

GREEN SYNTHESIS AND COMPARATIVE BIOLOGICAL EVALUATION OF ZINC OXIDE AND SELENIUM DIOXIDE NANOPARTICLES USING AEGLE MARMELOS LEAF EXTRACT

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ABSTRACT

The present study explored a simple plant-based route for synthesizing Zinc oxide nanoparticles (ZnONPs) and Selenium dioxide nanoparticles (SeO₂NPs) using Aegle marmelos leaf extract. The work combined phytochemical screening with physicochemical characterization and two biological assays, antioxidant and antibacterial activity. The synthesized materials were examined by UV-Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD) and zeta potential analysis. The leaf extract contained phenolic compounds, alkaloids, reducing sugars, saponins, terpenoids, flavonoids, steroids and glycosides. ZnONPs showed an absorption maximum in the 340–350 nm region, whereas SeO₂NPs showed a maximum around 240 nm. The zeta potentials were –30.3 mV for ZnONPs and –29.7 mV for SeO₂NPs. Total phenolic content was 6.99 ± 0.59 , 2.31 ± 0.17 and 2.92 ± 0.13 µg/mL for the plant extract, ZnONPs and SeO₂NPs, respectively. In the DPPH assay, SeO₂NPs gave the lowest IC₅₀ (45.82 µg/mL), followed by ZnONPs (50.52 µg/mL) and the plant extract (56.37 µg/mL). SeO₂NPs also produced the largest inhibition zones against *Bacillus subtilis* (30 mm) and *Escherichia coli* (22 mm). Taken together, the results suggest that A. marmelos leaf extract can serve as a useful biological medium for nanoparticle synthesis and that the resulting SeO₂NP preparation showed the strongest biological activity among the materials tested.

Keyword: *Pinus roxburghii*, Silver Nanoparticles, Green Synthesis, *Tribolium castaneum*, Biopesticide, Stored-Grain Protection.

INTRODUCTION

Nanoparticles have attracted sustained interest because their small size gives them surface and physicochemical properties that are quite different from those of bulk materials. These properties have opened opportunities in areas such as antimicrobial materials, drug delivery, diagnostics and environmental applications. At the same time, the way nanoparticles are produced matters. Conventional synthesis may require harsh chemicals or energy-intensive steps, which has encouraged interest in greener biological approaches.

Plant-mediated synthesis is particularly appealing because plant extracts contain a mixture of naturally occurring molecules that can participate in the reduction, stabilization and capping of newly formed nanoparticles. This approach can also be relatively simple and inexpensive. *Aegle marmelos* (L.) Correa, commonly known as bael, is a medicinal plant widely used in the Indian subcontinent. Its leaves contain several groups of secondary metabolites, including phenolics, flavonoids, alkaloids, terpenoids and saponins, which make the plant a promising source for biological synthesis.

Previous work has reported biological activity in *A. marmelos* extracts and has also explored plant-assisted synthesis of metal and metal oxide nanoparticles. However, comparing two different nanoparticle systems prepared from the same plant source provides a useful way of looking at how the nanoparticle type influences biological performance.

The present study therefore focused on the green synthesis of ZnONPs and SeO₂NPs using *A. marmelos* leaf extract. The synthesized materials were characterized by UV-Visible spectroscopy, FTIR, SEM, XRD and zeta potential measurements. Their phenolic and flavonoid contents, DPPH radical-scavenging activity and antibacterial activity against *B. subtilis* and *E. coli* were also compared.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh *A. marmelos* leaves were collected from Bilaspur district, Himachal Pradesh. The leaves were washed thoroughly, shade-dried for approximately 10–15 days and ground into a fine greenish-brown powder. Methanolic and aqueous extracts were prepared for phytochemical screening. The methanolic extract was obtained by extracting 10 g of powdered leaves with 100 mL methanol, followed by filtration and concentration.

Phytochemical screening

The extracts were screened qualitatively for phenolic compounds, alkaloids, reducing sugars, saponins, terpenoids, flavonoids, steroids and glycosides using standard colour and precipitation tests.

