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Original Article

Green Synthesis and *in vitro* Insights of Zinc Oxide Nanoparticles Using Nutmeg and Flaxseed Extracts

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ABSTRACT

Background: Among various nanomaterials, Zinc Oxide Nanoparticles (ZnO NPs) have gained significant attention due to their unique properties. Traditional methods for synthesizing ZnO NPs often involve hazardous chemicals and energy-intensive processes, raising environmental and health concerns. In response to these issues, green synthesis methods have been developed as an eco-friendly alternative. Nutmeg and flaxseed are 2 plant sources known for their rich phytochemical content. This study aims to assess the cytotoxic, antimicrobial, anti-inflammatory and antioxidant properties of ZnO NPs synthesized with nutmeg and flaxseed. **Materials and Methods:** 5 mg of nutmeg and flaxseed powder were dissolved in 0.1 L of distilled water. This mixture was boiled at 60ºC for 10 min and then filtered. 0.1 g of ZnO was added to 100 mL of filtered extract. After centrifugation, the concentrated nutmeg and flaxseed extract-mediated ZnO NPs were reduced to 100 mL. Various extract concentrations (25 µL, 50 µL and 100 µL) were then subjected to cytotoxic, antimicrobial, anti-inflammatory and antioxidant assays. **Results:** Nauplii lethality was assessed over 2 days. At 25 µL, no mortality was observed on either day. However, on day 2, mortality increased by 10% at both 50 µL and 100 µL extract concentrations. The antimicrobial action of nutmeg and flaxseed extract-mediated ZnO NPs against *S. aureus, S. mutans* and *E. faecalis* increased with concentrations from 25 µL to 100 µL. Additionally, the anti-inflammatory and antioxidant activities of the NPs also increased with higher concentrations, from 25 µL to 100 µL. **Conclusion:** The synthesized nutmeg and flaxseed extract-mediated ZnO NPs exhibited significant bioactivity, including antibacterial, anti-inflammatory and antioxidant effects and were non-cytotoxic.

Keywords: Green synthesis, Nanotechnology, Nanoparticles, Plant extracts, Phytotherapy.

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INTRODUCTION

Nanotechnology is an emerging field that is revolutionizing numerous industries by enabling the manipulation of matter at the atomic and molecular levels.[1] This capability allows scientists and engineers to create materials and devices with novel properties and functions that are not possible with conventional technologies. The unique characteristics of nanomaterials such as increased surface area, quantum effects and enhanced mechanical, electrical and optical properties open up a wide range of applications. In medicine, nanotechnology promises advancements in drug delivery systems that target specific cells, leading to more effective treatments with fewer side effects.[2]

Zinc Oxide Nanoparticles (ZnO NPs) have shown several advantages in medical research due to their unique properties. ZnO NPs exhibit strong antimicrobial properties against a wide range of bacteria, fungi and viruses.[3] This makes them useful in developing antimicrobial coatings for medical devices, wound dressings and other healthcare applications. ZnO NPs can reduce inflammation by decreasing proinflammatory cytokines.[4] This property is beneficial in treating inflammatory diseases and developing anti-inflammatory drugs. ZnO NPs can be used as carriers for targeted drug delivery due to their ability to penetrate biological membranes. They can enhance the bioavailability and efficacy of drugs, reduce side effects and provide controlled release of therapeutic agents.[5]

"Green synthesis" refers to the development of nanomaterials using environmentally friendly methods that minimize the use of toxic chemicals and energy consumption. This approach offers several advantages, particularly in the context of nanotechnology, including environmental friendliness and compatibility for biomedical applications.[6] Nanomaterials synthesized through green methods are generally more biocompatible, making them suitable for medical applications such as drug delivery, tissue engineering and diagnostics.[7-9] Many herbs are utilized in the

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green synthesis of nanoparticles due to their rich content of bioactive compounds, such as polyphenols, flavonoids, alkaloids and terpenoids, which act as reducing and stabilizing agents.[10]

Myristica fragrans, commonly known as nutmeg, is a tropical evergreen tree belonging to the Myristicaceae family. Myristica fragrans has been used in traditional medicine for its various health benefits, attributed to its rich composition of bioactive compounds such as myristicin, eugenol and elemicin, which have been shown to exhibit anti-inflammatory effects. Nutmeg's anti-inflammatory action involves the suppression of pro-inflammatory cytokines and enzymes like Cyclooxygenase-2 (COX-2), which play a role in inflammation.[11]

