

ANTI-INFLAMMATORY AND ANTIFUNGAL ACTIVITY OF ZINC OXIDE NANOPARTICLE USING RED SANDALWOOD EXTRACT

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ABSTRACT

Pterocarpus santalinus is valued for its strong, dark-purple, bitter heartwood. The gentle aroma of *P. santalinus* heartwood is caused by the presence of terpenoids, which gives it its color and fragrance. *P. santalinus* heartwood dye is used as a light microscopy stain, and as a coloring agent in pharmaceutical preparations. The Antibacterial, antimicrobial, and UV-blocking capabilities of ZnO NPs are outstanding compared with other metal oxides. The study aims to analyze the anti-inflammatory and antifungal activity of zinc oxide nanoparticles using red sandalwood extract. A plant extract sample was taken and 100ml of distilled water was added. Heated at 50°C for 5-10 minutes. The heated solution was filtered. 0.861g of zinc oxide was measured and dissolved in 70 ml of distilled water. The mixture was transferred to extract the sample. The solution was heated and labeled. The heating mantle was used with a temperature of 50-60°C and the time took was 6-8 minutes. The results obtained were statistically analyzed using spearman correlation analysis and using SPSS software. The sample shows an increasing absorbance rate and increases in the zone of inhibition with the increase in the concentration of the extract. It also showed an effective antifungal activity against *C.albicans*. The correlation analysis is statistically significant with p value<0.05. From the present study, it is concluded that red sandal-mediated zinc oxide nanoparticles possess anti-inflammatory and antifungal activity.

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Introduction

Natural herbal extracts of red sandalwood, botanical name *Pterocarpus santalinus*, have been used in therapeutics for centuries and are considered to have anti-diabetic, anti-inflammatory, antioxidant, and analgesic effects [1, 2]. In English, *Pterocarpus santalinus* L. f. (Fabaceae) is known as red sandalwood, but it also goes by other names in other languages. *Pterocarpus* is derived from the Greek words pteron (wing) and karpos (fruit), referring to the winged pod, while santalinus is derived from the Latin words sandal and inus (meaning similar to), referring to a plant that has characteristics similar to Indian sandalwood, Santalum album L [3, 4].

P. santalinus (also known as African or Nepalese sandalwood and Indian sandalwood) is valued for its strong, dark-purple, bitter heartwood. The active components of Red Sandalwood, such as anthocyanins, saponins, tannins, isoflavonoids, terpenoids, and associated phenolic compounds, beta-sitosterol, and lupeol, are considered to be multifunctional in the treatment of various diseases [5-7]. The gentle aroma of *P. santalinus* heartwood is caused by the presence of terpenoids, which gives it its color and fragrance. *P. santalinus* heartwood dye is used as a light microscopy stain, and a coloring agent in pharmaceutical preparations, and the food, leather, and textile industries [7-10]. Many studies have taken place using red sandalwood-mediated zinc oxide nanoparticles. Various activities such as anti-diabetic, antioxidant, cytotoxicity, and anti-cancer activities have been done by many authors. The previous studies have faced challenges mainly in the method that has been adopted to synthesize the nanoparticles. The chemical method of synthesis has its disadvantages such as the distribution of the size and control of the deposit parameters but it's also a widely used technique as it is very cost-efficient, requires no chemical purification, and large-scale production of the nanoparticle can be achieved through this method [11, 12].

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Due to their unique physical and chemical properties, zinc oxide nanoparticles (ZnO NPS), one of the most common metal oxide nanoparticles, are widely used in a variety of fields [13-16]. ZnO NPs were first used in the rubber industry to provide wear resistance to rubber composites, increase the durability and strength of high polymers, and provide anti-aging properties, among other things. ZnO is increasingly used in personal care products, such as cosmetics and sunscreen, due to its good UV absorption properties [17]. ZnO NPs also have outstanding antibacterial, antimicrobial, and UV-blocking properties. Zinc is commonly recognized as an important trace element that can be found in all body tissues, including the brain, muscle, bone, and skin [18-20].

Zinc participates in the body's metabolism and plays important roles in protein and nucleic acid synthesis, hematopoiesis, and neurogenesis as the key component of various enzyme systems. Because of the small particle size of Nano-ZnO, zinc is more readily absorbed by the body [21]. As a result, nano-ZnO is widely used in food. Furthermore, the US Food and Drug Administration has listed ZnO as a "GRAS" (generally accepted as safe) substance (FDA) [22, 23]. ZnO NPs have gotten a lot of attention in biomedical applications because of these properties. ZnO NPs, which are relatively inexpensive and less toxic than other metal oxide NPs, have a wide variety of biomedical uses, including anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bioimaging [24-27].