Green synthesis of ZnONPs

Zinc acetate dihydrate was used as the Zinc precursor and sodium hydroxide as the precipitating agent. Aqueous *A. marmelos* leaf extract was added gradually to the Zinc acetate solution with continuous stirring. The pH was adjusted to 8–10 using 2 M sodium hydroxide. The mixture was heated at 70°C for 1 h, producing a white precipitate. The precipitate was washed with distilled water and dried at 70°C overnight.

Green synthesis of SeO₂NPs

An aqueous leaf extract prepared from 20 g of dried leaf powder was used as the biological component. Selenium dioxide (0.013 g) dissolved in 10 mL deionized water was used as the precursor. The reaction mixture was diluted to 100 mL, maintained at 60°C under continuous stirring and supplemented with 200 µL of 40 mM ascorbic acid. A change from pale greenish-yellow to brick red was observed. The product was recovered by centrifugation, washed with deionized water and ethanol and dried at 50–60°C.

Nanoparticle characterization

UV-Visible spectra were recorded in the 200–800 nm range. FTIR was used to examine functional groups associated with the nanoparticles. SEM was used to examine particle morphology, while XRD was used to assess crystalline structure. Zeta potential measurements were carried out on diluted, sonicated nanoparticle suspensions at approximately 25°C, with measurements performed in triplicate.

Total phenolic and flavonoid content

Total phenolic content was estimated using the Folin-Ciocalteu method with gallic acid as the standard and absorbance measured at 765 nm. Total flavonoid content was estimated by an aluminium chloride colorimetric method using the reported standard curve and absorbance at 415 nm.

DPPH antioxidant assay

A 0.1 mM DPPH solution was used to evaluate radical-scavenging activity. Plant extract and nanoparticle samples were tested at 20, 40, 60, 80 and 100 µg/mL, with ascorbic acid used as the reference standard. Absorbance was measured at 517 nm and IC₅₀ values were determined from the reported dose-response relationships.

Antibacterial assay

Antibacterial activity was assessed by the agar well diffusion method against the Gram-positive bacterium *B. subtilis* and the Gram-negative bacterium *E. coli*. Plant extract, ZnONPs and SeO₂NPs were tested at the reported working concentration, and inhibition zones were measured in millimetres. Streptomycin served as the positive control.

RESULTS

The results are presented below in a form that combines the main observations with the corresponding experimental images and summary tables.

Plant extraction and phytochemical profile

The leaf material changed from fresh green leaves to a dry, greenish-brown powder after shade drying. The resulting methanolic extract was green and was used for the subsequent phytochemical and biological studies.

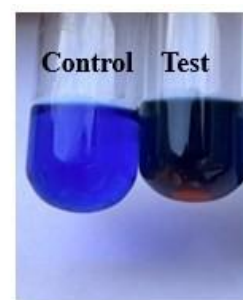
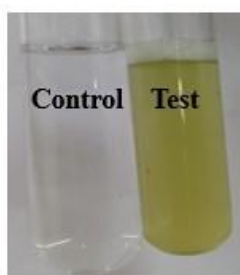
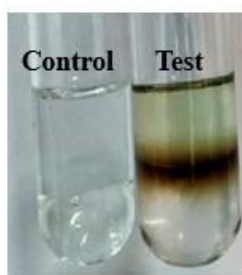
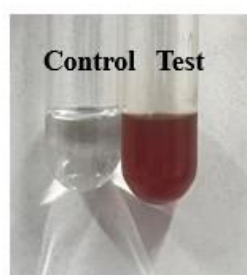
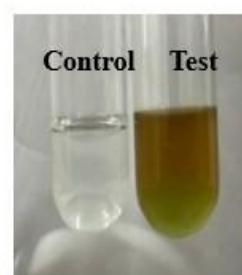


Fig. 1. Preparation of *A. marmelos* leaf material and crude extract

Table 1: Qualitative phytochemical screening of *A. marmelos* leaf extracts.

