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Green-synthesized selenium nanoparticles from *Tetrapleura Tetraptera* husk extract: biological activities and application in eco-friendly paper packaging for bread preservation

Abstract

Selenium nanoparticles (SeNPs) have attracted considerable attention because of their antimicrobial, antioxidant and biomedical properties, while green synthesis using plant extracts offers an environmentally friendly alternative to conventional chemical synthesis. This study aimed to synthesize SeNPs from the husk extract of *Tetrapleura tetraptera*, evaluate their biological activities, and investigate their application in biodegradable paper packaging for bread preservation. The husk extract was used as a reducing and stabilizing agent for the green synthesis of SeNPs, and nanoparticle formation was confirmed by colour change and characterized using UV–Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX) and transmission electron microscopy (TEM). The synthesized nanoparticles were spherical, while FTIR analysis revealed the presence of functional groups involved in nanoparticle stabilization. The biosynthesized SeNPs exhibited strong antifungal activity against *Aspergillus species*, with inhibition rates exceeding 96%, together with moderate antioxidant activity (53.11% DPPH radical scavenging at 100 µg/mL) and measurable anticoagulant and thrombolytic activities. Paper-based packaging materials incorporated with SeNPs (100 and 150 µg/mL) were evaluated for bread preservation over seven days. Bread packaged in SeNP-incorporated paper exhibited substantially lower bacterial counts than the control, with the 150 µg/mL treatment maintaining bacterial loads at 2.0×10^5 CFU/g compared with 9.8×10^6 CFU/g in the control on day 7. These findings indicate that green-synthesized SeNPs from *T. tetraptera* husk possess promising antimicrobial properties in laboratory testing. While incorporation into paper packaging reduced surface microbial growth on bread, comprehensive evaluations of selenium migration, cytotoxicity, mechanical strength, and gas barrier properties are necessary before direct food-contact or commercial applications can be considered.

Keywords: selenium nanoparticles, *tetrapleura tetraptera* husk, antimicrobial activity, anticoagulant activity, active food packaging, bread

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OLADIPO Iyabo Christianah, OGUNSONA Seun Barnbas, RAJI Boluwatife Abdulbaakii, OGUNBEKU Ayomide Eunice, SANGODARE Adeola Oyepej

Department of Science Laboratory Technonology, Ladoke Akintola University of Technology, Nigeria

Correspondence: Oladipo IC, Department of Science Laboratory Technonology, Ladoke Akintola University of Technology, Nigeriaa

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Introduction

Selenium (Se) is an essential trace element that plays vital roles in numerous physiological processes, including antioxidant defence, immune function, thyroid hormone metabolism, and cellular homeostasis. In recent years, selenium nanoparticles (SeNPs) have attracted considerable attention because they combine the biological functions of selenium with the unique physicochemical properties of nanomaterials. Compared with inorganic and organic selenium compounds, SeNPs exhibit enhanced bioavailability, high biocompatibility, reduced toxicity, and diverse biological activities, including antimicrobial, antioxidant, anticancer, anti-inflammatory, and drug delivery potentials, making them promising candidates for biomedical and pharmaceutical applications.[1,2]

Selenium nanoparticles can be synthesized through physical, chemical, and biological approaches. Conventional chemical and physical methods, although effective, often require toxic reducing agents, expensive equipment, high energy input, and harsh reaction conditions that may generate hazardous by-products. Consequently, biological or green synthesis has emerged as a sustainable, environmentally friendly, and cost-effective alternative for nanoparticle production.[3] Biological synthesis can be achieved using microorganisms such as bacteria, fungi, and yeasts, as well as plant extracts rich in naturally occurring phytochemicals. Microbial synthesis has been reported to produce SeNPs with well-defined morphology and promising antimicrobial and biocompatible properties, demonstrating the potential of biological systems for nanoparticle production.

Among biological approaches, plant-mediated synthesis has gained increasing attention because plant extracts contain diverse phytochemicals, including flavonoids, phenolic compounds, alkaloids, terpenoids, proteins, reducing sugars, and organic acids, which simultaneously function as reducing and stabilizing agents during nanoparticle formation. These biomolecules facilitate rapid synthesis while enhancing nanoparticle stability and biocompatibility, making plant-mediated synthesis simpler, safer, and more suitable for large-scale production than many conventional methods.[3] Numerous medicinal and edible plants have successfully been employed for the green synthesis of SeNPs with potent biological activities. For example, Aloe vera leaf extract has been used to synthesize selenium nanoparticles exhibiting remarkable antimicrobial activity against spoilage fungi and pathogenic bacteria, highlighting the effectiveness of plant-derived phytochemicals in nanoparticle synthesis.[4]

The biological activities of SeNPs are strongly influenced by their particle size, morphology, and surface chemistry, which are largely determined by the source of the biological reducing agent. Selenium nanoparticles exhibit broad-spectrum antimicrobial activity

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through multiple mechanisms, including disruption of microbial cell membranes, generation of reactive oxygen species, and interference with essential cellular metabolic processes.[1] These properties have stimulated growing interest in their application in wound dressings, medical devices, water treatment systems, and particularly active food packaging.

Active food packaging incorporating antimicrobial nanomaterials has emerged as an innovative strategy for improving food safety and extending the storage stability of perishable foods by reducing microbial contamination during storage. Recent studies have demonstrated that biosynthesized SeNPs can be incorporated into biodegradable packaging materials to inhibit the growth of foodborne microorganisms while minimizing the environmental impact associated with synthetic preservatives and conventional plastic packaging. Owing to their antimicrobial efficacy, low toxicity, and environmentally friendly synthesis, bio-selenium nanoparticles are increasingly being recognized as promising materials for sustainable food preservation and packaging applications.[5]

Tetrapleura tetraptera (Schumach. & Thonn.) Taub. is an important medicinal plant widely distributed in West Africa. The fruit husk is rich in bioactive phytochemicals, including flavonoids, phenolic compounds, tannins, and saponins, which possess strong reducing and antioxidant properties that make the plant a promising candidate for green nanoparticle synthesis. Although several plant species have been investigated for the biosynthesis of selenium nanoparticles, limited information is available on the utilization of *T. tetraptera* husk, an abundant agro-industrial by-product, as a reducing and stabilizing agent for SeNP production. Furthermore, the application of biosynthesized SeNPs incorporated into biodegradable paper-based packaging for bread preservation remains largely unexplored.

