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Sustainable Green Synthesis of Zinc Oxide Nanoparticles with Clove and Cinnamon: A Study on Antioxidant and Cytotoxic Properties

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Abstract

This study explores the green synthesis of zinc oxide nanoparticles (ZnO NPs) using clove and cinnamon extracts as natural reducing agents. The eco-friendly approach minimizes toxic chemicals, making it a sustainable alternative to conventional methods. The synthesized ZnO NPs were characterized using UV, SEM, FTIR, XRD, and DLS techniques. Antioxidant activity was assessed via DPPH and nitric oxide scavenging assays, revealing significant radical scavenging properties. Cytotoxicity analysis against HepG2 cells demonstrated dose-dependent effects, with an IC50 value of 147.44 µg/mL, suggesting potential anticancer applications. These findings highlight the dual benefits of green synthesis—environmental sustainability and biomedical potential—positioning ZnO NPs as promising agents in pharmaceuticals, cosmetics, and environmental applications.

Keywords: ZnO NP, Green synthesis, Antioxidant activity, Cytotoxicity assay

1. Introduction

Nanotechnology is the science, engineering, and application of materials and devices at the nanoscale, typically between 1 and 100 nanometers. At this scale, materials often exhibit unique propertiessuch as increased strength, lighter weight, or enhanced chemical reactivitythat differ from their larger counterparts(1). Nanotechnology enables advancements like targeted drug delivery systems in healthcare, more efficient solar panels in energy, and faster, smaller electronics in computing. Its potential to revolutionize technology and improve quality of life makes it a rapidly growing and highly interdisciplinary field(2).

Due to their nanoscale size, zinc oxide nanoparticles exhibit unique physical and chemical properties, making them highly useful across various industries. These nanoparticles have significant potential because of their strong antimicrobial, UV-blocking, and semiconducting properties(2).

ZnO nanoparticles are widely used in cosmetics (especially sunscreens), electronics, sensors, coatings, and pharmaceuticals. In medicine, they are being explored for drug delivery, cancer therapy, and as antimicrobial agents(3). Their biocompatibility and low toxicity also make them suitable for biomedical applications. Additionally, ZnO nanoparticles possess photocatalytic properties, enabling their use in environmental applications such as water purification and pollution control.

The diverse applications of ZnO NPs make them an important focus of research in nanotechnology, but their environmental impact and toxicity at higher concentrations are still under study(4).

The green synthesis of zinc oxide nanoparticles (ZnO NPs) is increasingly favored due to its eco-friendliness, sustainability, and reduced environmental impact. Traditional methods of synthesizing ZnO nanoparticles often involve toxic chemicals, high energy consumption, and hazardous byproducts, posing risks to both human health and the environment. Green

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synthesis offers a safer and more sustainable alternative by using natural, renewable resources and environmentally benign processes[5]. Thus, green synthesis of ZnO nanoparticles aligns with the principles of sustainability, offering an environmentally responsible approach while maintaining the desired properties of ZnO NPs for diverse applications[3].

The green synthesis of nanoparticles using clove (*Syzygium aromaticum*) offers an eco-friendly and sustainable approach to producing nanoparticles, avoiding the use of harmful chemicals. Clove is rich in bioactive compounds such as eugenol, flavonoids, and tannins, which act as reducing and stabilizing agents during the nanoparticle synthesis process[6].

The green synthesis of nanoparticles using cinnamon (*Cinnamomum cassia*) is a sustainable and eco-friendly approach that leverages the natural reducing and stabilizing agents present in cinnamon. Cinnamon contains bioactive compounds such as cinnamaldehyde, polyphenols, and flavonoids, which act as reducing agents, converting metal ions into nanoparticles without the need for harsh chemicals[7].

2. Materials and Methods

2.1. Preparation of Plant extract using clove and cinnamon

In this approach, clove flower buds and cinnamon bark were utilized to prepare the plant extract. A total of 25 g each of clove buds and cinnamon bark were collected, thoroughly washed under running tap water, and dried in the shade. The dried materials were ground into coarse powder, and 1 liter of distilled water was added to the powdered mixture. The solution was then subjected to shaking at 20°C with a speed of 100 rpm for 24 hours. Afterward, the mixture was filtered, and the obtained extract was stored at 4°C[8].