Phytoconstituent	Test/observation	Methanolic extract	Aqueous extract
Phenolic compounds	Ferric chloride; black precipitate	+	+
Alkaloids	Mayer's test; yellow colour	+	+

Alkaloids	Dragendorff's test; brownish-red precipitate	—	—
Reducing sugars	Benedict's/Fehling's tests	+	+
Saponins	Frothing test; stable foam	+	+
Terpenoids	Salkowski test	+	+
Flavonoids	Shinoda test	+	+
Steroids	Liebermann–Burchard reaction	+	+
Glycosides	Keller–Kiliani test	+	+

**Ferric chloride test****Mayer's test****Dragendorff test****Benedict's test****Fehling test****Frothing test****Salkowski test****Shinoda test****Liebermann-burchard
test****Keller- killani test***Fig. 2. Phytochemical screening of the methanolic A. marmelos leaf extract*

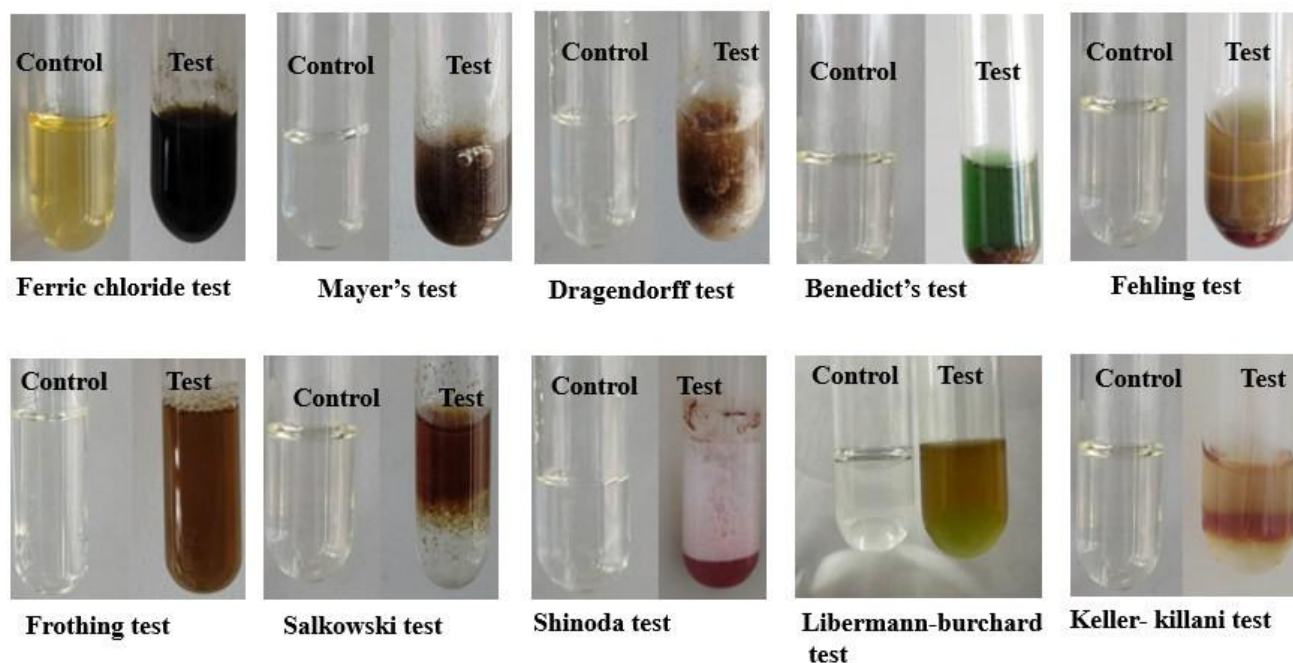


Fig. 3. Phytochemical screening of the aqueous *A. marmelos* leaf extract.

Green synthesis of nanoparticles

Nanoparticle formation was first apparent as a visible change during the reactions. In the ZnONP synthesis, the initially clear precursor mixture became pale yellow and subsequently produced a white precipitate. In the SeO₂NP reaction, the colour changed from pale greenish-yellow to brick red. These visual changes were followed by instrumental characterization.



