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# BIOGENIC ZINC OXIDE NANOPARTICLES FROM CITRUS FRUIT PEEL EXTRACT *(CITRUS LIMON)* AND ITS ANTIBACTERIAL ACTIVITIES

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**Abstract**

The study investigated the synthesis of zinc oxide nanoparticles (ZnO NPs) using citrus fruit peel extract and evaluated their antimicrobial properties. Citrus peel extract was combined with zinc oxide mixture and processed to obtain ZnO NPs, which were then characterized using SEM and XRD. Antimicrobial activity was assessed using the agar well diffusion method. Results demonstrated that ZnO NPs coated with citrus peel extract exhibited significant antimicrobial activity. Discussion highlighted previous research on the antimicrobial properties of citrus peel extract and the potential of nanoparticles as antimicrobial agents. The study concludes that biogenic ZnO NPs derived from citrus peel offer promising antimicrobial and antioxidant properties, suggesting potential applications in various medical fields. Moreover, the study suggests that these nanoparticles are comparable to standard antimicrobial agents like ascorbic acid. Overall, the findings underscore the potential of citrus peel-derived ZnO NPs as effective antimicrobial agents with wide-ranging medical applications.

**Keywords:** Public Health, Wellbeing, Novel, Citrus Limon, SEM, XRD, Antimicrobial Activity.

## INTRODUCTION

Man's requirements for food, clothing, shelter, flavorings, scents, and medicines have all been met by plants (Zhao et al. 2023). Ayurvedic, Unani, and Chinese, among other complex ancient medical systems, have their roots in plants. Some significant medications that are still in use today were developed by these medical systems (Jin et al. 2023). The African, Australian, Central, and South American medical systems, among others, are among the less well-known medical systems (Baer. 2015).

As old as humanity itself, herbal medicine has been used for healing. Written records, historical monuments, and even the original plant remedies provide enough proof of the relationship between man and his hunt for pharmaceuticals in nature dating back a very long time. Because of the many years spent fighting diseases, man has learned to look for pharmaceuticals in the bark, seeds, fruit bodies, and other parts of plants (Bhat et al. 2021).

As a consequence, he is now aware of the use of therapeutic plants(Raj, Martin et al. 2024). Citrus fruits are widely used because, in order to protect the host from harmful germs, the immune system is used. An immune system that isn't functioning properly makes a person more vulnerable to illnesses and permits diseases to worsen. Inflammation is one of the immune response's elements. Instances of severe or uncontrolled inflammation can harm host tissues and result in disease (Wong et al. 2023). Controlling oxidative stress is one method of reducing inflammation (Shakerinasab et al. 2023). Folate and Vitamin C, which both play important roles in maintaining the purity of immunological barrier and supporting the activity of several types of immune cells, including the phagocytes (cell eating), natural killer cells, B-cells, and T-cells, are both abundant in citrus fruit juices and are excellent sources of these nutrients. Aspects of the response to inflammation are diminished by antioxidant vitamin C (Kaya et al. 2023)(Khalid, Martin et al. 2024). The significance of oxidation in our bodies and in food has long been understood. The primary mechanism by which cells survive is oxidative metabolism. The development of free radicals and other reactive oxygen species, which exhibit oxidative alterations, determines the consequences of many diseases (Hasanuzzaman et al. 2019). Citrus fruit drinks include significant amounts of hesperidin, narirutin, and naringin bioactive polyphenols (Li et al. 2020). As narirutin and naringin are carbohydrates of naringenin, hesperetin and hesperidin are glycosides of hesperetin.