2.2. Green Synthesis of zinc oxide nanoparticles

A volume of 25 ml of the plant extract was heated to 60°C for 10 minutes. Subsequently, 3 g of zinc nitrate hexahydrate was added to the heated extract, and the mixture was allowed to sit for one hour until a white precipitate formed. Thep resulting solution was transferred to a crucible and subjected to aeration at 65°C for 12 hours to create a creamy paste. This paste was then washed multiple times with a distilled water and ethanol solution to remove impurities. Finally, the paste was dried in a furnace at 300°C for one hour to produce green zinc oxide nanoparticles. The green synthesized ZnO nanoparticles were further evaluated for characterisation using UV, SEM, FTIR, XRD and DLS which showed the confirmation of nanoparticles synthesized[9].

a) Antioxidant activity of green synthesized ZnO nanoparticles

Antioxidant Potential[10]: The green synthesized ZnO solutions with varying concentrations were subjected to a 30-minute dark incubation period at room temperature after the addition of 1 ml of DPPH solution. To create a control, combine 1 ml of DPPH solution with 1 ml of methanol. At 517 nm, the absorbance of the solutions was finally measured with a UV-spectrophotometer. Using a graph showing concentration versus percentage inhibition, the extract's 50% inhibitory concentrations (IC_{50} values) were determined. The percentage of inhibition for radical scavenging activity was reported. The concentration required to capture 50% of the free radicals caused by DPPH in a sample is known as its IC_{50} value. Triplicate measurements were made. The extract's corresponding concentration at which their 50% radical scavenging capacity is demonstrated is indicated by their IC_{50}. The extract and standards IC_{50} values were visually computed, and the formula was used to compute the percentage of inhibition:

$$I\% = \frac{AC - AO}{AC} \times 100$$

Where, AO is the sample solution's absorbance, I% is the percentage of

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inhibition, and AC is the control's absorbance (1 ml methanol + 1 ml DPPH solution). The extracts' ability to scavenge radicals is shown by their IC_{50} values. The data was presented as three mean values (n = 3)± standard deviation.

b) Nitric oxide scavenging activity of green synthesized ZnO nanoparticles

Preparation of Reagents[11]:

In phosphate buffer (pH-7.4), 1% sulphonilamide, 0.1% N 1-naphthylethylenediamine, and 2% orthophosphoric acid are combined to create Griess reagent. Phosphate buffer saline (PBS), which is obtained by dissolving 2.38 grams of sodium hydrogen phosphate, 0.19 grams of potassium dihydrogen phosphate, and 8 grams of sodium chloride in 1000 millilitres of distilled water. To make 10 mM sodium nitropruside, dissolve 298.04 mg of sodium nitropruside in 100ml PBS. Prepare the extracts and standard (ascorbic acid) at a concentration of1 mg/ml using distilled water.

2.3. Procedure

One millilitre of samples was extracted at different concentrations (100-1000 µg/ml). Samples were incubated for three hours at 30°C with 1 millilitre of 10 mM sodium nitroprusside produced in phosphate buffer saline (pH 7.4) added. The samples were then diluted using 1 millilitre of Griess reagent. Using a UV Spectrophotometer, the absorbance was measured at 550 nm. Standard ascorbic acid underwent the same process, and the outcomes were contrasted with the extracts.

$$I\% = \frac{AC - AO}{AC} \times 100\%$$

Here, AO stands for the absorbance of the sample solution, I% for the percentage of inhibition, and AC for the absorbance of the control solution, which is made up of 1 ml of DPPH solution and 1 ml of methanol.

c) Cell cytotoxicity assay of green synthesized ZnO nanoparticles[12,13]

The assay was carried out according to Mosmann, 1983 by using (3- (4, 5-dimethyl thiazol-2yl) - 2,5- diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial Succinate dehydrogenaseand reductase of viable cells, yielding a measurable purple product formazan. This formazanproduction is directly proportional to the viable cell number and inversely proportional to the degreeof cytotoxicity. Briefly, trypsinized cells were seeded in 96 well plate and incubated at 37°C for 24 h. After 24 h incubation, the HepG2 cells were treated with samples in different concentrations for 12 -24 hours. After treatment, media were removed and wells were added with MTT (5 mg/ml preparedin phosphate-buffered saline) and left for 4 hours at room temperature. Thus, the formazan crystalsformed were dissolved in 100 µl DMSO, and absorbance was read in a microplate reader at 570 nm. About 1 mg of green synthesized ZnO nanoparticles was mixed with sterile complete media and further diluted to produce the desired concentration.