Fig. 4. Green synthesis of ZnONPs using *A. marmelos* leaf extract



Fig. 5. Green synthesis of SeO_2NPs using *A. marmelos* leaf extract

Physicochemical characterization

The UV-Visible spectra showed distinct absorption features for both preparations. ZnONPs showed their highest absorbance in the 340–350 nm region, while SeO_2NPs showed a maximum around 240 nm.

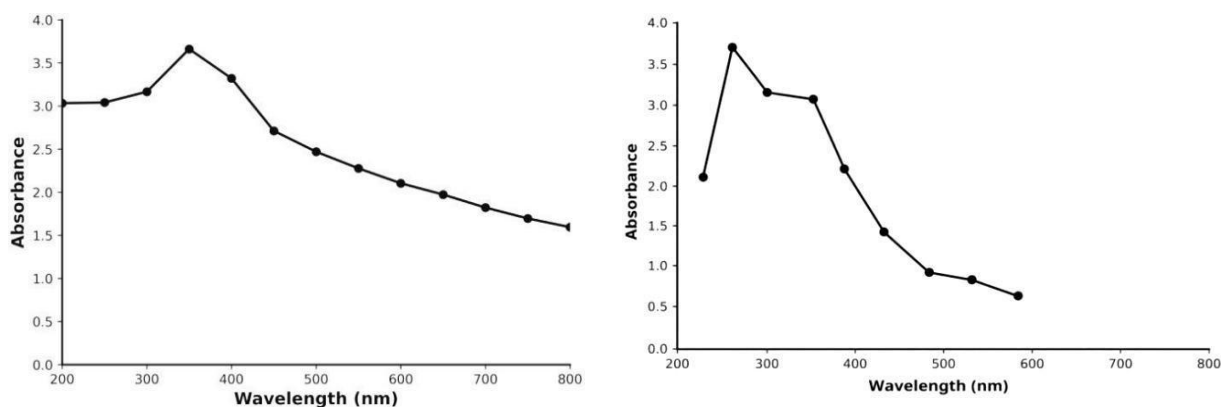


Fig. 6. UV-Visible spectra of (a) ZnONPs and (b) SeO_2NPs

FTIR spectra contained several bands attributable to hydroxyl, carbonyl, C–C, C–O and C–N-containing groups. For ZnONPs , the broad band near 3414 cm^{-1} and the low-wavenumber Zn–O-associated bands supported the involvement of plant-derived molecules in nanoparticle formation. For SeO_2NPs , bands around 3203 , 2922 – 2872 , 1629 – 1523 and 1379 – 1074 cm^{-1} were observed together with low-wavenumber bands assigned to Se–O/O–Se–O vibrations.

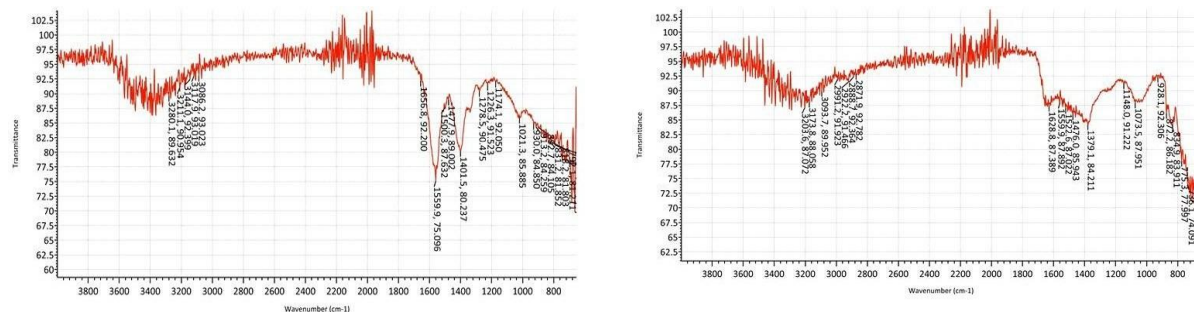


Fig. 7. FTIR spectra of (a) ZnONPs and (b) SeO₂NPs

SEM examination showed nanoscale particles with some aggregation. The images provide direct visual evidence of the irregular, clustered morphology observed in the synthesized preparations.