Nutmeg is rich in antioxidants, including phenolic compounds and essential oils. These antioxidants help to neutralize free radicals, which are unstable molecules that can damage cells and contribute to oxidative stress. By scavenging free radicals, nutmeg helps to protect cells from oxidative damage, which is linked to aging and various chronic diseases.[12] Flaxseed, derived from the plant *Linum usitatissimum*, is renowned for its numerous health benefits, which are attributed to its rich content of Alpha-Linolenic Acid (ALA), a type of omega-3 fatty acid. Omega-3 fatty acid is known to reduce inflammation, lower blood pressure and improve overall health. Also, flaxseed contains lignans, which have antioxidant and anti-inflammatory properties.[13]

Literature evidence indicates various studies on the characteristics of green-synthesized ZnO nanoparticles.[14-16] However, no studies have specifically assessed the properties of ZnO nanoparticles synthesized using nutmeg and flaxseed extracts. In this context, this study aimed to evaluate the cytotoxic, antimicrobial, anti-inflammatory and antioxidant properties of ZnO NPs synthesized with nutmeg and flaxseed.

MATERIALS AND METHODS

Preparation of nutmeg and flaxseed extract

Nutmeg and flaxseeds were commercially purchased and ground into powder. 5 mg of nutmeg powder and 5 mg of flaxseed powder were dissolved in 0.1 L of distilled water. The mixture was then boiled at 60ºC for 10 min until bubbles started to form. After boiling, the solution was filtered through filter paper into a conical flask. The filtered extract was then transferred to an airtight container and refrigerated overnight.

Green synthesis of ZnO NPs

0.1 g of ZnO was added to 100 mL of the filtered extract and the mixture was placed in a shaker. Changes in color were recorded every 2 hr using a UV spectrophotometer. After approximately 36 hr, the extract was subjected to centrifugation at 7000 rpm for 10 min. The nanoparticle synthesis was conducted in a dark environment. The resulted nanoparticle solution was first characterized using UV spectroscopy, scanning from 200 to 600 nm with a UV spectrophotometer. Following this, the final reaction mixture was centrifuged in a Lark refrigerated centrifuge at 800 rpm for 10 min. The concentrated mixture of nutmeg and flaxseed extract-mediated ZnO NPs was reduced to 100 mL and stored in an airtight Eppendorf tube.

Topography analysis

Nutmeg and flaxseed extract-mediated ZnO NPs were analyzed for their morphological features using Scanning Electron Microscope (SEM). To prepare the samples for imaging, a sputter-coater was employed to apply a thin layer of gold at room temperature. Following this gold coating process, the nutmeg and flaxseed extract-mediated ZnO NPs were subjected to SEM analysis using a field-emission scanning electron microscope (JEOL JSM-IT 800; JEOL USA, Peabody, MA), to assess their overall structure.

Cytotoxic assay

Cytotoxicity was evaluated using the brine shrimp assay. Brine shrimp eggs were incubated in artificial sea water, prepared with sea salt, dry yeast and oxygen supplied by an aquarium pump. After 2 days of incubation at 22-29ºC, a light source was used to attract the nauplii to one side of the tank and they were collected with a Pasteur pipette. The nauplii were separated from the eggs by pipetting them into small beakers filled with seawater. Ten nauplii were placed in each well containing a Sodium Chloride (NaCl) solution. Different volumes of nutmeg and flaxseed extract-mediated ZnO NPs (25 µL, 50 µL and 100 µL) were added to the wells. A control well, containing only nauplii and NaCl solution, was included for comparison. The wells were left undisturbed for one day, after which the number of nauplii in each well was counted and recorded to assess the lethality of the extract.

Antimicrobial activity

The antibacterial activity of nutmeg and flaxseed extract-mediated ZnO NPs was evaluated using the Agar well diffusion method. The study involved testing the extract-mediated ZnO NPs against *S. aureus, S. mutans, E. faecalis* at various concentrations (25 µL, 50 µL and 100 µL). The plates were incubated at 37ºC for 1 day. Zones of inhibition were observed for antibiotic controls, with amoxicillin serving as the positive control for *S. aureus, S. mutans* and *E. faecalis*.