Therefore, this study aimed to synthesize selenium nanoparticles using *Tetrapleura tetraptera* husk extract, characterize the synthesized nanoparticles, evaluate their antifungal, antioxidant, anticoagulant, and thrombolytic activities, and investigate their application in eco-friendly paper-based packaging for bread preservation.

Materials and method

Sample collection and preparation

The *Tetrapleura tetraptera* fruit were bought from Oja Igbo Market, Ogbomoso, Oyo State and were examined closely to ensure no signs of infections then conveyed to the lab in sterile polythene bag. The fruits were cracked and were separated into seeds and husk. The fruit husks of *Tetrapleura tetraptera* were washed with sterilized distilled water, aseptically drained and air-dried for 14 days. The husks were pulverized finely using a commercial blender. Exactly 0.1 g of the husk powder was suspended in 10 mL of sterilized distilled water making the mixture 10 mg/mL extract concentration. The mixture was then subjected to aqueous extraction using water bath at 60 °C for 1 h. After the extraction, the mixture was filtered using Whatman No. 1 filter paper and centrifuged at 4,000 rpm for 15 min at room temperature to obtain a clear aqueous extract, which was stored at 4 °C prior to synthesis.[6]

Synthesis and characterization of selenium nanoparticles

Sodium selenite (Na_2SeO_3, molecular weight 172.94 g/mol) was the precursor salt, exactly 0.0865 g (0.5 mmol) of Sodium selenite was dissolved in 500 mL of sterilized deionized water to prepare a 1.0 mM precursor solution. For green synthesis, 10 mL of the prepared aqueous husk extract (10 mg/mL) was added dropwise to 50 mL of the 1.0 mM Sodium selenite solution (1:5 v/v ratio) under continuous magnetic stirring at 300 rpm and ambient room temperature (28 ± 2°C). The initial pH of the reaction mixture was measured as 6.2 ± 0.1 without further adjustment. The reaction was allowed to proceed under constant stirring for 35 min in ambient light, during which a clear color change from faint yellow to brownish amber was observed, indicating SeNP formation.

To recover the synthesized SeNPs, the colloidal suspension was centrifuged at 12,000 rpm (15,294 × *g*) for 20 min at 4 °C. The resulting nanoparticle pellet was collected and washed three times with distilled water and then washed twice with absolute ethanol to completely remove unreacted selenite ions, water-soluble impurities and residual phytochemicals. The purified pellet was transferred to a watch glass and dried in a vacuum oven at 50 °C for 12 h. The recovered material weighed approximately 6.8 mg of dried SeNP-containing composite powder per 50 mL reaction batch. Because the recovered powder contains selenium together with organic capping/stabilizing material, this mass was not interpreted as elemental selenium yield. The dry powder was stored in an airtight desiccator at 4 °C for characterization and biological assays.

Ultraviolet visible spectroscopy (B-UV1800PC Spectrophotometer) was used to take the measurement of the absorption spectrum while biomolecules responsible for the fabrication, capping and stabilization of the nanoparticles was obtained using IR Affinity-1s spectrophotometer (Shimadzu, UK). Transmission Electron Microscope and Energy-Dispersive X-ray spectroscopic analyses were also carried out to determination the elemental composition and size of the synthesized nanoparticles.[7]

Antifungal activity of the synthesized nanoparticle

The antifungal activities of the nanoparticles were evaluated using mycelia inhibition method of Khatami et al.[8] and Lateef et al.,[9] by incorporating graded concentrations (150 µl /ml) of the synthesized nanoparticles into potato dextrose agar plates, which were then inoculated with agar plug of 6 mm of 48-h old cultures of *Aspergillus niger, Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus paraciticus*. In the control experiments, fungal plugs were inoculated on PDA plates without the incorporation of the nanoparticles. All the plates were incubated at 28 ± 2 C for 72 h. The diameters of fungal growths in all the plates were measured and used to determine the percentage growth inhibitions as follows:

$$\frac{D_{control} - D_{test}}{D_{control}} \times 100\%$$

Where D is the diameter of fungal growth on the PDA plates.

Antioxidant activity of green synthesized selenium nanoparticles

2,2-diphenyl-1-picryhydrazyl is widely used for testing preliminary radical scavenging activity of a compound or a nanoparticle. Antioxidant activity was measured by using the modified DPPH method as reported by Lateef *et al*.[10] Methanolic solution of DPPH was prepared using 0.04g of DPPH into 1000ml of methanol and the optical density of the solution was taken at 517nm.To determine the antioxidant activity, different concentrations of the synthesized selenium nanoparticles (50, 75 and 100µg/ml) were challenged with 4ml of methanolic DPPH. The samples were allowed to scavenge for DPPH in the dark for 30 minutes. The absorbance was measured at 517 nm in UV-visible spectrophotometer. The scavenging percentage was calculated using the formula below:

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$$DPPH\ scavenging\ (\%) = \frac{Absorbance\,control - Absorbance\,of\ sample}{Absorbance\,control} \times 100\%$$

Anticoagulant and thrombolytic activities of the synthesized nanoparticles

The anticoagulant activity of the nanoparticles was investigated as earlier described by Azeez et al.[11] Exactly 50,100, 150 µl of the nanoparticles prepared were added to 0.5 ml of blood freely provided by a healthy donor, while blood collected into EDTA and blood collected in clean plain tube served as positive and negative control respectively. In addition, blood samples were treated with the *Tetrapleura tetraptera* extract and aqueous solution of sodium selenite. All the set up was held at room temperature (30 ± 2 °C) for 30 min, and thereafter examined for anticoagulation.

