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Biosynthesis of Zinc Oxide Nanoparticles from Orange Peel Extract and their Antimicrobial Activity

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Abstract: The biosynthesis of zinc oxide nanoparticles (ZnO NPs) via an aqueous peel extract of *Citrus sinensis* represents a green and sustainable alternative to conventional synthesis methods, eliminating the need for toxic chemicals and diminishing energy consumption. Bioactive compounds present in the extract, such as flavonoids and polyphenols, serve as natural reducing and stabilizing agents during the nanoparticle formation process. Characterization via UV–VIS spectrophotometry confirmed the formation of crystalline and stable ZnO NPs. In this study, an aqueous orange-peel extract was employed as the biological reducing agent for the synthesis of ZnO nanoparticles from zinc nitrate as the precursor, and the influence of the precursor/extract ratio on the properties of the obtained particles was investigated. The antimicrobial activity of the synthesized ZnO NPs was tested against *Escherichia coli* and *Staphylococcus aureus*, with significant growth inhibition observed/particularly in the case of *E. coli*. The results obtained underscore the potential application of biosynthesized ZnO NPs in medicine, food preservation, and biodegradable packaging. This study confirms an efficient, eco-friendly and straightforward approach to the green synthesis of ZnO nanoparticles using orange peel extract.

Keywords: zinc oxide nanoparticles, orange peel extract, antimicrobial activity, green chemistry, Gram-positive bacteria, Gram-negative bacteria

INTRODUCTION

Nanotechnology, as one of the fastest-growing fields in contemporary science and engineering, has enabled the development of materials with exceptional physicochemical properties owing to structures of sub-100 nm dimensions. Nanomaterials manifest unique attributes in comparison to their macroscopic counterparts, rendering them applicable across a broad spectrum of industries - ranging from medicine, electronics and energy to food and environmental technologies (Kuzmec, 2017). Among them, zinc oxide nanoparticles (ZnO NPs) occupy a particularly significant role, characterised by high stability, chemical resistance and potent antimicrobial activity even at low concentrations. Unlike organic antibacterial agents, ZnO nanoparticles retain functionality under extreme conditions of temperature and pressure, which makes them suitable for applications in medicine, the food industry and environmental protection (*Brkiš, 2021*).

Traditional methods for the synthesis of ZnO nanoparticles frequently involve the use of toxic chemicals and demand high energy consumption, whereas green chemistry-based approaches offer an environmentally acceptable alternative. Plant extracts rich in polyphenols, flavonoids and terpenoids can function as natural reducing and stabilizing agents in the biosynthesis of nanomaterials. In this context, orange peel extract (*Citrus sinensis*) has proven particularly well-suited as a source of bioactive compounds for the synthesis of ZnO nanoparticles with pronounced antimicrobial properties.

The antimicrobial effect of ZnO nanoparticles is connected to their interaction with bacterial cell membranes, where structural differences between Gram-positive and Gram-negative bacteria influence microbial susceptibility. Gram-positive bacteria, with their thick peptidoglycan layer, and Gram-negative bacteria, which possess an additional outer membrane rich in lipopolysaccharides, respond differently to the action of metallic nanoparticles (*Steward, 2019*).

MATERIALS AND METHODS

The investigation comprised four main stages: the preparation of orange-peel extract, the biosynthesis of ZnO nanoparticles, the characterization of the synthesized nanoparticles and the assessment of their antimicrobial activity.

Preparation of Orange-Peel Extract

Fresh oranges of the Bahia variety were purchased at a local market in Sarajevo. The orange fruits were washed, dried and peeled. The peels were finely chopped to accelerate drying and then dried for 24 h at 60 °C. The dried peels were ground to a fine powder. For extract preparation, 3 g of the powder was extracted in 150 mL of distilled water while stirring at 60 °C for 2 h on a magnetic stirrer. The extract was subsequently filtered—first through qualitative filter paper and then through a blue-ribbon filter paper—yielding a clear extract. The extract was divided into three 50 mL portions.