Anti-inflammatory activity

Anti-inflammatory activity of the prepared nutmeg and flaxseed extract-mediated ZnO NPs was done using Bovine Serum Albumin (BSA) assay. BSA denatures and expresses antigens when heated. Different amounts (25 µL, 50 µL and 100 µL) of nutmeg and flaxseed extract-mediated ZnO NPs were combined with 2 mL of the 1% BSA. To bring the pH of the reaction mixture down to 6.8, 1N hydrochloric acid was then used. The reaction mixture was incubated for 20 min at room temperature. After letting the mixture cool to room temperature, the absorbance at 660 nm was measured. The control used was diclofenac sodium in various concentrations. Percentage of inhibition was calculated using the following formula:

% inhibition = (Absorbance of control - Absorbance of sample) / Absorbance of control x 100

Antioxidant activity

In order to evaluate the prepared nutmeg and flaxseed extract-mediated ZnO NPs' antioxidant activity, the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay was utilised. Nutmeg and flaxseed extract-mediated ZnO NPs at different concentrations (25 µL, 50 µL and 100 µL) were mixed with 1 mL of 0.1 mM DPPH in methanol solution and 450 µL of 50 mM Tris HCl buffer (pH 7.4). The mixture was then incubated for 30 min. Afterwards, the decrease in DPPH free radicals was gauged by measuring the absorbance at 517 nm. As a control, Butylated Hydroxytoluene (BHT) was used. The percentage of inhibition was determined using the following formula:

% inhibition = (Absorbance of control-Absorbance of sample) / Absorbance of control x 100

RESULTS

Topography analysis

The structural morphology of the prepared nutmeg and flaxseed extract-mediated ZnO NPs was examined using SEM at 12X magnification. The image confirmed the presence of consistent size and spherical shaped nanoparticles indicating successful synthesis and uniform nanoparticle formation. Also, the nanoparticles were evenly distributed and clustered in aggregates (Figure 1).

Cytotoxic assay

Figure 2 illustrates the lethality of nauplii at various extract concentrations (25 µL, 50 µL and 100 µL) over two days. At a concentration of 25 µL, no nauplii mortality was observed on either day. However, on day 2, the mortality rate increased by 10% at both 50 µL and 100 µL extract concentrations.

Antimicrobial activity

Figure 3 shows the zones of inhibition produced by different concentrations of nutmeg and flaxseed extract-mediated ZnO NPs, as well as a positive control, against *S. aureus, S. mutans* and *E. faecalis*. For *S. aureus*, 25 µL, 50 µL and 100 µL of the extract resulted in zones of inhibition of 5 mm, 7 mm and 10 mm, respectively. Against *S. mutans*, these concentrations produced zones of inhibition of 6 mm, 8 mm and 11 mm, respectively. For

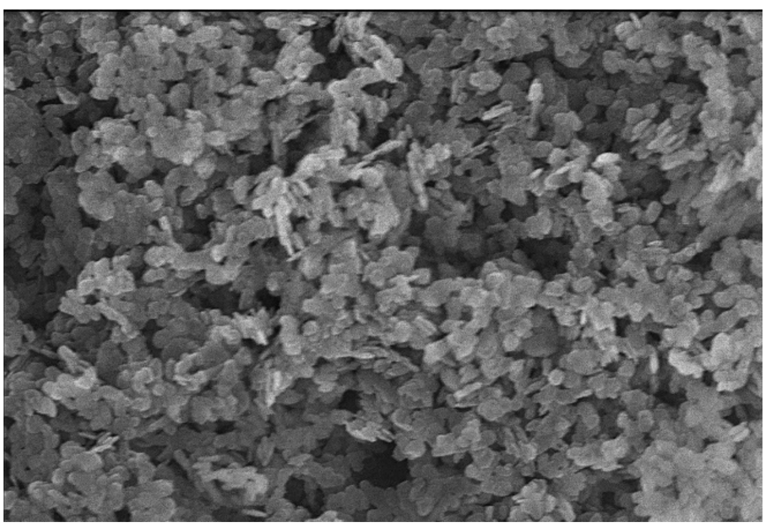

Figure 1: SEM image of nutmeg and flaxseed extract-mediated Zinc Oxide nanoparticles.

Table 1: Zone of inhibition against *S. aureus, S. mutans, E. faecalis*.