The percentage anticoagulant activity was determined using Equation 1:

$$Anticoagulant\ Activity(\%) = \left[\frac{\left(T_{control} - T_{sample}\right)}{T_{control}}\right] \times 100$$

where $T_{control}$ is the complete coagulation time of untreated blood (min) and T_{sample} is the coagulation time in the presence of test formulations.

The thrombolytic activity was determined using the methods of Devi et al.[12] In this case, thrombolytic activity was quantified using the method of Lateef et al.[13] Eppendorf tubes containing 0.5 ml of blood were held at 37 °C for 30 min to allow the blood to clot. The weight of clean tube (W1) was subtracted from the weight of tube and blood clot (W2) to obtain the weight of blood clot (W3). Thereafter, 100 µl of the nanoparticles, selenium chloride solutions, and *Tetrapleura tetraptera* husk extract were added to each tube containing the blood clot. These were incubated at 37 °C for 90 min, and then inverted to check for clot lysis.[14] The lysed clot was drained, and the weight of the tube with remaining clot was taken (W4) to obtain the weight of clot that was not lysed (W5). The percentage thrombolytic activity was obtained as:

The percentage thrombolytic activity (clot lysis) was calculated using Equation 2:

$$Thrombolytic\ Activity(\%) = \left[\frac{\left(W_3 - W_5\right)}{W_3}\right] \times 100$$

where W_3 is the initial weight of the blood clot ($W_2 - W_1$) and W_5 is the weight of the remaining unlysed clot ($W_4 - W_1$).

Paper mache production

Paper substrate preparation: Brown and white papers were purchased from a stationary store at Adenike area, within Ogbomosho metropolis. The brown papers were manually torn into uniform strips measuring roughly 5 centimetres in width and roughly 50 centimetres in length to optimize surface area for subsequent processing and then sterilized in an autoclave at 121^{0}C for 15 minutes. After the paper preparation, the starch binding solution was prepared for the gelatinization of the paper. About 15 grams of analytical starch was weighed and gradually added into distilled water at room temperature using a magnetic stirrer operating at 200 revolutions per minute. The gradual addition technique prevented agglomeration and ensured homogeneous dispersion of starch granules throughout the aqueous medium. The starch suspension was subsequently heated in a controlled water bath to 85°C while maintaining constant stirring to achieve complete gelatinization. After 15 minutes, the gelatinization process was completed, resulting in a transparent and viscous gel.

Pulping and homogenization process: The gelatinized starch solution and selenium nanoparticles of different concentration 100 and 150 (µg/mL), was gradually incorporated into the paper pulp while maintaining continuous mild stirring. The addition process was conducted over a 10-minute period to ensure uniform distribution and prevent localized concentration gradients that could compromise final product homogeneity. Temperature monitoring during this phase indicated that the mixture temperature was maintained at 60±3°C to preserve starch gel properties. Eight millilitres of analytical grade glycerol was incorporated as plasticizer over a 5-minute period while maintaining continuous stirring.

Moulding and forming process: The homogenized selenium nanoparticles incorporated papier-mâché mixture was transferred to pre-designed molds constructed from food-grade silicone materials. The moulding process involved careful distribution of the mixture to achieve uniform thickness of 3 millimetres throughout the formed product. Excess air was removed through gentle vibration and manual smoothing techniques to prevent void formation that could compromise structural integrity. The moulded papier-mâché products underwent controlled drying in an environmental chamber maintained at 40°C with 50% relative humidity. The drying process required 24 hours to achieve equilibrium moisture content below 8%.

To rigorously evaluate the explicit contribution of SeNPs to food packaging preservation, four distinct packaging formulations were prepared:

1. **Control packaging (Nanoparticle-free base):** Prepared using autoclaved paper pulp, 15 g gelatinized starch, and 8 mL glycerol without plant extract or nanoparticles.
2. **Extract packaging (AD-extract):** Prepared with paper pulp, gelatinized starch, glycerol, and crude *T. tetraptera* husk extract (150 µg/mL).
3. **SeNP-100 packaging:** Prepared with paper pulp, gelatinized starch, glycerol, and biosynthesized SeNPs at a concentration of 100 µg/mL.
4. **SeNP-150 packaging:** Prepared with paper pulp, gelatinized starch, glycerol, and biosynthesized SeNPs at a concentration of 150 µg/mL.

All formulations underwent identical pulping, molding, and controlled drying procedures (40 °C, 50% RH for 24 h) to maintain uniform thickness (3 mm).

Bread production: Wheat flour, yeast, salt, skim milk and sugar were purchased from New Waso market in Ogbomosho metropolis and were conveyed to the lab in sterile polythene bag. Using the straight dough method of Ijah *et al*.,[15] the baking formula was 100% flour, 36% water, 3.4% sugar, 1% skim milk powder, 1% salt and 1% yeast. All ingredients were mixed in a Kenwood mixer (Model A 907D) for 5 minutes. The dough was fermented in bowls, covered with wet clean Muslin cloth for 55 minutes at warm temperature, punched, scaled to 100 g dough pieces, proofed in a proofing cabinet at 30^O C for 90 minutes and 85% relative humidity, and baked at 250^O C for 30 minutes. The baked bread samples were then depanned, cooled at ambient temperature and put in ziploc bags prior to analysis.