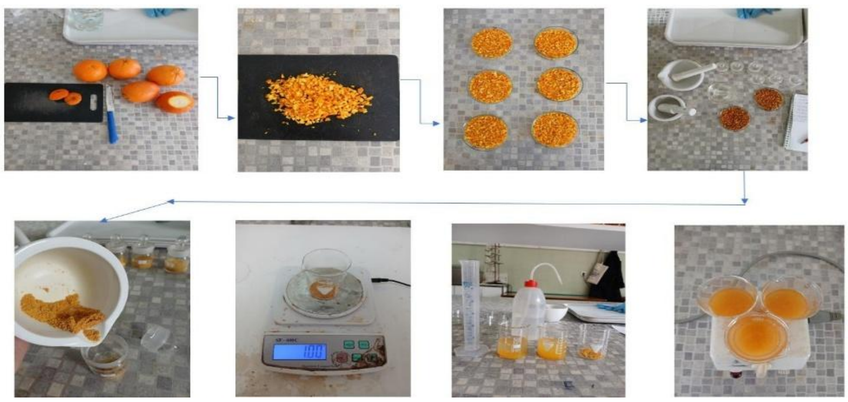


Figure 1: *Citrus sinensis* peel extract preparation

Biosynthesis of ZnO Nanoparticles

A 0.1 M solution of zinc nitrate hexahydrate was prepared. The zinc-nitrate solutions were mixed with orange-peel extract in volumetric ratios of 1:1, 1:2 and 1:4. The pH of the mixture was adjusted to 12 by addition of NaOH solution. The reaction mixtures were then stirred for 2 h at 60 °C. The synthesis of nanoparticles is based on the bio-reductive properties of the orange-peel extract.

Characterization of ZnO Nanoparticles

The UV-Vis spectra of the prepared nanoparticle suspensions were recorded on a Varian Cary 1E Spectrophotometer. The spectra were recorded in the range of 200–800 nm. Samples were measured in optical glass cuvettes with a 1 cm optical path length. Diluted orange-peel extracts in equivalent ratios were used as blanks, wherein the zinc-nitrate solution was substituted by distilled water.

Assessment of Antibacterial Activity

The antibacterial effect of the synthesized ZnO nanoparticles was determined using the disk-diffusion (Kirby–Bauer) method against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Mueller–Hinton agar medium was prepared by dissolving 38 g of agar in 1 L of distilled water, heated to boiling and poured into sterilized Petri dishes, then cooled to room temperature. The bacterial cultures were incubated for 24 h at 37 °C and standardized to 0.5 McFarland units. Sterile disks impregnated with nanoparticle suspensions were placed on the surface of the nutrient medium and incubated for 24 h at 37 °C. After incubation, the diameter of the inhibition zone around each disk was measured.

RESULTS AND DISCUSSION

Visual Confirmation

The synthesis of zinc-oxide nanoparticles was first confirmed by visual observation, based on the appearance of turbidity in the reaction mixture, which directly indicates the formation of ZnO nanoparticles. In the figure shown, the most pronounced turbidity was exhibited by sample A (ratio 1:1), suggesting the formation of a larger quantity of nanoparticles. It was observed that the quantity of nanoparticles formed was inversely proportional to the amount of orange extract used in the synthesis.

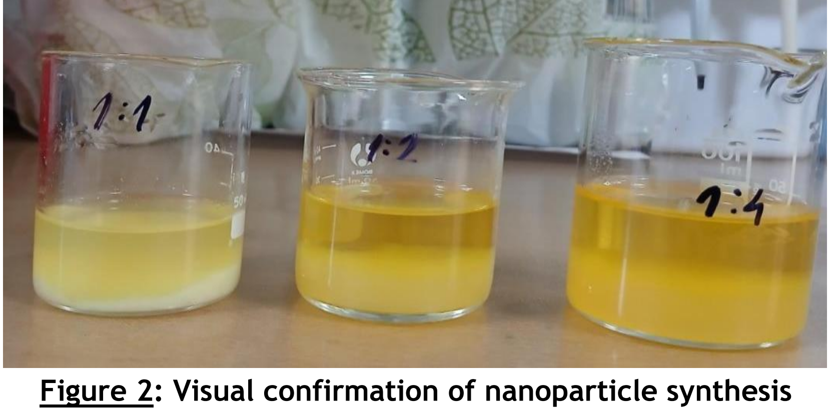
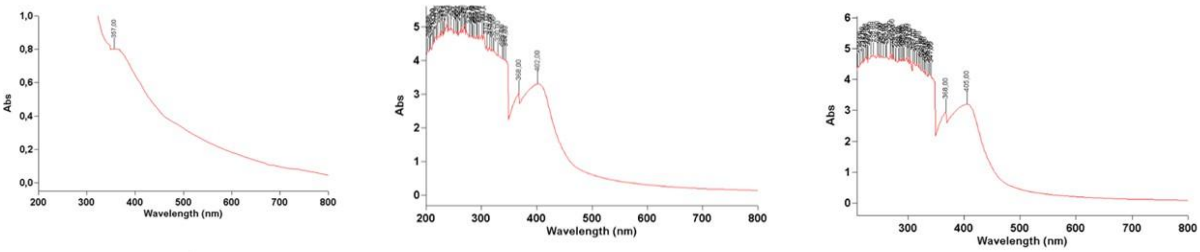


Figure 2: Visual confirmation of nanoparticle synthesis

Results of UV-VIS Spectrophotometry

Following visual confirmation, the synthesis of zinc-oxide nanoparticles was further verified by UV–VIS spectrophotometric analysis in the wavelength range of 200 to 800 nm. The spectra of the synthesised ZnO nanoparticles exhibited well-defined absorption maxima that varied depending on the reagent ratio used during the synthesis.