3. Results

3.1. Antioxidant activity of green synthesized ZnO nanoparticles

Table 1 anf Figure 1 depicts the results of DPPH assay. The radical scavenging activity (%RSA) increases with the concentration of the ZnO nanoparticles extract, indicating a dose-dependent antioxidative property. At 100 µg/mL, the RSA is 49.81%, which gradually increases to 84.87% at the highest concentration of 1000 µg/mL. At 800 µg/mL and 1000 µg/mL, the RSA values reach 79.13% and 84.87%, respectively. This suggests that the nanoparticles are highly effective in scavenging free radicals at higher concentrations. The highest RSA value of 84.87% at 1000 µg/mL suggests that these nanoparticles can serve as strong antioxidative agents at higher concentrations.

Figure 2 and Table 2 represents the nitric oxide scavenging assay. The RSA% increases steadily as the concentration of ZnO nanoparticles rises from 100 µg/mL to 1000 µg/mL. This indicates that the

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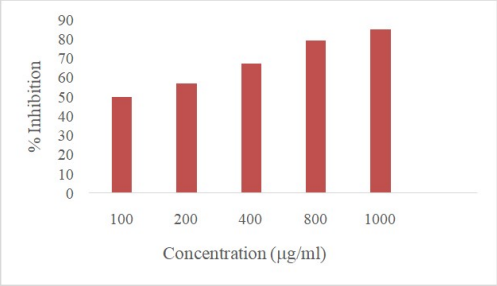

Fig. 1: DPPH Radical Scavenging Assay of green synthesized ZnO nanoparticles

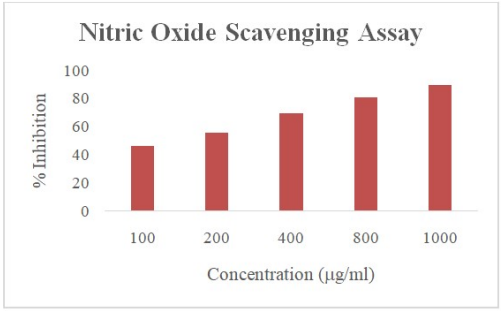

Fig. 2: Nitric Oxide Scavenging Assay of green synthesized ZnO nanoparticles

Table 1: Nitric Oxide Scavenging Assay of green synthesized ZnO nanoparticles	
Concentration of green synthesized ZnO nanoparticles (µg/ml)	RSA %
100	45.61
200	55.10
400	68.99
800	80.21
1000	89.00

Table 2: Nitric Oxide Scavenging Assay of green synthesized ZnO nanoparticles	
Concentration of green synthesized ZnO nanoparticles (µg/ml)	RSA %
100	45.61
200	55.10
400	68.99
800	80.21
1000	89.00

nanoparticles exhibit a dose-dependent ability to neutralize nitric oxide radicals. At 800 µg/mL and 1000 µg/mL, the RSA% values are close to or exceed 80%, showcasing strong radical scavenging potential. This result highlights the potential of these nanoparticles as antioxidative agents capable of neutralizing nitric oxide, a reactive species involved in oxidative stress and inflammation.

Table 3 depicts the cell cytotoxicity assay of Zinc Oxide Nanoparticles reinforced

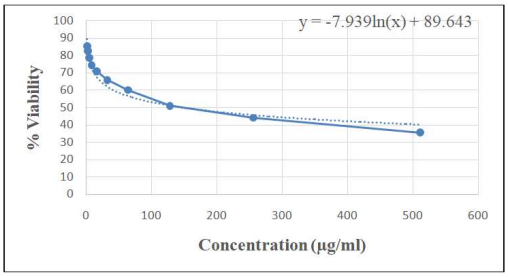

Fig. 3: Cell cytotoxicity assay of green synthesized ZnO nanoparticles

with clove and cinnamon extract. At the highest concentration (512 µg/mL), the average cell viability is approximately 35.50%, indicating substantial cytotoxicity. At 256 µg/mL, the viability increases to around 43.96%, showing a gradual reduction in cytotoxicity as concentration decreases. At 128 µg/mL, the average viability is about 50.98%, suggesting moderate cytotoxicity. This trend continues with increasing cell viability percentages (60.15%, 65.86%) at concentrations of 64 µg/mL and 32 µg/mL, respectively.

