented a narrow peak slightly shifted toward the negative side, suggesting a uniform and moderately stable colloidal system. Overall, the zeta potential values of the synthesized ZnONPs confirmed that the nanoparticles possess sufficient surface charge, to ensure colloidal stability, with some variability based on the nature of the peel extract used for synthesis.

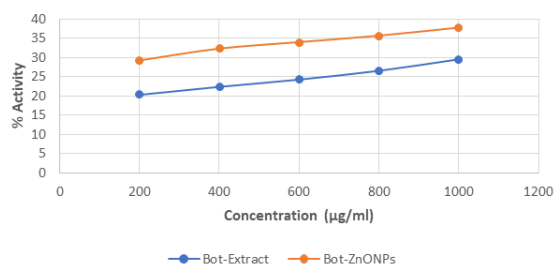
**Biological potential:**

In the current research, ZnONPs were prepared by using fruit peel extracts of plants belonging to the Cucurbitaceae family and were screened for their antibacterial, antifungal, antioxidant, antidiabetic, and anti-inflammatory activities. Biological activities of both the peel extracts and their respective ZnONPs were compared to determine the improvement in efficacy due to nanoparticle formation.

Anti-oxidant test:

Antioxidant potential was quantitated by employing the DPPH free radical scavenging assay. ZnONPs of bottle gourd extract had IC_{50} values ranging from 2187–2875 µg/ml, showing moderate antioxidant activity. With respect to antioxidant activity, as determined by DPPH radical scavenging assay, bottle gourd-ZnONPs also reflected a 23.99% increase in antioxidant activity.

Figure: % Free radical scavenging activity of the bottle guard extract and their nanoparticles.



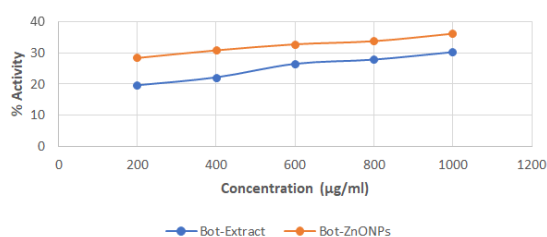
Alpha-amylase inhibitory assay showed that bottle gourd extract as well as respective ZnONPs presented moderate antidiabetic activities, with IC_{50} values in the range 2400–5900 µg/ml. For the antidiabetic activity determined through inhibition of alpha-amylase, bottle gourd-ZnONPs had no appreciable enhancement in antidiabetic activity with adverse values reflecting reduced efficacy.

Anti-diabetic test

Table: Alpha amylase inhibitory potential of different fruit peel extracts and ZnONPs synthesized.

Name of samples	Anti-diabetic activity at different concentrations (µg/ml)					IC ₅₀ value (µg/ml)
	200	400	600	800	1000	
Extract	19.63±0.20	22.18±0.22	26.52±0.20	27.88±0.25	30.31±0.17	2424.73±18.36
ZnONPs	28.40±0.22	30.87±0.17	32.81±0.29	33.82±0.20	36.23±0.20	2489.68±23.77

Figure 4.10: % alpha amylase inhibitory activity of the bottle guard extract and their nanoparticles.



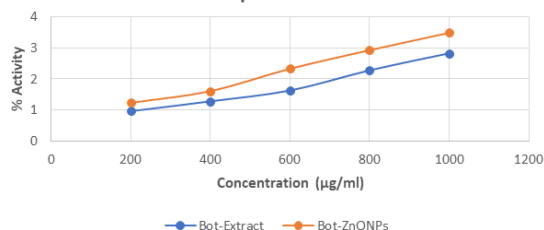
The anti-inflammatory activity was determined by inhibiting protein denaturation. Bottle gourd extract exhibited mild to low anti-inflammatory activities, and a slight improvement was observed following nanoparticle synthesis. Anti-inflammatory activity, determined by protein denaturation inhibition, indicated moderate enhancements in all ZnONPs. Bottle gourd ZnONPs had good enhancement with a 18.78% reduction in IC_{50} value over the extract.

Anti-inflammatory test

Table: Anti-inflammatory potential of different fruit peel extracts and ZnONPs synthesized.

Name of samples	Anti-diabetic activity at different concentrations (µg/ml)					IC ₅₀ value (µg/ml)
	200	400	600	800	1000	
Extract	0.96±0.28	1.28±0.21	1.63±0.23	2.28±0.21	2.83±0.15	20996.41±889.58
ZnONPs	1.23±0.19	1.61±0.18	2.33±0.13	2.93±0.10	3.49±0.19	17058.24±538.88

Figure 4.15: % anti-inflammatory inhibitory activity of the bottle guard extract and their nanoparticles.



with heightened inhibition by their ZnONPs. Antibacterial activity against *Escherichia coli* was improved considerably following the synthesis of ZnONPs in contrast with the crude peel extracts. Bottle gourd ZnONPs exhibited higher antibacterial effectiveness with 13.33% and 14.29% enhancement in zones of inhibition, respectively. All these results assure that nanoparticle synthesis significantly improved antibacterial performance against *E. coli*. Therefore, ZnONPs demonstrated a high rise in antibacterial activity from the crude extracts.