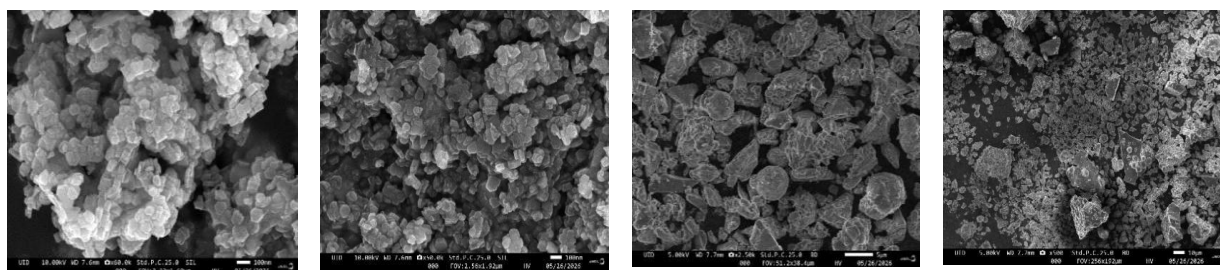


Fig. 8. SEM micrographs of ZnONPs and SeO₂NPs synthesized using A. marmelos leaf extract

XRD analysis produced sharp, well-defined diffraction peaks, indicating crystalline materials. ZnONPs showed reflections consistent with a hexagonal wurtzite structure. The SeO₂NP pattern also showed distinct crystalline reflections.