For the sample with ratio 1:1, an absorption maximum was detected at 357 nm, whereas the samples with ratios 1:2 and 1:4 exhibited maxima at 402 and 405 nm. These results confirm the presence of ZnO nanoparticles, while the wavelength shift indicates differences in particle size between the samples.



1:1 1:2 1:4

Figure 3: UV-VIS Spectra of ZnO manoparticles synthesized at different reactant ratios (1:1, 1:2, and 1:4)

The obtained absorption maximum of 357 nm for the 1:1 sample suggests smaller particle size. According to Chandrasekhar et al. (2016), ZnO nanoparticles synthesised without surfactant display an absorption maximum at 330 nm, which corresponds to a size below 10 nm. Given that in this investigation the maximum is somewhat higher, it is presumed that the particles are somewhat larger than 10 nm, yet still within the nanoscale. Similarly, Singh et al. (2019) report that a maximum at 380 nm corresponds to particles of size 30–40 nm. Therefore, this nanoparticle sample likely contains particles between 10 and 20 nm.

The maxima at 402 and 405 nm for the 1:2 and 1:4 samples indicate larger particles, aggregation or the presence of more oxygen defects in the crystal lattice. The absorption peaks in the 400-410 nm range suggest that the formed particles are larger than 50 nm (Abebe, Zereffa and Murthy, 2020).

The blue shift of the absorption maximum in the 1:1 sample can be attributed to quantum confinement, which arises when particle size becomes comparable to the excitonic Bohr radius of ZnO (~2.34 nm). With increasing particle size in the 1:2 and 1:4 samples, a red shift occurs, which further corroborates changes in dimensions.

In addition to wavelength, the intensities of absorption peaks were analyzed. Higher intensities in the samples with larger reagent ratios indicate higher concentrations of synthesized particles, i.e. more efficient synthesis. The width of the absorption peak (FWHM) was more pronounced in the 1:2 and 1:4 samples, which suggests greater polydispersity and the presence of particles of varying sizes. It should be noted that UV–VIS spectrophotometry does not enable direct determination of nanoparticle size, but only indirect conclusions based on optical properties. Accurate determination of dimensions and morphology requires additional characterization techniques (e.g., TEM, DLS, XRD).

Antimicrobial Activity

The results of the UV–VIS analysis indicated differences in the optical properties of the synthesised ZnO nanoparticles depending on the reagent ratio, which implies possible differences in biological activity. Accordingly, the antimicrobial activity of the samples was further investigated to assess their potential.

The largest inhibition zone for E. coli was shown by the 1:1 sample (average 26.5 mm), indicating strong antimicrobial effect. Samples with ratios 1:2 and 1:4 exhibited smaller inhibition zones — 15.5 mm and 10.5 mm respectively — which by standards are classified as bacterial resistance to the tested samples. As a standard for comparison the antibiotic ceftriaxone (CTX) was used, with values above 21 mm considered to denote strong inhibitors.

In comparison with ciprofloxacin (CIP), a reference antibiotic, it was confirmed that the 1:1 sample display pronounced antimicrobial activity, while the 1:2 and 1:4 samples remain below the threshold of moderate activity (16–21 mm).

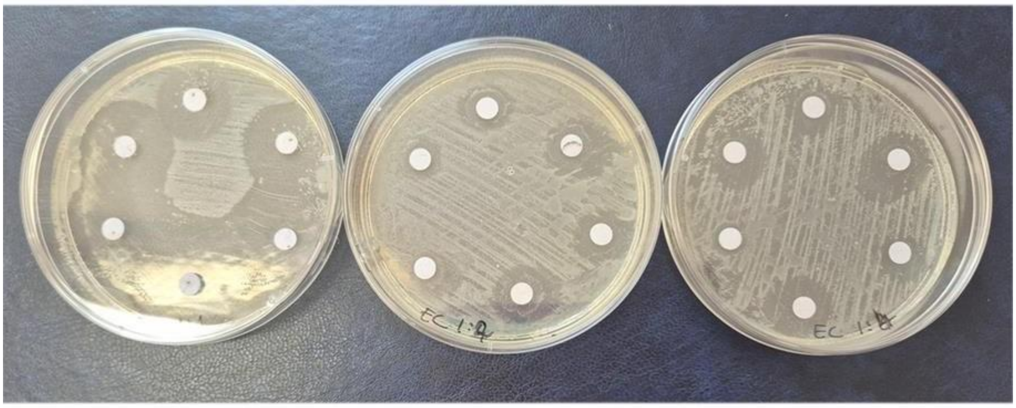


Figure 4: *E. coli* - inhibition zones

No measurable inhibition zones were recorded for *S. aureus,* which indicates that the synthesised ZnO nanoparticles do not exert inhibitory action on the growth of Gram-positive bacteria.