Concentration (µL)	Zone of inhibition (mm)		
	S. aureus	*S. mutans*	*E. faecalis*
25	5	6	5
50	7	8	9
100	10	11	11
Antibiotic	26	20	18

E. faecalis, the zones of inhibition were 5 mm, 9 mm and 11 mm for the same respective concentrations. The control antibiotic showed zones of inhibition of 26 mm, 20 mm and 18 mm against *S. aureus, S. mutans* and *E. faecalis*, respectively (Table 1).

Anti-inflammatory activity

Figure 4 demonstrates the significant anti-inflammatory effects of nutmeg and flaxseed extract-mediated ZnO NPs. The inhibition percentages for the NPs at concentrations of 25 µL, 50 µL and 100 µL were 40%, 55% and 70%, respectively, closely matching the control's inhibition rates of 45%, 60% and 80%. The anti-inflammatory activity of the NPs increased with higher concentrations, from 25 µL to 100 µL.

Antioxidant activity

According to the DPPH assay, the NPs were initially tested at a concentration of 25 µL, then subsequently increased to 50 µL and 100 µL. The antioxidant activity of the NPs increased with the rise in concentration from 25 µL to 100 µL (Figure 5).

DISCUSSION

The current study aimed to evaluate plant extract-mediated zinc oxide nanoparticles' cytotoxic, antimicrobial, anti-inflammatory and antioxidant properties. Nutmeg and flaxseeds were used in their synthesis. In recent years, modern advancements in nanoparticle synthesis have emerged, contrasting with methods used over the past few decades. Previously, nanoparticles were primarily produced using physio-chemical techniques. While traditional physical and chemical methods can generate large quantities of nanoparticles rapidly, they often rely on hazardous chemicals as capping agents for stability, leading to environmental toxicity.[17] Green nanotechnology, which utilizes plants, is increasingly recognized as an environmentally friendly alternative due to the cost-effectiveness of plant extract-mediated nanoparticle production.[18]

In the present study, the synthesized nutmeg and flaxseed-extract mediated ZnO NPs showed antimicrobial activity against *S. aureus, S. mutans, E. faecalis*. Thummaneni C *et al*., investigated the antibacterial effect of NPs generated from aqueous seed extract of nutmeg and suggested that the NPs exhibited significant antibacterial effect against gram-positive *Bacillus subtilis* and

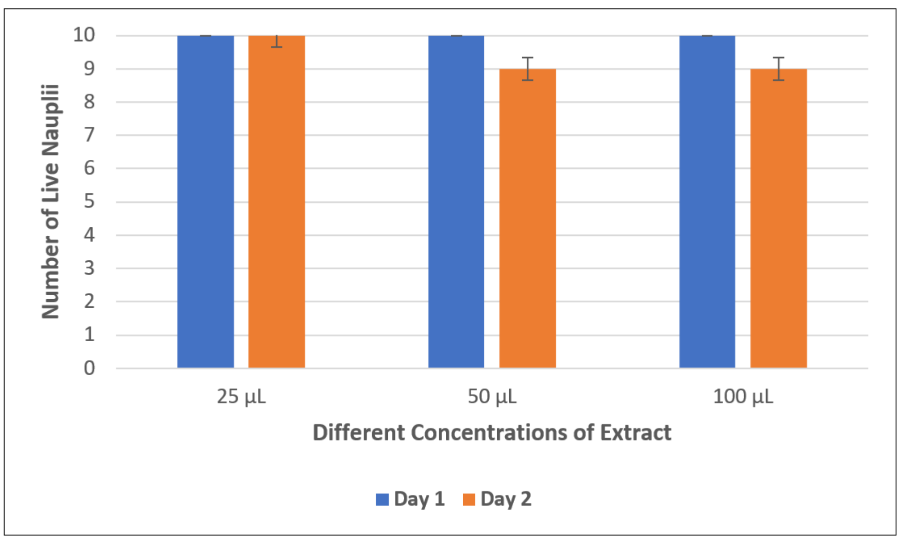

Figure 2: Lethality of the nauplii at different concentrations of extract.

Figure 3: Zone of inhibition using different concentrations of nutmeg and flaxseed extract-mediated ZnO NPs against *S. aureus, S. mutans, E. faecalis*.