In model systems, the anti-inflammatory properties of all of the following hesperidin, hesperetin, naringenin, naringin, and narirutin have been discovered (Tomás-Barberán et al. 2020). Human trials with hesperidin have also shown decreased levels of inflammatory markers. The action of different antioxidants provides defense mechanisms against the consequences of excessive oxidation, and the requirement to evaluate antioxidant activity is well documented (Ebenezer et al. 2020; Priyaa. 2020; Thota & Crans, 2018). Because of their widespread biological properties and potential uses in medicine, biomedical nanomaterials have recently attracted increased attention. Metal oxide nanoparticles have a vast range of potential uses in the biomedical area, including the delivery of antibacterial and anticancer drugs and genes, cell imaging, biosensing (Ebenezer et al., 2020)(Prathap and Lakshmanan 2022). Zinc oxide nanoparticles (ZnO-NPs), which are produced using nanotechnology and have an antibacterial effect, have drawn a lot of attention from researchers throughout the world. There are numerous microorganisms in the size range of a few micrometers to hundreds of nanometers (Asif et al., 2023)(Ambika, Manojkumar et al. 2019).

Due to their high specific surface area and higher particle surface reactivity brought on by their smaller particle size, ZnO-NPs have appealing antibacterial properties (Asif et al., 2023; Ramzan et al., 2023). ZnO is a biosafe substance that has effects on biological and chemical species through photo-oxidizing and photocatalysis. This review discussed ZnO-NPs antibacterial activity, testing procedures, the effects of UV irradiation, and ZnO particle characteristics (Arif et al., 2023)(Sundaram, Bupesh et al. 2022).

## MATERIALS AND METHODOLOGY

### 1. Preparation of Citrus extract

The extract of citrus fruit peel to reduce and stabilize zinc oxide nanoparticle's synthesis was done under aseptic conditions. Approximately 5g of powdered citrus peel was poured in a 500 mL of beaker which contained 250 mL of double distilled water. The extraction was accomplished by swirling continuously for 20 minutes at 60 °C on a hot plate with a magnetic stirring device. After cooling, Whatman No. 1 filter paper was used for a double filtration of the solution. Zinc oxide nanoparticles (ZnO NPs) were created using the yellow, clear, and aqueous citrus extract that was obtained.

### 2. 1,1, diphenyl-2-picrylhydrazyl assay (DPPH)

DPPH was used to assess the ability of plant extracts to scavenge free radicals. Different quantities of 0.1M (acetic acid) $CH_3COOH$ buffer with pH of 5.5 were added to plant extracts (0–1.0% (volume/volume)). Vitamin C (ascorbic acid) is used as a

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positive control (50-250 µg/mL). In a 6-well plate, sample of 40 µL were combined with 60 µL of ethanol (0.1 mM DPPH) for 30 minutes at 37°C and at 517 nm the absorbance was measured (Pažarauskaitė et al., 2023)(BABU and MOHANRAJ 2020). The computation of DPPH radical scavenging was done by the given formula:

Radical Scavenging effect (%) =[(Ao-A1)/Ao] x100

Absorbance

A0 - Control

A1 - Test sample.

## 3. Phyto-based fabrication of ZnO nanoparticles

20 mL of citrus peel extract and 20 mM zinc nitrate hexahydrate [Zn (NO3)] with volume of 80 mL were aggressively agitated in order to produce ZnO NPs. 6H20] was incubated for 24 hours after being stirred at 600 rpm on a magnetic agitator at 60°C for 4 hours. Centrifuging was done for 20 minutes at 10,000 rpm on the settled, muddy-colored precipitate. In order to get rid of contaminants attached to the nanoparticles, the pellet was thrice washed with distilled water. The pellet of pure zinc oxide nanoparticles was heated to 400°C in a muffle furnace for two hours, ground into a fine powder, and dried in an oven at 70 to 80°C. The obtained zinc oxide nanoparticles from *A. Citrus* peel extracts were referred to as ZnO NPs and kept in a antiseptic bottle. Advanced analytical and microscopic technologies were used to carry out the physicochemical characterization of nanoparticles (Abd Halim et al., 2023)(Velumani, Arasu et al. 2023).

## 4. Physicochemical characterization of ZnO NP’s

### 4.1. ZnO NP’s UV-visible spectroscopy

Citrus peel extract and zinc nitrate were well blended, and the color conversion was evaluated visually. The aqueous reaction solution's biological reduction of zinc ions into ZnO NP’s was examined by routine sampling of aliquots (3 to 4 mL). In a UV-Visible spectrophotometer, the greatest optical density was observed from 200 to 800 nm(Akshaya and Ganesh 2022).