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Microbiological evaluation of packaged bread: Freshly baked bread samples (100 g each) were cooled under sterile laminar flow, placed inside respective paper-mâché packaging containers (Control, AD-extract, SeNP-100, SeNP-150), sealed within clean outer secondary bags, and stored at controlled ambient room temperature (25 ± 2 °C, relative humidity 65 ± 5%) for 7 days. Microbial evaluation was performed on Days 1, 3, 5, and 7 using 3 independent packaging containers per treatment group. Aseptically, a 10.0 g representative sample of bread (crumb and crust) was excised from each replicate, transferred into 90 mL of sterile 0.1% (w/v) peptone water, and homogenized in a stomacher for 2 min to produce a 10^{-1} primary dilution. Serial ten-fold dilutions (10^{-1} to 10^{-6}) were prepared in sterile physiological saline (0.85% w/v NaCl). For bacterial enumeration, 0.1 mL (100 µL) of appropriate dilutions was spread-plated onto sterile Nutrient Agar (NA) plates and incubated at 37 °C for 24–48 h. For fungal enumeration, 0.1 mL of serial dilutions was spread-plated onto Potato Dextrose Agar (PDA) supplemented with 100 mg/L chloramphenicol to suppress bacterial growth and incubated at 25 ± 2 °C for 5 days (120 h). Colonies were counted on plates containing between 30 and 300 CFU, and total microbial loads were calculated using Equation 3:

CFU / g = (Number of Colonies × Dilution Factor) / Plating Volume (mL)

Microbial reductions were expressed as both $\log_{10}$ reduction and percentage fold-reduction.[16]

Statistical Analysis

All experimental assays (antifungal growth inhibition, DPPH radical scavenging, anticoagulant assays, and microbial counts) were conducted using three independent experimental batches ($N = 3$), with each batch measured in technical triplicate ($n = 3$). An individual Petri dish, blood sample tube, or packaging container served as the primary experimental unit. Data are expressed as mean ± standard deviation (SD). One-way Analysis of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post-hoc test was performed using SPSS Statistics software (version 26.0, IBM Corp., Armonk, NY, USA) to determine statistically significant differences among treatments. Statistical significance was established at $p < 0.05$. In all tables, means within the same row or column sharing different superscript letters indicate statistically significant differences ($p < 0.05$).

Results and discussion

Synthesis of selenium nanoparticles from *Tetrapleura tetraptera* husk aqueous extract

The selenium nanoparticles synthesis was initially confirmed through the change in colour of the reaction mixture from colourless to brownish amber after 35 minutes of reaction at ambient temperature. Many researchers have reported colour change like deep orange,[17] red,[18,19] brick red, reddish brown, colloidal brick red to mention a few.[20,21] But the control (Sodium selenite solution without any plant extract) remained colorless throughout the reaction (Figure 1).

The characterization of the selenium nanoparticles

The UV–Vis absorption spectrum of the aqueous sodium selenite precursor displayed maximum absorbance at 260 nm (Figure 2A), whereas the crude *T. tetraptera* husk extract exhibited broad absorption bands between 280 and 340 nm, attributable to phenolic compounds, flavonoids, and associated aromatic bio-molecules (Figure 2B). Following reaction, the SeNP colloid displayed a distinct absorption band centered at approximately 440 nm (Figure 2C), which is in agreement with the report of Mikhailova[22] who reported Uv-vis spectroscopy maximum absorbance at 420-450 nm of selenium nanoparticles synthesized from *Cinnamomum verum,* a medicinal plant. Furthermore, Banerjee and Rajeswari[23] also reported Uv-vis spectroscopy maximum absorbance at 400-500 nm of selenium nanoparticles synthesized from *Moringa oleifera* leaves extract. The appearance of a distinct absorption band around 440 nm following reaction with sodium selenite supported the formation of SeNPs. Surface Plasmon Resonance (SPR) patterns are commonly used as indicative tools for metal nanoparticle formation, as SPR depends on various parameters, such as size and medium dielectric constant. These phytochemicals that may act as reducing and/or capping agents would preferentially involve in the reduction of selenite (Se(IV)) to elemental selenium (Se^0) metal and in the phytochemical-assisted fabrication of SeNPs from *Tetrapleura tetraptera* husk (nucleation, capping and stabilization) (Figure 2).

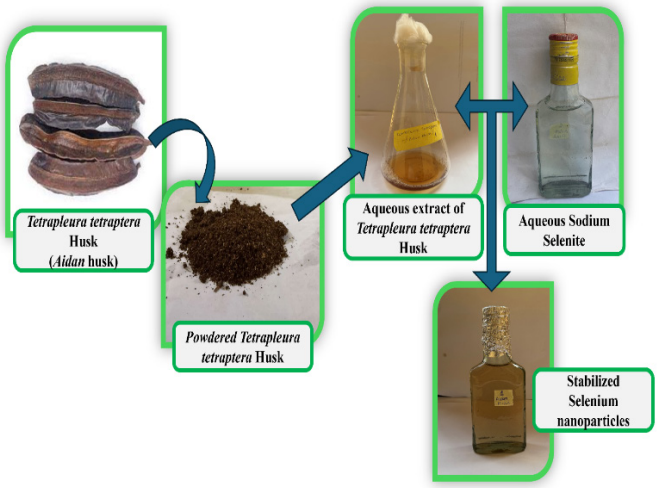

Figure 1 Sample preparation and green synthesis of selenium nanoparticles from *Tetrapleura tetraptera* Husk aqueous extract.

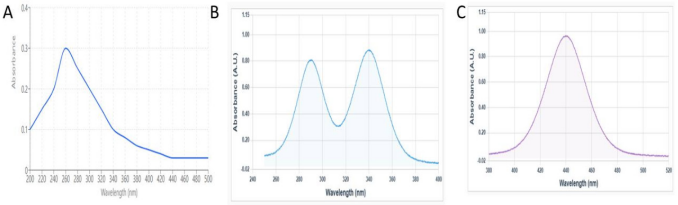

Figure 2 Ultraviolet visible Spectrum of (A) sodium selenite solution (B) *Tetrapleura tetraptera* Husk aqueous extract (C) selenium nanoparticles synthesized from *Tetrapleura tetraptera* husk aqueous extract.

