

Green synthesis of selenium nanoparticles using *Ficus platyphylla* leaf and bark extracts: antimicrobial activity against mrsa and other pathogens, anticoagulant and antioxidant properties

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Abstract

The rise of antibiotic-resistant bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella oxytoca*, underscores the need for novel antimicrobial strategies. This study synthesized selenium nanoparticles (SeNPs) for the first time using *Ficus platyphylla* leaf (FPL) and bark (FPB) extracts, employing a sustainable green synthesis approach. The nanoparticles were characterized using UV-visible spectroscopy, FTIR, TEM, XRD, and EDX. Polydispersed spherical, oval, and square-shaped SeNPs from bark (FPBS) exhibited maximum absorption at 327 nm, while monodispersed, oval-shaped SeNPs from leaves (FPLS) absorbed at 338 nm. FTIR confirmed the involvement of phenolic compounds and proteins in nanoparticle synthesis. At 200 µg/ml, FPLS and FPBS showed significant antibacterial activity, with inhibition zones of 33.3 ± 0.88 mm and 32.3 ± 0.33 mm against MRSA and *Klebsiella oxytoca*, respectively. The SeNPs demonstrated antifungal activity (97.21–100% inhibition) against *Aspergillus* and *Penicillium* species, as well as antioxidant activity, scavenging DPPH (37.76–79.99%) and H₂O₂ (17.32–77.40%) at 1–50 mg/ml. They also showed anticoagulant potential, comparable to EDTA. This study highlights the alignment with circular bioeconomy principles by utilizing sustainable, renewable plant resources to synthesize SeNPs, minimizing the environmental impact. It also supports the United Nations Sustainable Development Goals, particularly Goal 3 (Good Health and Well-being) and Goal 12 (Responsible Consumption and Production). These findings showcase *F. platyphylla*-derived SeNPs as eco-friendly, multifunctional nanoparticles with significant biomedical applications, advancing green nanotechnology.

Introduction

The rise of antibiotic-resistant bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella oxytoca*, has become a global health crisis, necessitating the urgent development of alternative treatment strategies. MRSA, a variant of *Staphylococcus aureus*, is notorious for causing severe infections in both healthcare and community settings, often complicated by biofilm formation, which enhances its resistance to conventional antibiotics and increases healthcare costs [1, 2]. Factors such as the misuse and overuse of antibiotics have exacerbated the prevalence of resistant strains [3, 4]. Similarly, *Klebsiella oxytoca*, a significant pathogen in hospital-acquired infections, is associated with multidrug resistance, particularly due to the presence of extended-spectrum beta-lactamases (ESBLs) and biofilm formation, which further limits treatment options [5, 6]. Given these challenges, there is an increasing interest in the identification of novel antimicrobial agents, especially those sourced from natural origins. SeNPs have attracted considerable interest owing to their extensive antibacterial characteristics and potent antioxidant powers. The distinctive physicochemical characteristics of SeNPs, such as a high surface area-to-volume ratio and increased reactivity, render them more potent against resistant bacteria and beneficial in mitigating oxidative stress in cells [7, 8]. Selenium, a vital trace element, is crucial in cellular defense by reducing oxidative stress and bolstering immunological responses. SeNPs demonstrate enhanced bioavailability, decreased toxicity, and amplified biological activity, making them viable candidates for therapeutic applications [9, 10].

Green synthesis, utilizing plant extracts as natural reducing and stabilizing agents, has emerged as a sustainable and environmentally acceptable alternative for nanoparticle creation. Phytochemicals, including flavonoids, phenolics, tannins, and terpenoids found in plant extracts, not only promote nanoparticle production but also augment their biological activity (Ravi *et al.*, 2023). This method reduces the ecological footprint of traditional synthesis techniques by utilizing renewable and biocompatible materials (Khan *et al.*, 2022). The eco-friendly synthesis of SeNPs using plant extracts as natural reducing and stabilizing agents has become a viable alternative to conventional synthesis methods. This method utilizes the abundant phytochemical constituents of plant extracts, such as flavonoids, phenolics, tannins, and terpenoids, which promote nanoparticle synthesis and augment their biological efficacy [11]. The manufacture of SeNPs utilizing *Bacillus subtilis* has been recorded, demonstrating their potential for antibacterial applications [12]. The characterization of SeNPs has demonstrated their capacity to interact with bacterial cells, resulting in improved antibacterial actions [13]. Research has shown that SeNPs can significantly impede the proliferation of MRSA and other and carbapenem-resistant *Klebsiella* species as well as antifungal activity against genera such as *Aspergillus* and *Penicillium*, underscoring their potential as alternative treatment agents [14, 15]. Furthermore, green-synthesized SeNPs possess antioxidant capabilities associated with their capacity to scavenge free radicals, presenting therapeutic potential for the management of oxidative stress-related illnesses (Wang *et al.*, 2024). The dual antibacterial and antioxidant properties of SeNPs render them interesting candidates for many biological applications. The capacity of SeNPs to neutralize free radicals and bolster cellular antioxidant defenses renders them significant possibilities for therapeutic strategies designed to alleviate oxidative stress [16]. The environmental advantages of green synthesis technologies are significant.