This study aims to determine the anti-inflammatory and antifungal activity of red sandalwood-mediated zinc oxide nanoparticles.

Materials and Methods

Preparation of Plant Extract

Plant samples such as *Pterocarpus santalinus* were extracted from fresh or dried plant material. There was no bias and a random sampling method was adopted. Since the plant extract was already available in powdered form, the extract was directly prepared. 100ml of distilled water was taken in a measuring cylinder 1.008g of *Pterocarpus santalinus* was measured and taken using a weighing machine. (Figure 1) Then, the weighted powder is mixed with the distilled water that is taken in a conical flask. The solution is labeled and heated by using a machine called the heating mantle. The temperature of the heating mantle was set to about 50°C and the time is taken to heat is about 5-10 minutes. The heating solution is taken out of the heating mantle when there is an appearance of small bubbles. After the heating process, the heated solution is filtered using filter paper (Figure 2).

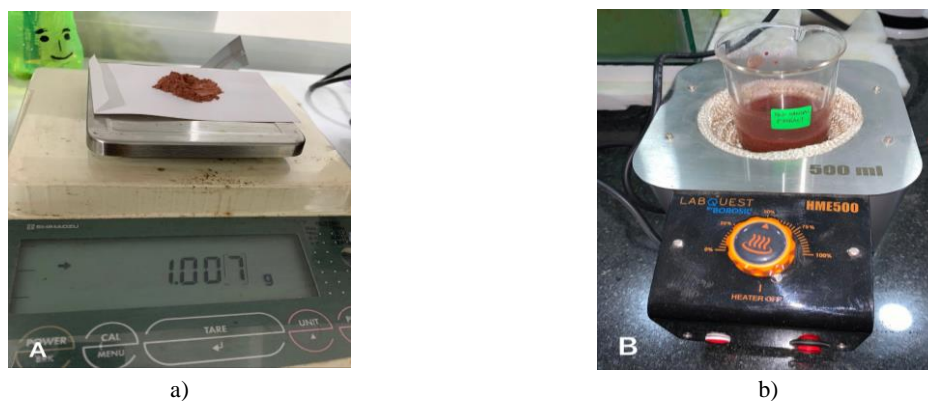


Figure 1. Image A depicts the measurement of red sandalwood powder and image B depicts the heating to prepare the red sandalwood extract.

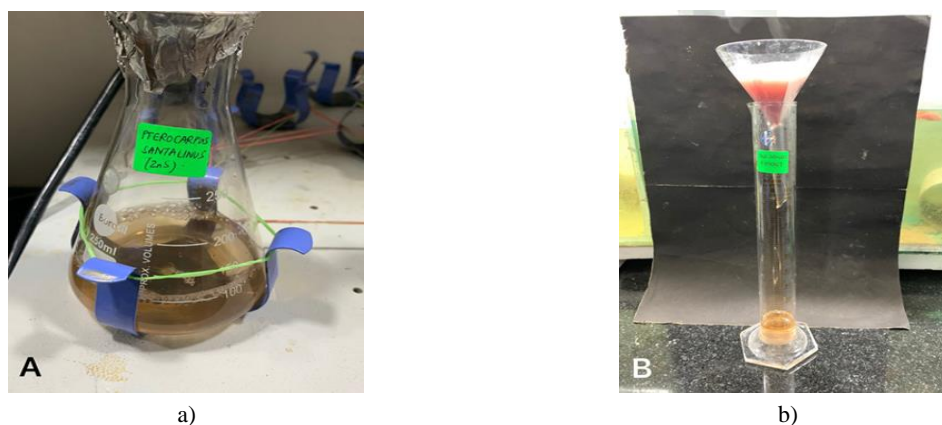


Figure 2. Image A depicts the extract being placed in the orbital shaker. Image B Depicts the filtration of the extract.

Preparation of Zinc Oxide Nanoparticle

0.861g of zinc oxide was measured and dissolved in 70 ml of distilled water (**Figure 3**). Now the calculated amount of zinc oxide and distilled water has been applied to the previously prepared plant extract. The solution was to be red. The measured powder which was mixed with distilled water was taken in a conical flask. The solution was heated and labeled. The heating mantle was used with a temperature of 50-60 degrees Celsius and the time taken was 6-8 minutes. The annealed powder was used as a sample for analysis.



Figure 3. Image A depicts the zinc oxide powder. Image B depicts 0.861gms of zinc oxide that was measured to prepare the nanoparticle.