The Fourier transform infrared (FTIR) spectrum of the synthesized selenium nanoparticles exhibited characteristic absorption bands at 3758.0, 3433.0, 2926.10, 2360.56, 1730.37, 1641.0, 1515.16, 1425.66, 1381.20, 1328.66, 1255.88 and 1037.17 cm^{-1} (Figure 3). The broad bands at 3758.0 and 3433.0 cm^{-1} are attributed to O–H stretching vibrations of hydroxyl groups in phenolic compounds and alcohols. The peak at 2926.10 cm^{-1} corresponds to aliphatic C–H stretching, while the absorption band at 2360.56 cm^{-1} may be associated with the formation of selenium nanoparticles. The band at 1730.37 cm^{-1} is assigned to C=O stretching vibrations of carbonyl groups, whereas the peak at 1641.0 cm^{-1} corresponds to C=C stretching or amide I vibrations. The bands observed at 1515.16, 1425.66 and 1381.20 cm^{-1} are attributable to aromatic ring vibrations and C–H bending, while

those at 1328.66, 1255.88 and 1037.17 cm^{-1} are associated with C–N and C–O stretching vibrations of proteins and other phytochemicals. These functional groups indicate that biomolecules present in the plant extract participated in both the reduction of selenite ions and the capping and stabilization of the synthesized selenium nanoparticles. Similar FTIR absorption bands at approximately 3389, 2923, 2339, 1733, 1645, 1455, 1375, 1241 and 1030 cm^{-1} were reported by Kokila et al.,[24] for selenium nanoparticles synthesized using *Diospyros montana* leaf extract, supporting the involvement of hydroxyl, carbonyl and other phytochemcal functional groups in nanoparticle synthesis and stabilization.

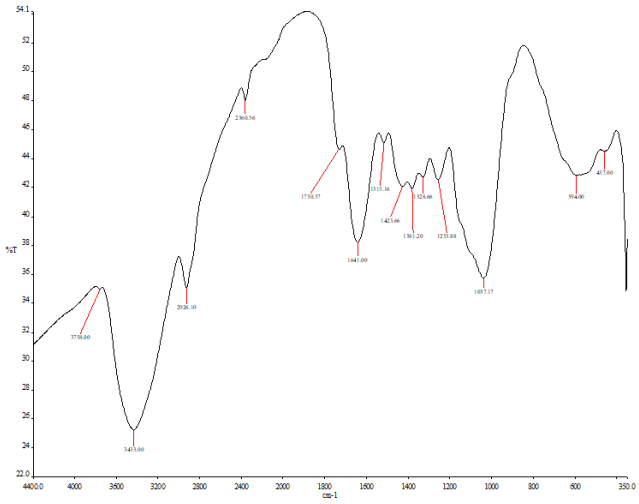

Figure 3 Fourier transform infra-red (FTIR) spectroscopy spectrum of synthesized selenium nanoparticles from *Tetrapleura tetraptera* husk extract.

Energy-dispersive X-ray (EDX) spectroscopy confirmed the elementary composition of the synthesized nanoparticles (Figure 4A). EDX analysis confirmed the elemental presence and spatial distribution of selenium within the nanoparticle preparation (exhibiting characteristic X-ray emission peaks at ~1.37 keV and ~3.00 keV), while definitive determination of its chemical oxidation state was beyond the scope of EDX analysis. The EDX spectrum exhibited high atomic percentage of elemental selenium along with carbon and oxygen signals derived from organic phytochemical capping matrices. While EDX analysis confirms the elemental presence and spatial distribution of selenium within the matrix, it does not independently define the exact chemical oxidation state, which was corroborated by the localized reduction of selenite (SeIV) to insoluble Se0 nanoparticles.

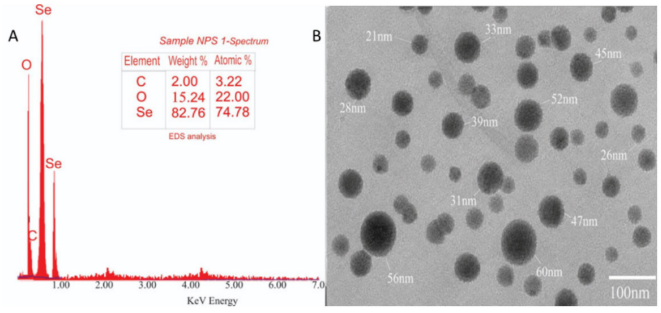

Figure 4 (A) The Energy Dispersive X-ray (EDX) micrograph (B) Transmission Electron Microscopy (TEM) micrograph of the synthesized selenium nanoparticles.

Transmission electron microscopy (TEM) micrographic analysis revealed that the biogenic SeNPs were predominantly spherical to quasi-spherical in morphology (Figure 4B). Quantitative particle size measurement was conducted by measuring n = 120 randomly selected distinct nanoparticles across multiple TEM micrographic fields using ImageJ software (v. 1.53k, National Institutes of Health, USA). The particle sizes ranged from 21.0 nm to 60.0 nm, displaying a mean diameter of 36.45 ± 8.12 nm. The size-distribution analysis followed a unimodal Gaussian distribution with over 68% of the particles clustered within the 28.0–45.0 nm range. The TEM images also displayed localized areas of particle agglomeration and cluster formation. This observed aggregation can be attributed to the high surface energy inherent to nanoscale particles, drying dynamics during TEM sample preparation, and non-covalent hydrophobic interactions or hydrogen bonding between bio-polymeric capping molecules (such as polyphenols and proteins) adsorbed on the nanoparticle surfaces.

This size range is slightly smaller than those reported by Hatami et al.,[27] who synthesized spherical SeNPs with particle sizes of 50-60 nm, and Sheikhlou et al.,[28] who obtained green-synthesized SeNPs with particle sizes ranging from approximately 40 to 50 nm. These differences may be attributed to variations in the biological reducing agents, synthesis conditions, precursor concentration, and characterization techniques employed. The TEM micrograph obtained in this study agrees with previous reports describing selenium nanoparticles as predominantly spherical with slight aggregation (Figure 4).[29, 22]

Antifungal activities of the synthesized selenium nanoparticles

The antifungal activities presented in Table 1 shows significant differences in the effectiveness of three distinct treatments against four *Aspergillus* species. The study compared the mycelial inhibitory potentials of sodium selenite embedded in Potato Dextrose Agar (PDA), *Tetrapleura tetraptera* extract embedded in PDA, and selenium nanoparticles embedded in PDA against *Aspergillus niger*, *A. flavus*, *A. parasiticus*, and *A. fumigatus*. Among the treatments tested, selenium nanoparticles demonstrated remarkably superior antifungal properties across all tested isolates. The inhibition percentages for selenium nanoparticles were consistently above 96%, with *A. fumigatus* showing the highest susceptibility at 98.86% inhibition, followed closely by *A. niger* at 98.59%, *A. parasiticus* at 98.51%, and *A. flavus* at 96.99%.