Figure 3 depicts that at the lowest concentrations (1–16 µg/mL), cell viability ranges from 70.88% to 85.51%, indicating minimal cytotoxic effects.The data suggests that the ZnO nanoparticles exhibit cytotoxic activity in a dose-dependent manner, with higher concentrations causing greater cell death and lower concentrations showing reduced effects.

Table 4 represents the IC_{50} value against HepG2 cell line. The IC_{50} value,

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Table 4: Cell cytotoxicity assay of green synthesized ZnO nanoparticles

Concentration (µg/ml)	% Viability	% Viability (Duplicate value)	% Viability (Triplicate value)	Average % Viability	Standard Deviation
512	35.562	35.470	35.457	35.496	35.496 ± 0.057
256	43.918	43.957	44.003	43.959	43.959 ± 0.042
128	50.984	50.938	51.017	50.980	50.980 ± 0.039
64	60.191	60.125	60.145	60.153	60.153 ± 0.033
32	65.818	65.857	65.916	65.864	65.864 ± 0.049
16	70.856	70.889	70.908	70.884	70.884 ± 0.026
8	74.468	74.520	74.553	74.514	74.514 ± 0.042
4	78.845	79.238	78.322	78.802	78.802 ± 0.459
2	82.313	82.640	82.902	82.619	82.619 ± 0.295
1	85.539	85.493	85.513	85.515	85.515 ± 0.022

Table 3: IC_{50}value against HepG2 cell line

Sample	Concentration (µg/mL) IC_{50}
Green synthesized ZnO nanoparticles	147.44

representing the concentration at which 50% of the cells are viable, is approximately between 128 and 256 µg/mL, based on the data provided. This value aligns with the reported cytotoxic potential of the nanoparticles in previous studies.The variability (indicated by standard deviations) is minimal, reflecting consistent results across replicates.

4. Discussion

The green synthesis of zinc oxide nanoparticles (ZnO NPs) using clove and cinnamon extracts provides an eco-friendly and sustainable alternative to conventional synthesis methods. The bioactive compounds present in clove (eugenol, flavonoids, tannins) and cinnamon (cinnamaldehyde, polyphenols, flavonoids) acted as natural reducing and stabilizing agents during nanoparticle synthesis, leading to the successful production of ZnO NPs with desired properties. This approach minimizes the use of hazardous chemicals, thereby reducing environmental and health risks, while aligning with the principles of green chemistry.

The antioxidant activity of the synthesized ZnO NPs was confirmed through DPPH and nitric oxide scavenging assays. The results demonstrated significant radical scavenging activity, with inhibition percentages increasing with concentration. The IC_{50} values calculated from the assays reflect the high antioxidant potential of the nanoparticles, supporting their potential applications in fields requiring antioxidative agents, such as biomedicine and cosmetics.

The cytotoxicity assay against the HepG2 cell line revealed a dose-dependent reduction in cell viability, indicating that the nanoparticles exhibit effective cytotoxic activity at higher concentrations. The IC50 value of 147.44 µg/mL highlights the potential of these nanoparticles as anticancer agents. This property, combined with their biocompatibility, suggests their applicability in cancer therapy and drug delivery systems.

The study highlights the dual benefits of green synthesis: environmental sustainability and the production of nanoparticles with significant biological activities. Furthermore, the bio-synthesized ZnO NPs offer a cost-effective and scalable solution for applications in pharmaceuticals, agriculture, and environmental remediation.

5. Conclusion

This study successfully synthesized ZnO nanoparticles using clove and cinnamon extracts, demonstrating an eco-friendly and sustainable approach. The nanoparticles exhibited promising antioxidant and cytotoxic activities, with potential applications in

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biomedicine, particularly in cancer treatment and antioxidative therapies. The findings underscore the importance of green synthesis as a sustainable alternative to conventional methods, aligning with global efforts to minimize environmental impact while addressing industrial and biomedical needs. Future studies could explore further functionalization and real-world applications of these nanoparticles to expand their utility across diverse sectors.

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