Anti-Inflammatory Activity

Bovine serum albumin was used for the assay. 2 ml of bovine albumin was mixed with 400 microliters of zinc oxide nanoparticles in different concentrations was used as standard and then incubated at 55°C and then the results were analyzed spectrometrically.

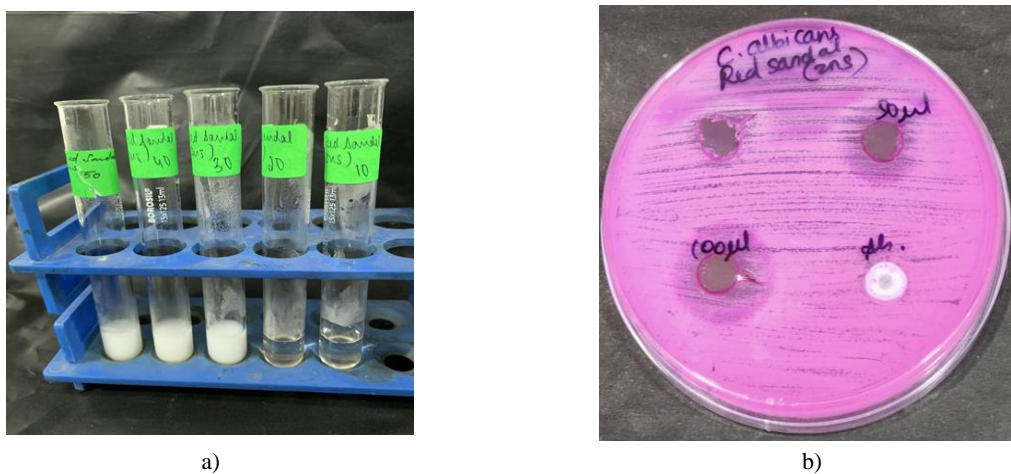


Figure 4. Image depicting samples with different concentrations of red sandalwood zinc oxide nanoparticles with bovine serum albumin to show anti-inflammatory activity (left side). Plate depicting Zone of inhibition of *C. albicans* with different concentrations of red sandalwood zinc oxide nanoparticle to show antifungal activity (right side).

Antifungal Activity

Candida albicans were used as a tested pathogen by agar well diffusion assay (**Figure 4**). Sabouraud's Dextrose Agar is used to prepare the medium. The prepared and sterilized medium was swabbed with test organisms and nanoparticles with different concentrations were added to the well. The plates were incubated at 28° C for 48-72hours. After the incubation time, the zone of inhibition was measured.

The results obtained were tabulated accordingly and their graphs were plotted using correlation analysis in the statistical software "SPSS version 23". The graphs thus collected were studied and analyzed. The data collected and studied were validated by the guide and the nanoparticle researcher.

Results and Discussion*Anti-Inflammatory*

Table 1. The table depicts the concentration and the absorbance rate obtained for the activity.

CONCENTRATION	ABSORBANCE
10 microlitre	0.092
20 microlitre	0.104
30 microlitre	0.195
40 microlitre	0.215
50 microlitre	0.265

Anti Fungal (C albicans)

Table 2. The table depicts the concentration and the zone of inhibition obtained for the activity.

CONCENTRATION	ZONE OF INHIBITION
25 microlitre	9mm
50 microliter	14mm
100 microliter	20mm
standard	12mm

Anti-Inflammatory

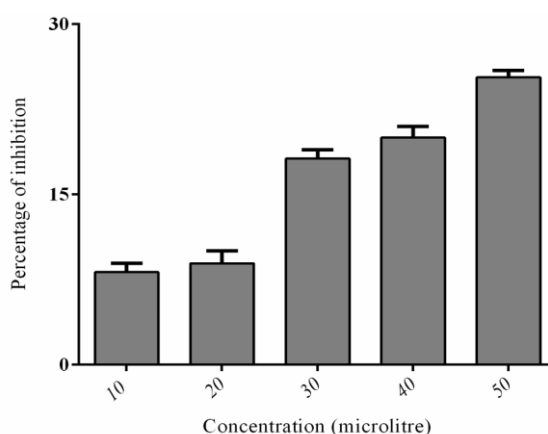


Figure 5. The above bar graph represents the increase in absorbance rate with an increase in concentration. The X-axis denotes concentration and Y axis denotes the percentage of inhibition, with a positive spearman correlation ($r=1$) with the rise in concentration

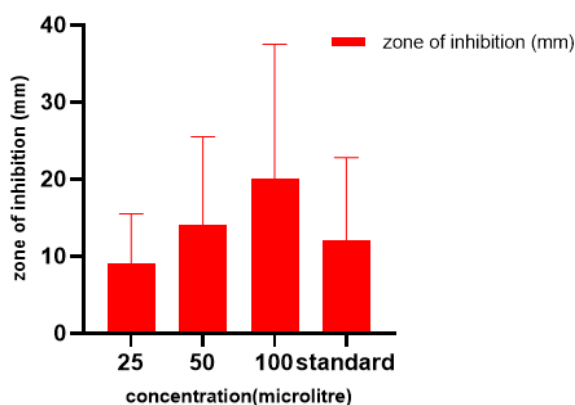


Figure 6. The above bar graph denotes the relation between concentration and increase in the zone of inhibition of *Candida albicans*.