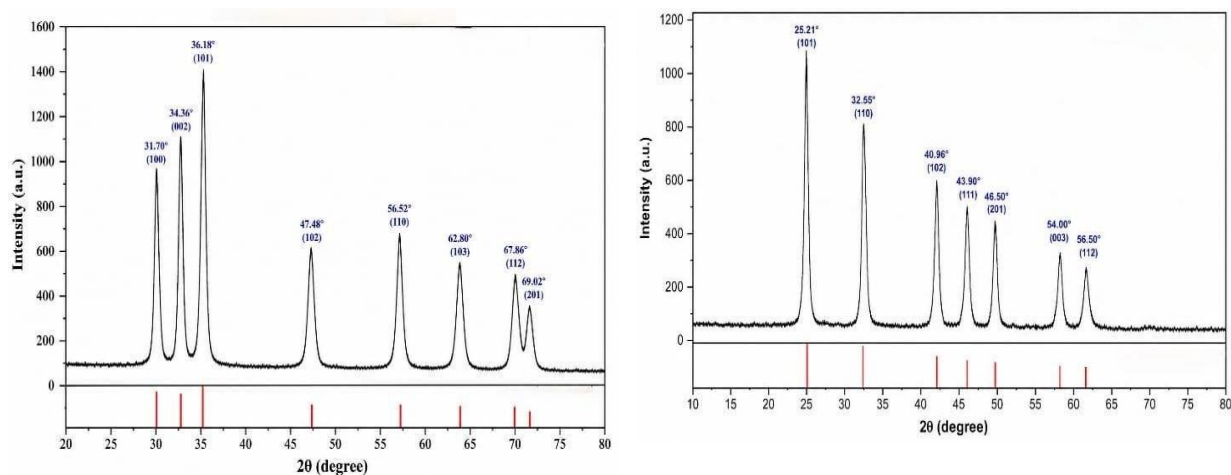


Fig. 9. XRD patterns of (a) ZnONPs and (b) SeO₂NPs

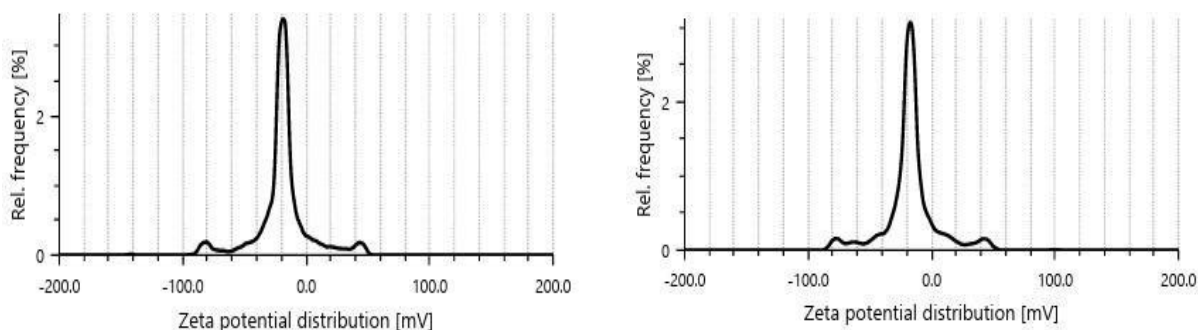


Fig. 10. Zeta-potential distributions of (a) ZnONPs and (b) SeO₂NPs

Table 2. Zeta potential of the synthesized nanoparticles.

Sample	Zeta potential (mV)
ZnONPs	-30.3
SeO ₂ NPs	-29.7

Phenolic and flavonoid contents

The crude leaf extract contained more measurable phenolics and flavonoids than either nanoparticle preparation. The total phenolic content was 6.99 ± 0.59 $\mu\text{g/mL}$ in the plant extract, 2.31 ± 0.17 $\mu\text{g/mL}$ in ZnONPs and 2.92 ± 0.13 $\mu\text{g/mL}$ in SeO₂NPs. The corresponding flavonoid values were 4.34 ± 0.50 , 1.38 ± 0.09 and 2.39 ± 0.09 $\mu\text{g/mL}$.

Table 3. Total phenolic and flavonoid contents.

Sample	Total phenolic content ($\mu\text{g/mL}$)	Total flavonoid content ($\mu\text{g/mL}$)
<i>A. marmelos</i> leaf extract	6.99 ± 0.59	4.34 ± 0.50
ZnONPs	2.31 ± 0.17	1.38 ± 0.09
SeO ₂ NPs	2.92 ± 0.13	2.39 ± 0.09

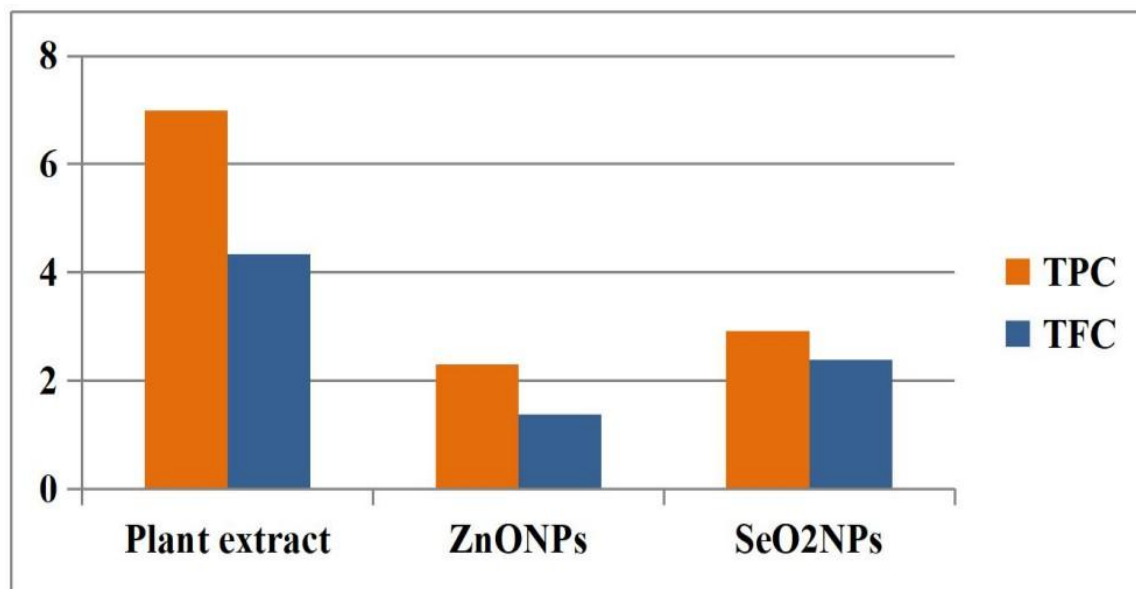


Fig. 11. Comparison of total phenolic content (TPC) and total flavonoid content (TFC)