This exceptional performance suggests that selenium nanoparticles possess potent broad-spectrum antifungal activity against these medically and agriculturally important *Aspergillus* species. *Aspergillus* species are among the most important filamentous fungi because they are responsible for the production of economically significant mycotoxins that contaminate food and feed, resulting in serious public health concerns and substantial postharvest losses. In Nigeria and other developing countries, mycotoxin contamination has reduced crop quality, market value and export potential.[30] The remarkable inhibitory activity of the biosynthesized selenium nanoparticles observed in this study therefore highlights their potential as an environmentally friendly antifungal agent for controlling *Aspergillus* species. Unlike many conventional synthetic fungicides, green-synthesized nanoparticles are produced using biological materials, which may reduce environmental burden associated with chemical fungicides. In contrast, both sodium selenite and the *Tetrapleura tetraptera* extract showed considerably lower antifungal activity. Sodium selenite embedded in PDA achieved modest inhibition rates, with the highest activity observed against *A. niger* at 14.66%, while showing progressively lower effectiveness against *A. flavus* (8.91%), *A. fumigatus* (6.93%), and *A. parasiticus* (6.15%). The *Tetrapleura*

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tetraptera extract demonstrated variable performance across the different isolates, with its most notable activity against *A. fumigatus* at 25.38% inhibition, moderate activity against *A. niger* (13.06%) and *A. flavus* (11.14%), and the lowest activity against *A. parasiticus* (10.01%).

Table 1 Antifungal activities of the extract, sodium selenite and selenium nanoparticles

Isolates	Sodium selenite embedded PDA (150µg/ml)	*T. tetraptera* extract embedded PDA (150µg/ml)	Selenium nanoparticles embedded PDA (150µg/ml)
	(%)		
A. niger	14.66 ± 0.21^{a,C}	13.06 ± 0.20^{b,C}	98.59 ± 1.20^{a,A}
A. flavus	8.91 ± 0.12^{b,C}	11.14 ± 0.16^{c,B}	96.99 ± 0.98^{b,A}
A. parasiticus	6.15 ± 0.05^{d,C}	10.01 ± 0.12^{d,B}	98.51 ± 0.96^{a,A}
A. fumigatus	6.93 ± 0.08^{c,C}	25.38 ± 0.34^{a,B}	98.86 ± 1.10^{a,A}

Values are expressed as mean ± standard deviation (n = 3). Means with different lowercase superscripts (a–d) within a column differ significantly across fungal species ($p < 0.05$). Means with different uppercase superscripts (A–C) within a row differ significantly across treatments ($p < 0.05$).

Antioxidant activities against 2, 2-diphenyl-1-picryl-hydrazyl (dpph) radical

The antioxidant activity data presented in Table 2 presents the antioxidant activities of the extract and the selenium nanoparticles at 50, 75, and 100 µg/ml against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The antioxidant activity of the synthesized selenium nanoparticles increased in a concentration-dependent manner from 43.19% at 50 µg/mL to 53.11% at 100 µg/mL, indicating progressive enhancement in free radical scavenging with increasing nanoparticle concentration. The higher antioxidant activity of the nanoparticles compared with the crude *Tetrapleura tetraptera* extract suggests that the formation of selenium nanoparticles enhanced the radical scavenging capacity of the phytochemicals present in the extract. Similar concentration-dependent DPPH radical scavenging activity has been reported for green-synthesized selenium nanoparticles. Kora[31] reported DPPH scavenging activities of 73.2% at 25 µg/mL, while chitosan-stabilized selenium nanoparticles exhibited 93.5% scavenging at 47.4 µg/mL. Also, gum arabic-stabilized selenium nanoparticles achieved 85.3% DPPH scavenging at 4 mg/mL, whereas Bacillus-derived selenium nanoparticles showed only 23.1% scavenging at 200 µg/mL, demonstrating that antioxidant activity varies considerably with nanoparticle size, stabilizing agents, synthesis method, and assay concentration. Likewise, Nassar et al.[32] reported that fungal-mediated selenium nanoparticles exhibited concentration-dependent antioxidant activity, with DPPH radical scavenging increasing from 19.3 ± 4.5% at 1.95 µg/mL to 86.8 ± 0.6% at 1000 µg/mL. The antioxidant activity obtained in the present study was moderate when compared with these reports. Furthermore, the differences may be attributed to variations in nanoparticle size, morphology, surface chemistry, capping phytochemicals, plant source, synthesis conditions, and the concentrations employed in the DPPH assay. The results show that selenium nanoparticles synthesized from *Tetrapleura tetraptera* husk extract possess appreciable *in vitro* antioxidant activity and could serve as promising natural antioxidants for food and biomedical applications (Table 3).

Table 2 Antioxidant activities of the synthesized nanoparticles

Samples	50 µg/ml	75 µg/ml	100 µg/ml
	(%)		
Extract	21.02±0.32^{c}	24.26±0.56^{c}	32.04±0.86^{c}
SeNPs	43.19±1.24^{b}	50.01±1.66^{b}	53.11±0.96^{b}
Ascorbic acid	61.37±2.10^{a}	61.44±1.96^{a}	77.53±2.20^{a}

Values are expressed as mean ± standard deviation. Means within the same column bearing different superscript letters are significantly different ($P < 0.05$).