The test for anti-inflammatory activity was assessed using bovine serum albumin. 2 ml of the bovine albumin was added to the red sandalwood ZnO nanoparticle extract with the extract concentration ranging from 1 microlitre to 5 microlitres and the result was seen after incubation. The study showed a positive increase in the absorbance rate with the increase in the

concentration of the nanoparticle extract (**Figure 5**). The test for antifungal activity was assessed using the fungal pathogen, *Candida albicans* (**Figure 6**). The test was done using Sabouraud's dextrose agar in a sterile medium and the plates were incubated for 48-72 hours at 28 degrees Celsius and the zone of inhibition was obtained. The study showed positive results as the zone of inhibition of *Candida Albicans* showed a positive increase with an increase in the concentration of the extracted sample. From the results obtained from our study, it has been observed that the red sandalwood-mediated zinc oxide nanoparticle has a positive effect on anti-inflammatory and antifungal activity.

Nanomaterials are particles with nanoscale dimensions, are extremely small particles with increased catalytic reactivity, thermal conductivity, nonlinear optical efficiency, and chemical stability due to their wide surface area to volume ratio [28]. Nanoparticles have been incorporated into many consumer industries, including industrial, health, food, feed, space, chemical, and cosmetics, necessitating a green and environmentally friendly approach to their synthesis [29, 30]. Biosynthesis of nanoparticles is a method of synthesizing nanoparticles for biomedical applications using microorganisms and plants. This method is eco-friendly, cost-effective, biocompatible, safe, and environmentally friendly. Plants, bacteria, fungi, algae, and other species are all used in green synthesis [31]. They allow the development of ZnO NPs on a large scale without the addition of impurities. Biomimetic NPs have higher catalytic activity and needless costly and toxic chemicals to manufacture [32].

Similar studies have been done earlier with different fungal pathogens that have shown positive results. In the study by Saquib, because of the large-scale development, easy downstream processing, and economic viability, extracellular synthesis of NPs from the fungus is extremely useful. Fungal strains are favored over bacteria due to their higher resistance and ability to bioaccumulate metals [33-35]. SEM, TEM, and XRD analysis confirmed that NPs synthesized with *Candida albicans* had a similar size range of 15–25 nm. For the synthesis of ZnO NPs, *Aspergillus* species have been commonly used, and NPs synthesized from these fungal strains were mostly spherical [36, 37]. In the study by MD Jayappa, the anti-inflammatory activity of the ZnO NPs was assessed using the human red blood cells membrane stabilization method where the blood samples were collected without nonsteroidal and anti-inflammatory drugs (NSAIDs). This study showed positive absorbance of 560 nm for the anti-inflammatory activity [38, 39]. Several other studies have shown that the zinc oxide nanoparticle has been very effective and significant in antioxidant, anti-diabetic, and anti-cancer activities.

Other green sources for the synthesis of nanoparticles include biocompatible chemicals. It is a simple and cost-effective process that removes the production of any side products during the nucleation and synthesis of nanoparticles. With its well-dispersed existence, it results in the creation of nanoparticles of regulated shape and size [40]. The limitations of this study were mainly the sample size and the errors that might've been caused in the measurement of the concentrations in the extract. In the last decade, the biosynthesis of nanoparticles using an environmentally friendly approach has been a subject of study. For the synthesis of shape and size-controlled nanoparticles, green sources act as both a stabilizing and a reducing agent. Plant-based nanoparticles have a wide range of applications in the food, pharmaceutical, and cosmetic industries, and have thus become a major research subject [41, 42].

Conclusion

From the present study, it is concluded that red sandal-mediated zinc oxide nanoparticles possess anti-inflammatory and antifungal activity. In summary, this is a simple, effective biosynthetic method that could be used to substitute chemical and physical methods for large-scale ZnO-NP development. The use of such environmentally-friendly ZnO-NPs in a variety of fields, including medicine, catalysis, and drug delivery systems, makes this bioreduction method an excellent choice for large-scale synthesis.

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Conflict of interest: None

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Ethics statement: None

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