Antibacterial test

Against *E. coli*

The antibacterial activity of bottle gourd peel extracts and their respective ZnONPs was tested against *E. coli* at four different concentrations (25, 50, 75, and 100 µg/ml). Extracts of bottle gourd demonstrated moderate antibacterial activity but

Against *S. aureus*

Against *S. aureus*, Bottle gourd extracts contained IZs of 9–15 mm. Their ZnONPs were more effective, with inhibition zones as high as 16 mm and corresponding AI values > 0.42. For *Staphylococcus aureus*, a significant enhancement in antibacterial activity was noted after nanoparticle

formation. Bottle gourd ZnONPs also revealed a rise of 18.18%.

Against *P. aeruginosa*:

Bottle gourd exhibited relatively lower activity than their respective ZnONPs, with ZnONPs exhibiting IZs of 14–16 mm. ZnONPs synthesized from fruit peels also exhibited higher activity against *Pseudomonas syringae*. Bottle gourd ZnONPs also recorded increases of 42.86%. The data show that nanoparticle synthesis considerably enhanced the antibacterial activity against *Pseudomonas*

syringae.

Against *B. cereus*:

Against *Bacillus cereus*, Bottle gourd-ZnONPs also exhibited notable increases in inhibition zones with 30.00% improvements, respectively. The uniform increase for all samples tested indicates the effectiveness of green-synthesized ZnONPs in inhibiting *Bacillus cereus* growth. These findings validate that ZnONPs are more potent antibacterial agents against *Bacillus cereus* than peel extracts.

Table: Antibacterial activity of fruit peel extracts and the synthesized ZnONPs.

Name of bacteria		Activity at different concentrations (µg/ml)							
		25		50		75		100	
		IZ	AI	IZ	AI	IZ	AI	IZ	AI
<i>E. coli</i>	Extract	8	0.3333333	11	0.3666667	13	0.4193548	15	0.46875
	ZnONPs	10	0.4166667	13	0.4333333	15	0.483871	17	0.53125
	Std.	31		36		38		41	
<i>S. aureus</i>	Extract	10	0.32	14	0.4	15	0.41	16	0.42
	ZnONPs	13	0.41	18	0.51	19	0.52	20	0.52
	Std.	31		35		36		38	
<i>Pseudomonas syringae</i>	Extract	7	0.32	8	0.35	9	0.33	11	0.41
	ZnONPs	8	0.36	9	0.39	12	0.44	14	0.52
	Std.	22		23		27		27	
<i>Bacillus cereus</i>	Extract	7	0.23	8	0.22	10	0.26	11	0.27
	ZnONPs	9	0.29	11	0.31	15	0.39	16	0.39
	Std.	31		36		38		41	

Antifungal test:

Against *A. alternata*:

Antifungal efficacy against *Alternaria alternata* was evaluated at four concentrations (25, 50, 75, 100 µg/ml). Bottle gourd peel extract exhibited very little antifungal activity (7–9 mm IZ), but bottle gourd ZnONPs enhanced it to 8–12 mm IZs with AI of up to 0.52. The antifungal efficacy against *Alternaria alternata* was significantly enhanced following ZnONP synthesis. bottle gourd ZnONPs showed a 33.33% increase. These results indicated that ZnONPs were far more effective against *A. alternata* than the native plant extracts. Generally, ZnONPs prepared from peels had much better antifungal activity than the crude extracts.

Against *Penicillium chrysogenum*:

Bottle gourd extract did not have significant activity (7–9 mm IZ), whereas ZnONPs increased it to 8–11 mm IZs. Likewise, pumpkin ZnONPs exhibited increased inhibition up to 15 mm. For *Penicillium chrysogenum*, ZnONPs exhibited significant enhancement in antifungal activity.

Against *Aspergillus niger*

Against *Aspergillus niger*, Bottle gourd ZnONPs exhibited the most dramatic results, with inhibition zones of 32 mm at 100 µg/ml concentration and AI values of 1.45, the highest in all antifungal assays. Against *Aspergillus niger*, the improvement in antifungal activity was quite impressive. Bottle gourd ZnONPs registered a maximum 113.33% improvement in the inhibition zone value in comparison with bottle gourd extract.

Against *Fusarium solani*

The antifungal against *Fusarium solani* revealed that Bottle gourd and pumpkin ZnONPs also showed enhanced inhibition, pointing towards the fact that green-synthesized ZnONPs have improved antifungal activity than peel extracts.

The antifungal efficacy against *Fusarium solani* also enhanced following nanoparticle synthesis. Bottle gourd ZnONPs had a 44.44% improvement. Pumpkin ZnONPs also showed a 40.00% improvement. These findings indicate that green-synthesized ZnONPs significantly enhanced the antifungal activity against *Fusarium solani*.