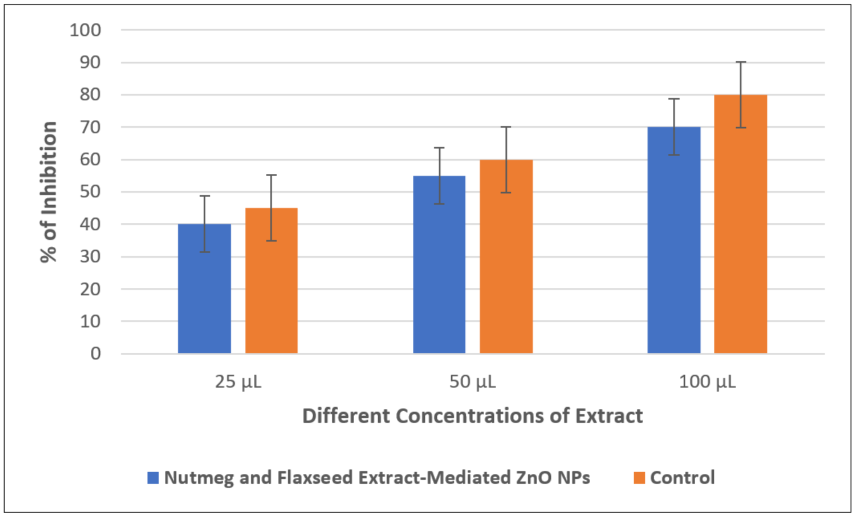

Figure 4: Comparison of anti-inflammatory activity between nutmeg and flaxseed extract-mediated zinc oxide nanoparticles and control.

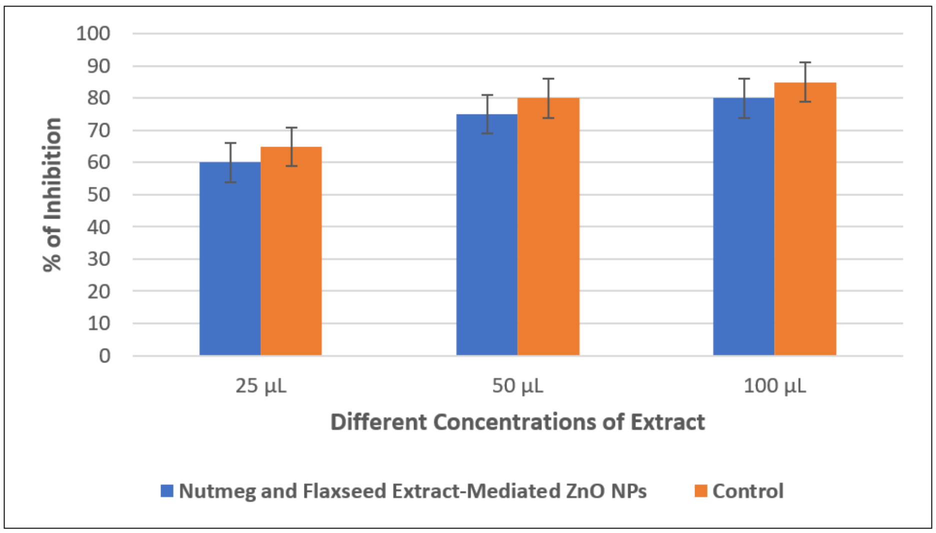

Figure 5: Comparison of antioxidant activity between nutmeg and flaxseed extract-mediated zinc oxide nanoparticles and control.

the gram-negative *Escherichia coli* bacteria.[19] In another *in vitro* analysis, using *Myristica fragrans* seed extract, metal nanoparticles were synthesised and their antibacterial efficacy evaluated against gram-positive and gram-negative pathogens. The results showed that *Myristica fragrans* seed extract-derived silver nanoparticles exhibited strong antibacterial activity.[20] Using the agar disc diffusion method and the free radical scavenging activity assay, edible films based on chitosan-flax seed were evaluated for their antibacterial and antioxidant properties, respectively. The outcomes demonstrated that it possessed strong antioxidant properties and showed potent antibacterial activity against pathogenic bacteria.[21] Similarly, utilising the disc diffusion method, the antibacterial properties of flaxseed extracts were assessed against four distinct oral pathogens: *Streptococcus pyogenes, Streptococcus mutans, Lactobacillus casei* and *Enterococcus faecalis*. By assessing the inhibition zone diameter, the inhibitory activities of flaxseed extracts against the tested oral pathogens were investigated. The study's findings indicated a considerable antibacterial activity.[22] Green synthesised zinc oxide nanoparticles made using flaxseed extract have demonstrated possible anticancer effects, according to *in vitro* research.[23] Furthermore, Sharbidre AA *et al.*, demonstrated a notable antibacterial efficacy of flaxseed-mediated silver nanoparticles against gram positive and negative bacteria.[24] The current investigation showed that nutmeg and flaxseed extract-mediated zinc oxide nanoparticles exhibited potent antibacterial activity comparable to the commercially available antibiotic. The present study's finding suggests that nutmeg and flaxseed extract-mediated zinc oxide nanoparticles might be used as an alternative for antibacterial drugs that are sold commercially.