Antioxidant activity

The DPPH assay showed a concentration-dependent increase in radical-scavenging activity. At 100 $\mu\text{g/mL}$, the reported scavenging activities were 66.79% for the plant extract, 73.38% for ZnONPs, 78.45% for SeO₂NPs and 61.23% for ascorbic acid.

Table 4. IC₅₀ values from the DPPH radical-scavenging assay

Sample	IC ₅₀ ($\mu\text{g/mL}$)
Ascorbic acid	71.63
<i>A. marmelos</i> leaf extract	56.37
ZnONPs	50.52
SeO ₂ NPs	45.82

The lowest IC₅₀ was observed for SeO₂NPs, indicating the strongest radical-scavenging activity among the tested samples under the conditions of this assay.

Antibacterial activity

Both nanoparticle preparations inhibited the growth of *B. subtilis* and *E. coli*. The inhibition was greater against *B. subtilis*, and SeO₂NPs produced the largest zones among the synthesized materials. Streptomycin remained the most active control.

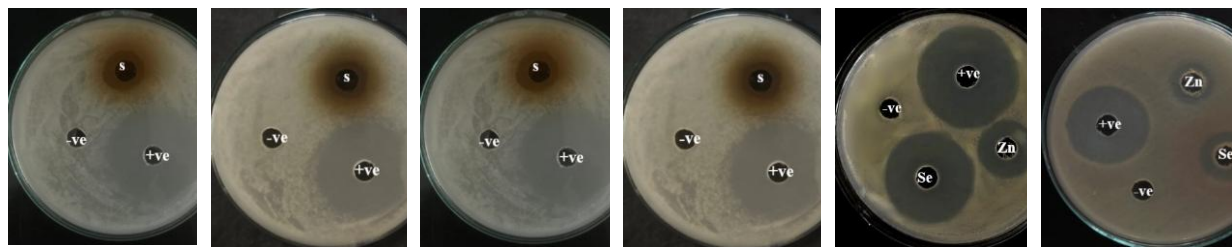


Fig. 12. Representative agar-well diffusion plates used to assess antibacterial activity

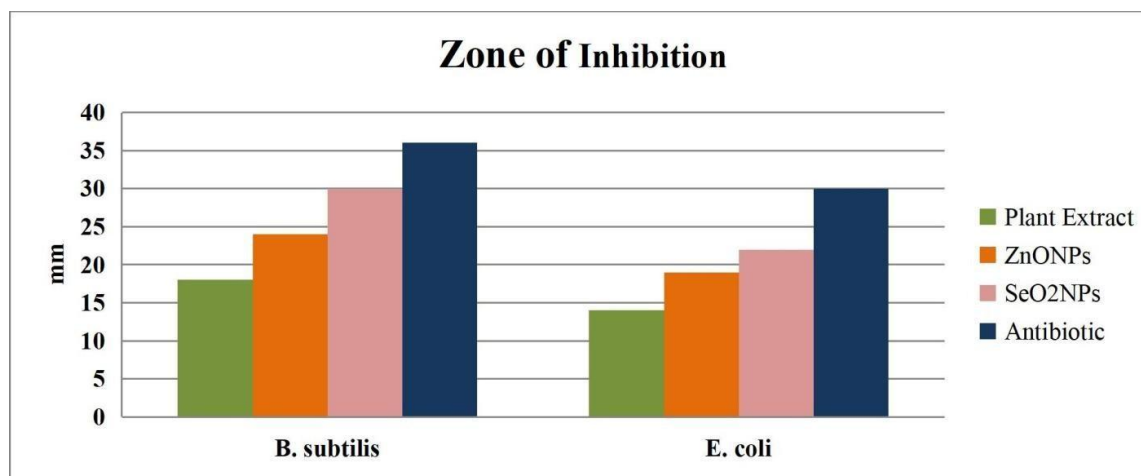


Fig. 13. Antibacterial efficacy of *A. marmelos* leaf extract, ZnONPs, SeO₂NPs and the standard antibiotic