### 4.2. FT-IR analysis of ZnO NP’s (Fourier transform infrared spectroscopy)

The distinct FTIR spectra created by the phytometabolites were used to confirm the existence of the functional groups, phytometabolites, and chemical bonds in Anilotica that reduced and stabilized the AN-ZnO NPs. Thermo Fischer Scientific's Nicolet iS50 FTIR was used to scan the sample with a wavelength range of 400 cm-1 to 4000 cm-1 at a resolution of 4 cm-1 after potassium bromide and zinc oxide nanoparticles were mixed to create the pellet in a hydraulic pellet machine.

### 4.3. X-ray diffraction

Through powdered XRD, the purity phase and crystallographic fingerprint of ZnO NPs were carefully evaluated. Using the previously described Debye-Scherrer formula, D = 0.892/Bcos, the crystallite dimension of the synthesized ZnO NPs was identified. Here, the full width at half maximum, wavelength of the used X-ray, crystallographic size (nm) of the nanoparticles, and Bragg's angle of diffraction were all indicated as B, B, and D, respectively.

### 4.4. SEM analysis of ZnO Nanoparticles

SEM JOEL 6390LA was used to analyze the surface textural characteristics of engineered ZnO NPs. A thin coating of ZnO NPs was applied on a copper grid that had been coated with carbon. This layer was allowed to dry under a mercury lamp, and then imaging was carried out under magnification.

### 4.5. Antimicrobial activity:

The antimicrobial activity was measured using previously reported methos (Ali et al., 2020). The agar well diffusion method was used to determine the antimicrobial activity. The 5g of nutrient agar was dissolved in 250 mL of distilled water and then autoclaved for 10 minutes. The nutrient agar medium was poured in the petriplates and kept it for some time for solidification. The microbial species *Candida albicans* and *E.coli* were spread on the agar medium and then well made with a well borer. The citrus extract sample was added in different concentrations (50 µL, 100 µL, 150 µL, 200 µL, and 250 µL). The petri plates were kept in a incubator for 24 hrs. Following 24 hr incubation, the zone of inhibition was determined.

## RESULTS

### DPPH radical scavenging activity

The assay of antioxidant activity using DPPH radicals as the basis for optical detection. This is a result of the fact that DPPH has been frequently employed to calculate the antioxidant activity of phytochemicals like polyphenols and flavonoids. The antioxidant in this experiment reduces the purple chromogen radical (DPPH) into the equivalent pale-yellow hydrazine (DPPH-H). When hydrogen-donating antioxidants lower the chromogenic purple radical (DPPH•), the radical's color on the antioxidant sensor changes from purple to a light yellow. This displayed antioxidant activity as a result of the OH group and effective hydrogen donors that stabilized the unpaired electrons and scavenged free radicals as a result.