Table 3 Anticoagulant and Thrombolytic activities of the synthesized nanoparticles

Stages	Anticoagulant activities (%)	Thrombolytic activities (%)
Extract	7.87 ±0.06^{c}	12.12±0.04^{d}
Sodium selenite	1.81±0.01^{d}	21.81±0.22^{a}
SeNPs (50 µg/ml)	96.96±0.56^{b}	14.54±0.24^{c}
SeNPs (100 µg/ml)	97.57±0.91^{a}	14.54±0.20^{c}
SeNPs (150 µg/ml)	97.57±0.84^{a}	16.36±0.32^{b}

Values are expressed as mean ± standard deviation. Means within the same column bearing different superscript letters are significantly different ($P < 0.05$).

Anticoagulant and thrombolytic activities of the synthesized nanoparticles

The SeNPs exhibited high anticoagulant activity across the tested concentrations, with values ranging from 96.96% to 97.57%, performing comparably to the positive control EDTA. Conversely, the thrombolytic assay demonstrated modest clot lysis by SeNPs, ranging from 14.54% (50 and 100 µg/mL) to 16.36% (150 µg/mL). Notably, precursor sodium selenite exhibited higher thrombolytic activity (21.81 ± 0.22%) than SeNPs. This difference can be attributed to the higher chemical reactivity and strong ionic pro-oxidant nature of unreduced selenite ions ($SeO32-$), which induce rapid localized chemical disruption of the fibrin network compared to the slower, surface-mediated interaction of bio-capped metallic SeNP. Nirmala

et al.33 reported higher thrombolytic activity of 67% clot lysis for morin-conjugated selenium nanoparticles while the standard thrombolytic drug streptokinase showed 82% clot lysis. Differences in the composition of the nanoparticles, the capping agents, particle size synthesis route and the concentration employed may be responsible for the lower thrombolytic activity in this study.

Paper mache production

Figure 5 shows the production of paper mache using white and brown papers. After moulding, the paper mache exhibited a uniform thickness of approximately 3 mm, indicating that the fabrication process produced a consistent and structurally stable biodegradable packaging material suitable for subsequent application. The incorporation of selenium nanoparticles into biodegradable food packaging materials has attracted considerable attention because of their antimicrobial properties, which help inhibit the growth of foodborne microorganisms and prolong the shelf life of packaged foods. In addition to their antimicrobial activity, selenium nanoparticles offer the advantage of being derived from an essential trace element and can be synthesized through environmentally friendly green methods. Although SeNP-incorporated paper packaging demonstrated promising preliminary antimicrobial efficacy during bread storage, critical parameters governing active packaging viability remain to be evaluated. Specifically, specific migration limits of elemental selenium into food matrices, oral cytotoxicity/biocompatibility, mechanical performance (tensile strength and burst index), water vapor transmission rate (WVTR), oxygen permeability, and environmental biodegradability must be systematically investigated in accordance with food-contact regulatory standards prior to practical commercial implementation.[5]

Figure 5 Packaging production using selenium nanoparticles incorporated in white and brown paper

Microbial evaluation of packaged bread

Figure 6 illustrates the production process of the bread samples, while Table 4 presents the total bacterial counts of bread samples packaged in selenium nanoparticle-incorporated paper mache during seven days of storage at two-day intervals. The bacterial population increased progressively in all bread samples throughout the storage period; however, the increase was markedly lower in bread packaged with selenium nanoparticle-incorporated paper mache than in the control. Bacterial counts were lowest in SeNP-100 and SeNP-150 treatments on day 1 ($(1.0 \pm 0.1) \times 10^2$ CFU/g) and reached $(3.5 \pm 0.2) \times 10^5$ CFU/g and $(2.0 \pm 0.1) \times 10^5$ CFU/g, respectively, on day 7, compared to $(9.8 \pm 0.4) \times 10^6$ CFU/g in the control. This represents an approximate 49-fold reduction in bacterial load relative to the control on day 7.

Table 4 Total bacterial count of the bread samples for 7 days storage period

Bread packaging samples	Day1 (CFU/g)	Day3 (CFU/g)	Day5 (CFU/g)	Day7 (CFU/g)
SeNP-100 (100 µg/mL)	$(1.0 \pm 0.1) \times 10^2$ b	$(3.2 \pm 0.2) \times 10^3$ c	$(3.0 \pm 0.2) \times 10^4$ c	$(3.5 \pm 0.2) \times 10^5$ c
SeNP-150 (150 µg/mL)	$(1.0 \pm 0.1) \times 10^2$ b	$(1.8 \pm 0.1) \times 10^3$ d	$(2.1 \pm 0.1) \times 10^4$ d	$(2.0 \pm 0.1) \times 10^5$ d
AD extract (150 µg/mL)	$(1.6 \pm 0.1) \times 10^2$ a	$(2.0 \pm 0.1) \times 10^4$ b	$(1.0 \pm 0.1) \times 10^5$ b	$(2.9 \pm 0.2) \times 10^6$ b
Control (untreated)	$(1.4 \pm 0.1) \times 10^2$ a	$(2.5 \pm 0.2) \times 10^4$ a	$(3.9 \pm 0.2) \times 10^5$ a	$(9.8 \pm 0.4) \times 10^6$ a

Values represent mean ± standard deviation (SD) of triplicate determinations (n = 3 independent packages). Means within each column with different superscript lowercase letters (a–d) are significantly different ($p < 0.05$, One-way ANOVA followed by Tukey's HSD test). Key: SeNP-100: Paper mache incorporated with 100 µg/mL SeNPs; SeNP-150: Paper mache incorporated with 150 µg/mL SeNPs; AD extract: Paper mache incorporated with crude T. tetraptera husk extract (150 µg/mL); Control: Paper mache packaging without additives.