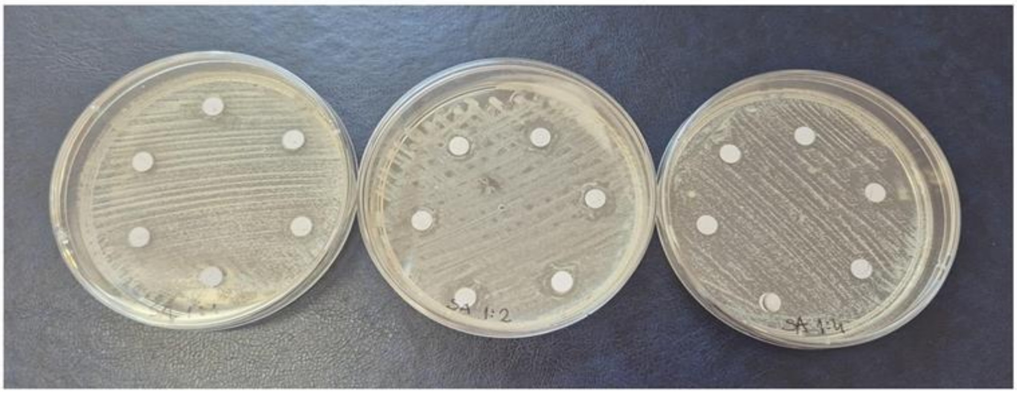


Figure 5: S. aureus - inhibition zones

According to Doan Thi et al. (2020), examined ZnO nanoparticles exhibited strong activity against *E. coli* and *S. aureus*, which is partially consistent with the results of this work for the 1:1 sample. Hirota et al. (2010) also demonstrated that ZnO NPs possess sustained antibacterial effect on E. coli even in the absence of light. These results suggest the presence of additional mechanisms, such as generation of reactive oxygen species (ROS) or direct interaction with the cell membrane.

On the other hand, Li et al. examined the antibacterial activity of ZnO NPs in relation to ROS production and the presence of Zn^{2+} ions. It was observed that Zn^{2+} concentrations around 1 mg/L do not inhibit *E. coli* growth, while increased ROS levels significantly reduce the growth rate, which suggests that the principal mechanism of action is associated with oxidative stress, rather than zinc-ion release.

Table 1*: Results of bacterial inhibition zone E. coli*

Disc	1:1	1:2	1:4
D1	24 mm	13 mm	11 mm
D2	27 mm	18 mm	9 mm
D3	29 mm	18 mm	10 mm
D4	26 mm	17 mm	10 mm
D5	25 mm	15 mm	11 mm
D6	28 mm	12 mm	12 mm
Average	26,5 mm	15,5 mm	10,5 mm

CONCLUSIONS

Based on the conducted research, whose objective was the synthesis of zinc-oxide nanoparticles (ZnO) from orange-peel extract using the principles of green chemistry and the examination of their antimicrobial activity on bacterial species Escherichia coli and Staphylococcus aureus, it can be concluded that this approach successfully achieved an environmentally acceptable, non-toxic and chemically simpler method of obtaining nanoparticles. The phytochemicals present in the orange-peel extract played a key role in the synthesis process, acting as reducing and stabilizing agents. The change in solution color from transparent to pale-yellow-white and the appearance of turbidity at pH 12 confirmed the formation of ZnO nanoparticles, which was further substantiated by UV–VIS spectrophotometry. The obtained absorption maximum in the interval of 357–368 nm is close to literature values for ZnO nanoparticles, which indicates their successful synthesis and stability. Antimicrobial testing revealed that the synthesized ZnO nanoparticles possessed pronounced inhibitory activity against *Escherichia coli*, however did not exhibit measurable inhibition zones *against Staphylococcus aureus*. Comparison with standard antibiotics ceftriaxone (CTX) and ciprofloxacin (CIP) demonstrated that ZnO nanoparticles exhibit comparable antimicrobial activity against *E. coli*, thereby confirming their potential as alternative antimicrobial agents obtained by employing green technologies.

Conflict of interest

The data that support the findings of this study are available on request from the corresponding author.

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