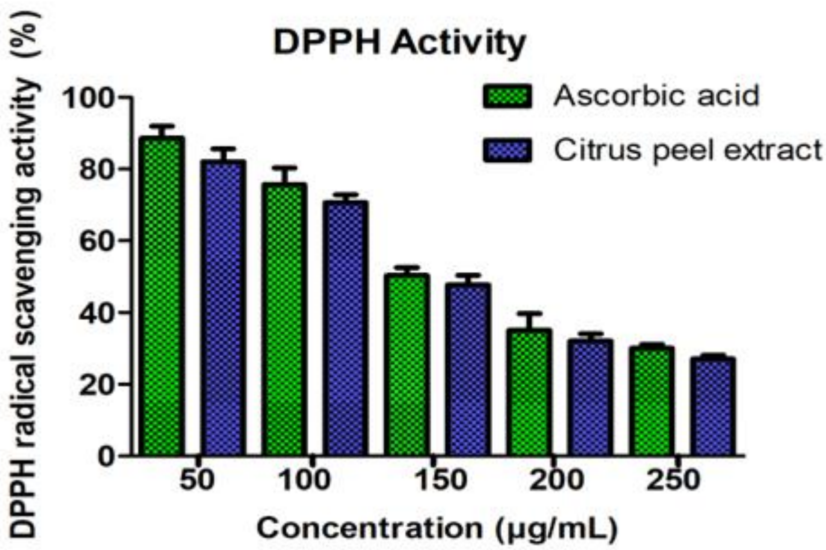


**Figure 1: The DPPH radical scavenging activity of citrus peel extract. Ascorbic acid is used as a standard**

According to the findings, ascorbic acid has more radical scavenging ability than the tested complexes at all doses. The concentration at which 50% of DPPH radicals are effectively scavenged is known as the IC50 value. The compound's capacity to operate as a DPPH scavenger is high when the IC50 value is low.

### Phytochemical analysis

A phytochemical test showed the different types of plant compounds (Thajuddin & Mathew, 2020) present in the extract, like tannins, phenolic compounds, saponins, protein and amino acids, carbohydrates, and alkaloids.

**Table 1: Qualitative analysis of the citrus peel extract**

| S.No | Phytochemical Analysis | Citrus peel extract |
|---|---|---|
| 1 | Tannins | Positive (+) |
| 2 | Phenolic compounds | Positive (+) |
| 3 | Saponins | Positive (+) |
| 4 | Oil and Fat | Absent |
| 5 | Protein & Amino acid | Present |
| 6 | Flavonoids | Absent |
| 7 | Carbohydrates | Present |
| 8 | Alkaloids | Present |