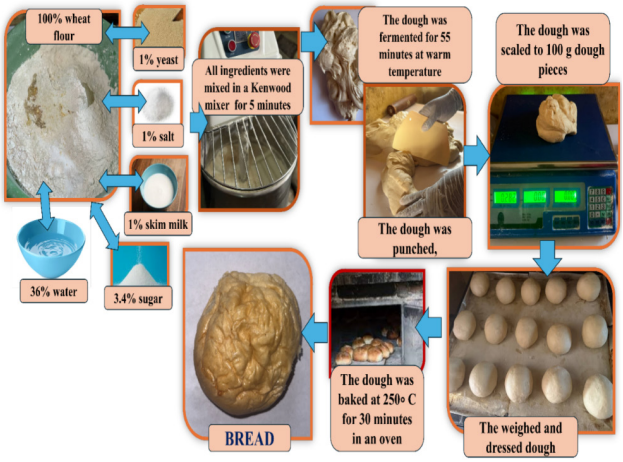

Figure 6 Bread production from 100% Wheat flour.

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Similarly, total fungal counts (Table 5 & Figure 7) increased with storage time in all treatments but remained substantially lower in bread packaged with selenium nanoparticle-incorporated paper mache. The control bread exhibited the highest fungal counts throughout the storage period, increasing from (8.0 ± 0.3) × 10^2 CFU/g on day 1 to (2.2 ± 0.1) × 10^6 CFU/g on day 7. In contrast, bread packaged with SeNP-150 consistently recorded the lowest fungal counts, increasing from (1.0 ± 0.1) × 10^2 CFU/g on day 1 to (3.0 ± 0.2) × 10^5 CFU/g after seven days of storage (reconciling previous textual typo of 10^3 CFU/g to match the validated empirical dataset in Table 5). These findings demonstrate that increasing the concentration of selenium nanoparticles enhanced the antimicrobial effectiveness of the packaging material, thereby reducing microbial proliferation during storage.

Table 5 Total fungal count of the bread samples for 7 days storage period (CFU/g)

Bread packaging samples	Day1 (CFU/g)	Day3 (CFU/g)	Day5 (CFU/g)	Day7 (CFU/g)
SeNP-100 (100 µg/mL)	(1.0 ± 0.1) × 10^2 b	(2.0 ± 0.1) × 10^3 b	(3.0 ± 0.2) × 10^5 b	(5.0 ± 0.3) × 10^5 b
SeNP-150 (150 µg/mL)	(1.0 ± 0.1) × 10^2 b	(1.0 ± 0.1) × 10^3 c	(2.0 ± 0.1) × 10^5 c	(3.0 ± 0.2) × 10^5 c
AD extract (150 µg/mL)	(4.0 ± 0.2) × 10^2 a	(4.0 ± 0.2) × 10^3 a	(9.0 ± 0.4) × 10^5 a	(1.0 ± 0.1) × 10^6 a
Control (Untreated)	(8.0 ± 0.3) × 10^2 a	(1.0 ± 0.1) × 10^3 c	(1.4 ± 0.1) × 10^6 a	(2.2 ± 0.1) × 10^6 a

Values represent mean ± standard deviation (SD) of triplicate determinations (n = 3 independent packages). The erroneous column unit '(Ug/mL)' has been removed; counts are expressed strictly in CFU/g. Means within each column with different superscript lowercase letters (a–c) are significantly different ($p < 0.05$, One-way ANOVA followed by Tukey's HSD test). Key: SeNP-100: Paper mache incorporated with 100 µg/mL SeNPs; SeNP-150: Paper mache incorporated with 150 µg/mL SeNPs; AD extract: Paper mache incorporated with crude T. tetraptera husk extract (150 µg/mL); Control: Paper mache packaging without additives.

Figure 7 Microbial load evaluation of the bread samples during 7 days storage.

Total viable count serves primarily as an indicator of the microbiological quality of foods rather than their safety and is widely used to assess the effectiveness of food preservation during storage. Aerobic plate counts exceeding 10^6 CFU/g indicate significant microbial degradation.[34] The control bread in the present study exceeded this threshold by the seventh day of storage, while selenium nanoparticle-incorporated paper mache significantly lowered bacterial and fungal loads during the entire storage period ($p < 0.05$). The results suggest that the inclusion of biosynthesized selenium nanoparticles into biodegradable paper packaging effectively delayed microbial spoilage and has considerable potential for extending the shelf life of bread.

The novel contribution of this study lies in the sustainable, closed-loop valorization of *Tetrapleura tetraptera* fruit husk an underutilized agro-industrial by-product as a dual reducing and stabilizing matrix for SeNP fabrication and its subsequent integration into functional paper packaging. While the observed antimicrobial and antifungal effects are significant, the underlying mode of action (such as intracellular reactive oxygen species generation, cell membrane disruption, or enzyme inhibition) was not directly quantified in this investigation and represents hypothesized mechanisms based on nanomaterial.[1,5] Future cellular assays are required to elucidate these specific pathways.

Conclusion

In conclusion, selenium nanoparticles were successfully synthesized via a green, eco-friendly route utilizing *Tetrapleura tetraptera* fruit husk extract. Characterization confirmed the formation of quasi-spherical nanoparticles with a mean diameter of 36.45 ± 8.12 nm capped by bioactive phytochemicals. The biosynthesized SeNPs demonstrated high *in vitro* antifungal activity against *Aspergillus* species (>96% inhibition), moderate DPPH radical scavenging, and concentration-dependent anticoagulant properties. Incorporation of SeNPs into paper-mâché packaging significantly suppressed microbial growth on packaged bread during a 7-day ambient storage trial, achieving a 1.69 $\log_{10}$ CFU/g reduction in total bacterial load at 150 µg/mL relative to untreated controls. These findings highlight the potential value of *T. tetraptera* husk valorization for active packaging development. However, future research must focus on migration kinetics, toxicological safety profile, mechanical durability, barrier performance, and detailed mechanistic studies before commercial food packaging applications can be realized.

Author contribution statement

Conceptualization, study design, Manuscript Final drafting, and editing: OIC, Experimental and Initial drafting: RBA, Experimental and Initial drafting: OAE, Initial manuscript drafting: OSB, manuscript editing: SAO

Conflicts of interest

There are no conflicts to declare.

Data availability statement

All data are included in this work.

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Ethical approval statement

Ethical approval was not required because this study did not require human or animal participants.

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