According to the study's findings, nutmeg and flaxseed extract-mediated zinc oxide nanoparticles displayed strong anti-inflammatory effect. Myristicin is a molecule that may be responsible for nutmeg's anti-inflammatory properties. It functions by blocking growth factors, cytokines and chemokines that are triggered by macrophages.[25] Additionally, nutmeg seed extract compounds have the ability to reduce inflammation by inhibiting the release of proinflammatory cytokines.[26] Flaxseed is one of the best sources of polyunsaturated fatty acids.[27] Supplementation of flaxseed or its derivatives has been shown to result in decreased production of prostaglandin E2, thromboxane B2, 5-hydroxyeicosatetraenoic acid and leukotriene E4 by inflammatory cells. Also, flaxseed and its derivatives reduced the C-Reactive Protein (CRP) concentration, which is an indicator of inflammation.[28] Additionally, researchers demonstrated that flaxseed extract showed dose-dependent suppression of vascular permeability caused by protein exudation, which was similar to that of aspirin.[29]

The present study also demonstrated that the nutmeg and flaxseed extract-mediated zinc oxide nanoparticles possessed strong antioxidant activity and also non-cytotoxic. Rizwana H *et al.*, demonstrated a robust antibacterial, antifungal and cytotoxic activity of nutmeg-mediated NPs indicating that it might be used in the agrochemical industry, pharmaceutical industry and biomedical applications.[30] Faisal S *et al.*, suggested that the biosynthesized zinc oxide nanoparticles prepared from aqueous fruit extracts of*Myristica fragrans* were found to be excellent antioxidant and biocompatible nanomaterials. Biosynthesized ZnO NPs were also used as photocatalytic agents. Owing to their eco-friendly synthesis, nontoxicity and biocompatible nature, ZnO NPs synthesized from*M. fragrans* can be exploited as potential candidates for biomedical and environmental applications.[31] Additionally, the abundance of phenolic compounds such as lignan, Secoisolariciresinol Diglucoside (SDG) and ferulic acid in flaxseed contributes to its well-known antioxidant properties.[32]

Collectively, green synthesis of ZnO NPs using nutmeg and flaxseed extracts eliminates the need for harmful chemicals typically used in nanoparticle synthesis. This synergistic approach not only enhances the functional properties of ZnO NPs but also paves the way for innovative applications in healthcare. Also, this method is environmentally friendly, reduces chemical waste and aligns with sustainable practices.

CONCLUSION

In conclusion, the green synthesis and characterization of nutmeg and flaxseed extract-mediated zinc oxide nanoparticles were successfully demonstrated in this *in vitro* study. The nanoparticles exhibited promising structural and functional properties, as confirmed by various characterization techniques. The synthesized ZnO NPs showed significant bioactivity, including antibacterial, antioxidant, anti-inflammatory effects and non-cytotoxic. These findings suggest that nutmeg and flaxseed extract-mediated ZnO NPs hold potential for various biomedical applications, highlighting their importance in the development of eco-friendly and effective nanomaterials.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ZnO NPs: Zinc oxide nanoparticles; **COX-2:** Cyclooxygenase-2; **ALA:** Alpha-linolenic acid; **SEM:** Scanning electron microscope; **BSA:** Bovine serum albumin; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **BHT:** Butylated hydroxytoluene.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by Saveetha Dental College and Hospitals' Institutional Review Board.

SUMMARY

The study successfully demonstrated the green synthesis of ZnO NPs using nutmeg and flaxseed extracts. Characterization confirmed their promising structural and functional properties. The nanoparticles exhibited significant bioactivities, including antibacterial, antioxidant and anti-inflammatory effects, while being non-cytotoxic. These findings underscore their potential for eco-friendly biomedical applications.

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