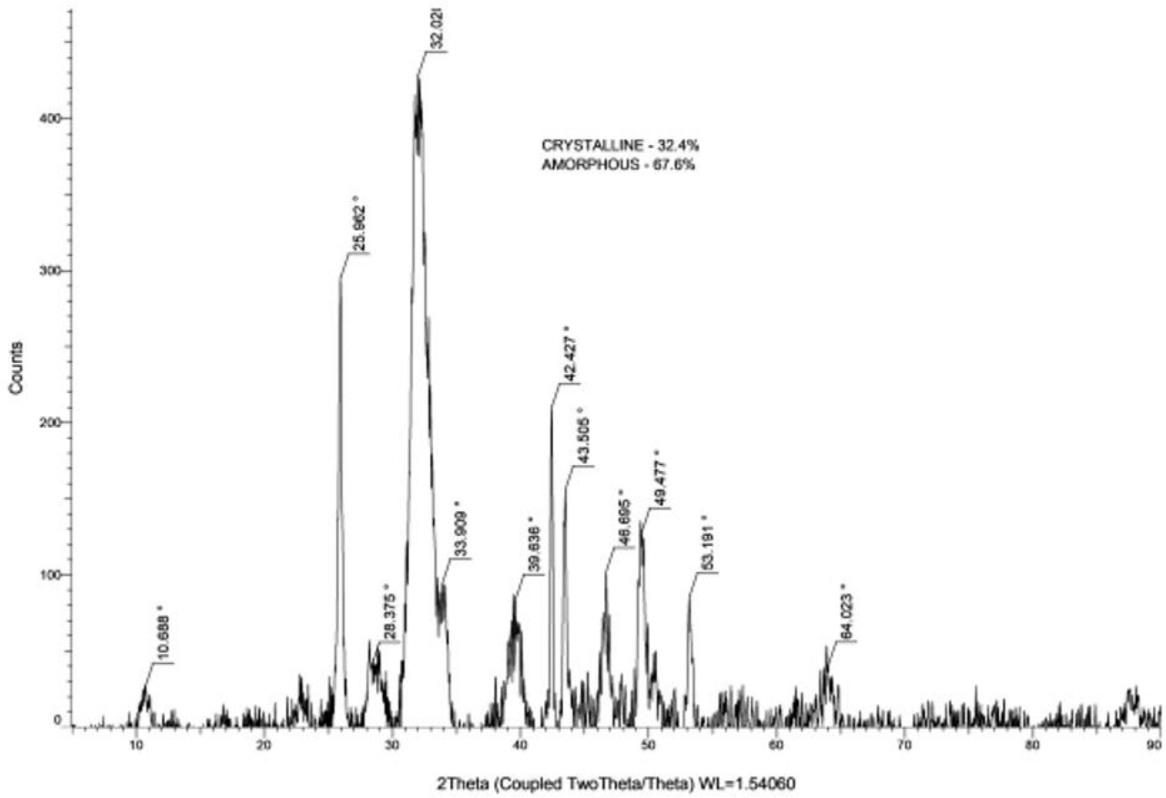


**Figure 2: XRD pattern of green synthesized ZnO nanoparticles in citrus peel extract**

The obtained diffraction patterns increased understanding of the ZnO NPs' phase chemistry, purity, and crystalline properties. Figure 1 represents 2θ degrees of 10.65, 25.9, 28.3, 32.02, 33.9, 39.83, 42.42, 43.5, 46.6, 49.4, 53.19 and 64.02, respectively.

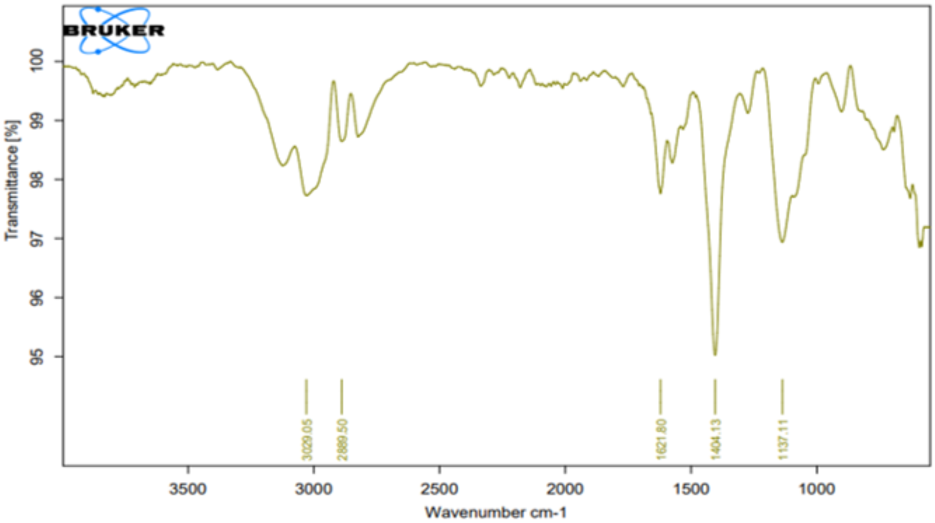


**Figure 3: FTIR analysis of ZnO NPs in citrus peel extract**

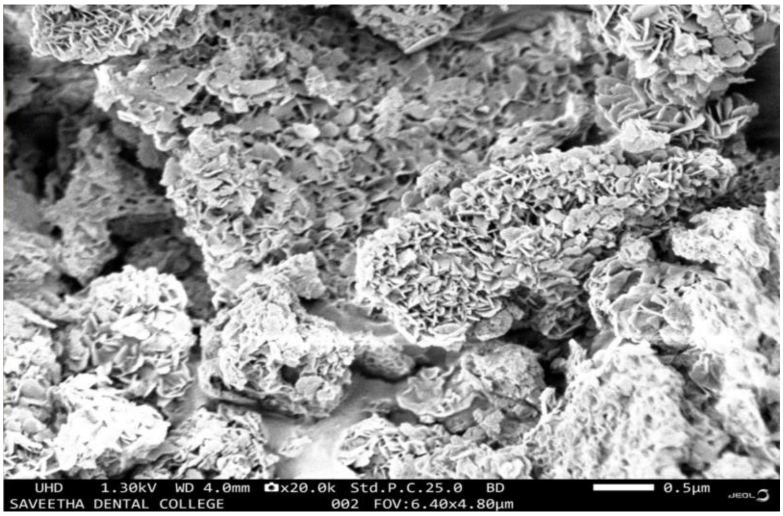


**Figure 4: SEM analysis of ZnO NPs in citrus peel extract**

Scanning electron microscope evaluation has been used to determine the morphology and size of ZnO nanoparticles. SEM examination shows a nanoplate structure with an average size of 33.5 nm for ZnO NPs.