Table: Antifungal activity of fruit peel extracts and the synthesized ZnONPs.

Name of fungus		Activity at different concentrations (µg/ml)							
		25		50		75		100	
		IZ	AI	IZ	AI	IZ	AI	IZ	AI
<i>Alternaria alternata</i>	Extract	NA	NA	7	0.35	8	0.38	9	0.39

	ZnONPs	8	0.44	9	0.45	11	0.52	12	0.52
	Std.	18		20		21		23	
<i>Penicilliumchrysogenum</i>	Extract	7	0.875	7	0.388889	8	0.380952	9	0.409091
	ZnONPs	7	0.875	8	0.444444	9	0.428571	11	0.5
	Std.	20		21		23		24	
<i>Aspergillus niger</i>	Extract	8	1	12	0.666667	13	0.619048	15	0.681818
	ZnONPs	9	1.125	21	1.166667	29	1.380952	32	1.454545
	Std.	8		18		21		22	
<i>Fusarium solani</i>	Extract	NA	NA	7	0.37	8	0.38	9	0.36
	ZnONPs	7	0.47	8	0.42	11	0.52	13	0.52
	Std.	15		19		21		25	

DISCUSSION:

The current study aimed to evaluate the antibacterial, antifungal, antioxidant, antidiabetic and anti-inflammatory activity of fruit peel extract as well as its biosynthesized zinc oxide nanoparticles (ZnONPs). For this, green nanoparticles were biosynthesized from bottleguard and the activities of the fruit peel extract was compared with their biosynthesized green nanoparticles. The first part of the study evaluated antibacterial activity of the fruit extracts and their biosynthesized ZnONPs against four different bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas syringae*, and *Bacillus cereus*. The results of the study showed that in all these cases, biosynthesized ZnONPs exhibited significantly greater antibacterial activity compared to their corresponding crude extracts.

Various phytochemicals such as flavonoids, terpenoids, alkaloids, and phenolics are adsorbed onto the nanoparticle surfaces during green synthesis of nanoparticles using plant extracts. Apart from stabilizing the nanoparticles, these groups contribute to antibacterial activity of the nanoparticles. They can enhance the interaction between nanoparticles and bacterial cells, leading to ROS generation, and possess their own antimicrobial properties.

Plant-derived phytochemicals such as flavonoids, phenolic acids, terpenoids, and alkaloids become adsorbed onto the nanoparticle surface. They facilitate efficient binding of nanoparticles to fungal cells, aid in ROS generation, and exhibit direct antibacterial and antifungal effects by inhibiting spore germination, impairing cell wall synthesis, and interfering with fungal enzyme activity (12-17).

Some plant-based nanoparticles exhibit catalytic activity, which can be leveraged to reduce oxidative stress. For example, the presence of certain metals in nanoparticles can catalyze the conversion of superoxide radicals into less harmful molecules such as hydrogen peroxide and oxygen, thereby mitigating oxidative stress (18-22).

Ability to inhibit alpha-glucosidase, another

enzyme involved in carbohydrate digestion. Inhibition of alpha-glucosidase slows down carbohydrate absorption in the intestines. Some plant extracts may improve lipid metabolism, helping to lower elevated levels of triglycerides and cholesterol, which are often associated with diabetes (23-25).

Presence of bioactive compounds that inhibit enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), which inhibit the production of pro-inflammatory mediators such as prostaglandins and leukotrienes. Plant extracts may suppress the expression of genes involved in inflammation, such as those encoding for COX-2, iNOS (inducible nitric oxide synthase), and various cytokines (26-28).

The findings of the current study demonstrate that zinc oxide nanoparticles (ZnONPs) synthesized from fruit peel extracts exhibit significantly greater bioactivity compared to their crude extracts across all tested parameters—antibacterial, antifungal, antioxidant, antidiabetic, and anti-inflammatory activity. The enhanced efficacy of ZnO NPs can be attributed to their nanoscale size, increased surface area, and improved cellular interactions, which collectively boost their biological performance. These findings not only highlight and validate the therapeutic potential of plant-based ZnONPs but also promote the sustainable use of fruit waste in the development of cost-effective, eco-friendly biomedical applications.

CONCLUSION:

This study demonstrated the potential of fruit peels in green nanotechnology by effectively synthesizing zinc oxide nanoparticles (ZnONPs) using musk melon peel extract. In comparison to the crude extract, the produced ZnONPs shown improved biological activities, such as anti-inflammatory, antibacterial, antifungal, antidiabetic, and antioxidant qualities. The findings imply that environmentally friendly ZnONP synthesis has potential for use in environmental and biomedical applications. In vivo testing and production scaling for industrial and pharmaceutical applications may be the main topics of future studies.

CONFLICT OF INTEREST:

Authors declare that there is no conflict for interest for the manuscript.

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