Table 5. Antibacterial activity expressed as diameter of the inhibition zone

Test organism	Streptomycin (mm)	Plant extract (mm)	ZnONPs (mm)	SeO ₂ NPs (mm)
<i>B. subtilis</i>	36	18	24	30
<i>E. coli</i>	30	14	19	22

DISCUSSION

The results give a fairly consistent picture of the role of *A. marmelos* in the nanoparticle synthesis process. The leaf extracts contained several classes of phytochemicals, and these same biological molecules provide a plausible source of reducing and surface-capping activity during nanoparticle formation. The visible colour changes during synthesis were therefore useful as an initial indication, but the spectroscopic and structural measurements provided the stronger evidence.

The optical profiles of the two materials were clearly different. ZnONPs showed their main absorption in the 340–350 nm region, whereas SeO₂NPs showed a maximum near 240 nm. FTIR results further suggested that plant-derived functional groups remained associated with the particles. This is relevant to green

synthesis because the plant extract is not simply a reaction medium; its constituents can become part of the surface chemistry of the resulting material.

The XRD patterns showed well-defined crystalline peaks, with the ZnONP pattern corresponding to the characteristic hexagonal wurtzite phase. The negative zeta potentials of -30.3 and -29.7 mV for ZnONPs and SeO₂NPs, respectively, also indicate that the particles carried a substantial negative surface charge. In practical terms, this suggests a reasonably stable suspension, although zeta potential alone does not describe every aspect of colloidal stability.

An interesting observation was that the crude extract had the highest measured phenolic and flavonoid contents, yet the nanoparticle preparations showed stronger DPPH activity in the reported assay. SeO₂NPs gave the lowest IC₅₀ value, followed by ZnONPs. This suggests that antioxidant behaviour was not determined simply by the amount of measurable phenolic material. Surface chemistry and the interaction between the inorganic component and plant-derived molecules may also have contributed.

The antibacterial results followed a similar pattern. SeO₂NPs produced inhibition zones of 30 mm against *B. subtilis* and 22 mm against *E. coli*, while ZnONPs produced 24 and 19 mm, respectively. The stronger response observed with *B. subtilis* may reflect differences between the cell envelopes of Gram-positive and Gram-negative bacteria. The present results support the biological activity of the synthesized materials, but they should not be interpreted as evidence of clinical efficacy because only two bacterial strains were examined.

Overall, the comparison suggests that the type of nanoparticle mattered: under the experimental conditions used in this study, SeO₂NPs consistently showed stronger antioxidant and antibacterial activity than ZnONPs. The plant extract appears to have provided both the biological chemistry needed for synthesis and an important contribution to the surface characteristics of the final particles.

CONCLUSION

This study demonstrates that *A. marmelos* leaf extract can be used as a biological component for the preparation of ZnONPs and SeO₂NPs. The synthesized materials showed characteristic optical, functional-group, crystalline and surface-charge features. Both preparations displayed antioxidant and antibacterial activity, with SeO₂NPs giving the lowest DPPH IC₅₀ and the largest inhibition zones among the synthesized nanoparticles. The findings are encouraging for further investigation of plant-mediated nanomaterials, particularly studies that include particle-size distribution, more bacterial strains, mechanistic experiments, cytotoxicity testing and standardized synthesis conditions.

Study limitations

The present work was an exploratory laboratory study. The biological evaluation was limited to a DPPH assay and two bacterial strains, and the thesis does not provide a complete particle-size distribution or a

detailed mechanistic analysis. These limitations should be kept in mind when considering future applications.

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