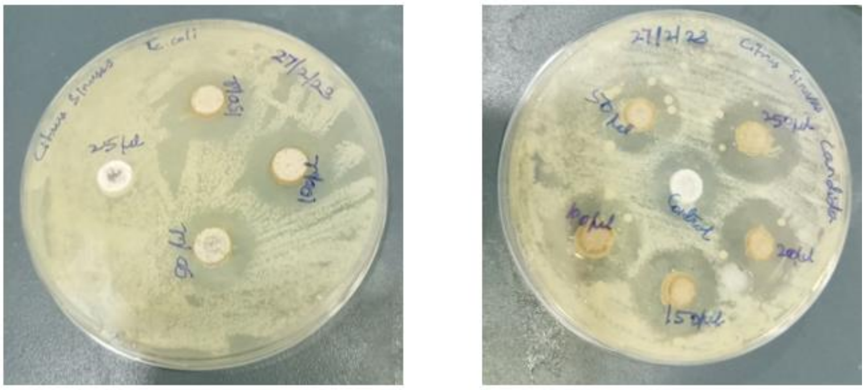

**Figure 3: Antimicrobial activity of green synthesized ZnO nanoparticles of citrus peel extract**

## DISCUSSION

Nanotechnology is a field of study that focuses on the design, characterization, manufacture, and development of materials that range in size from 1 to 100 nm by looking at them from an atomic and molecular perspective (Thajuddin & Mathew, 2020). By incorporating nanoscale structures into massive material components and systems, new goods and materials have been generated as a result of the widespread use of nanotechnology in all spheres of life.

Many disciplines, including chemistry, physics, and materials science, depend heavily on metal oxides (Sakthi Devi et al., 2022; . Tomás-Barberán et al., 2020). Aluminum oxide, titanium oxide, silica oxide, iron oxide, silver, and zinc oxide are just a few examples of the metal and metal oxide nanoparticles that are increasingly being used. Due to their vast use and unique nanotechnological characteristics, zinc oxide (ZnO) nanoparticles are generated by researchers in a variety of ways (Bhat et al., 2021; Khyrun et al., 2022).

A study on the antimicrobial activity of ZnO NPs was done by (Asif et al., 2023; Li et al., 2020; Varshan & Prathap, 2022), and their SEM results showed that the shape of ZnO NPs was found to be rod like structures, whereas in this study, ZnO NPs were combined with citrus fruit peel extract and had a nano plate like structure (Li et al., 2020; Willander, 2014). And to check the antimicrobial properties of the prepared substance two types of microbes were used and those were *Escherichia coli* and *Candida albicans*. And showed very good results proving that the prepared substance had excellent antimicrobial activity. There can be further studies done on the biocompatibility, in vitro studies, clinical trials based on these zinc oxide nanoparticles. The properties of this compound have vast scope in the medical field as it is antimicrobial, antioxidant, its biosensing properties, has the ability to deliver drugs etc(USHANTHIKA and MOHANRAJ 2020).

## CONCLUSION

This study concludes that citrus fruit peel extract coated with zinc oxide nanoparticle has DPPH radical scavenging activity and presence of phytochemicals like tannins, phenolic compounds, saponins, protein and amino acids, carbohydrates, and alkaloids. It also shows the antimicrobial property of this compound on *Escherichia coli* and *Candida albicans.* SEM shows the presence of zinc oxide nanoparticles which are found to have nano plate-like structures. XRD shows the amorphous nature of this compound. FT-IR shows the presence of different functional groups.

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