Comprehensive characterization is needed to completely understand the structural and functional features of SeNPs for their potential applications. Techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Ultraviolet-Visible Spectroscopy (UV-Vis) elucidate the chemical bonding and optical characteristics of nanoparticles. Transmission Electron Microscopy (TEM) and X-ray Diffraction (XRD) provide insights into their dimensions, shape, and crystalline structure, which are essential for forecasting and improving their biological activities (Alvi *et al.*, 2021; Ganapathy, 2021; Garza-García *et al.*, 2023; Lazcano-Ramírez *et al.*, 2023). These characterization approaches not only validate the effective synthesis of SeNPs but also provide a basis for enhancing their efficacy in certain applications. The analysis of SeNPs via FTIR enables researchers to discern the functional groups implicated in the synthesis process, thereby validating the presence of phytochemicals that facilitate the reduction and stability of the nanoparticles [17]. UV-Vis spectroscopy is essential for tracking the synthesis of SeNPs by detecting the distinctive surface plasmon resonance peaks that signify nanoparticle production [18]. TEM research offers comprehensive insights into the dimensions and shape of SeNPs, which are critical factors affecting their biological activity [19]. XRD analysis enhances these techniques by elucidating the crystalline characteristics of the nanoparticles, which may influence their stability and reactivity [20].

F. platyphylla, referred to as the Gutta-percha tree, is a significant medicinal plant historically employed in traditional medicine, especially in Nigeria. This species is noted for its diverse phytochemical makeup,

which supports its several medicinal uses. In Northern Nigeria, infusions of *F. platyphylla* leaves and bark are traditionally utilized for their antiepileptic effects, highlighting the plant's importance in seizure management [21]. The effectiveness of *F. platyphylla* in seizure management has been recorded, with local populations depending on its therapeutic properties for centuries [21]. Besides its antiepileptic properties, *F. platyphylla* demonstrates significant antibacterial action, especially against multidrug-resistant bacterial strains. Recent research has validated its efficacy against multiple bacterial infections, including those induced by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [22]. The antibacterial characteristics of *F. platyphylla* are ascribed to its abundant phytochemical composition, encompassing flavonoids, tannins, and saponins that enhance its medicinal efficacy [22]. These findings underscore the significance of *F. platyphylla* as a crucial asset in combating antibiotic resistance, an escalating issue in both clinical and community environments [22].

The anti-inflammatory characteristics of *F. platyphylla* are also significant. The presence of tannins and saponins in the plant is thought to regulate inflammatory pathways, rendering it a viable option for addressing inflammatory diseases [21]. The conventional uses of *F. platyphylla* in wound healing highlight its therapeutic flexibility. The flavonoids and terpenes included in the plant facilitate tissue repair, rendering it a favored option for wound management in traditional medicine [23]. This corresponds with the widespread acknowledgment of the plant's capacity to facilitate healing and recuperation from injuries. Furthermore, the antioxidant potential of *F. platyphylla* is substantial, as its phenolic and flavonoid components are helpful in mitigating oxidative stress linked to chronic diseases, such as neurological disorders and cancer [22]. The plant's capacity to scavenge free radicals renders it a prospective candidate for the development of natural antioxidants that may enhance conventional treatments for oxidative stress-related disorders [22]. This facet of *F. platyphylla*'s pharmacological profile is especially pertinent given the growing interest in natural products for health enhancement and illness prevention.

F. platyphylla has historically been employed in the management of metabolic diseases, including diabetes. The antidiabetic qualities of the plant have been acknowledged in traditional therapies, where its extracts are utilized to improve glycemic control and mitigate diabetes-related symptoms [21]. Emerging scientific research corroborates this traditional knowledge, highlighting the potential of *F. platyphylla* in the management of metabolic health [21]. The plant's function in diabetes care exemplifies the overarching trend of merging ancient medicinal practices with contemporary health strategies, especially in areas with restricted access to mainstream therapy. Besides its applications in human health, *F. platyphylla* exhibits potential in aquaculture and veterinary medicine. Plant extracts exhibit antibacterial effects against fish illnesses, underscoring their significance in enhancing fish health and mitigating disease outbreaks in aquaculture environments [21]. The dual application of *F. platyphylla* in human and animal health emphasizes its adaptability and the necessity of investigating its complete therapeutic potential. The antiparasitic effectiveness of *F. platyphylla*, especially against apicomplexan infections, enhances its potential for broader therapeutic applications [21]. This facet of the plant's pharmacological profile is especially pertinent in areas where parasitic illnesses are common, and traditional treatments are frequently pursued for their treatment.

The historical application of *F. platyphylla* for respiratory conditions, especially cough, is ascribed to its bioactive components, including flavonoids and saponins, which enhance its therapeutic properties [21]. The bioactive compounds of *F. platyphylla*, such as flavonoids, tannins, phenolics, and saponins, provide it a superior option for the synthesis of green nanoparticles. These phytochemicals serve as natural reducing and capping agents, promoting the environmentally sustainable synthesis of SeNPs [23, 24]. Recent studies have shown the effectiveness of green-synthesized SeNPs in treating multidrug-resistant infections and mitigating oxidative stress-related diseases [23, 24]. The incorporation of *F. platyphylla* in nanoparticle production not only improves the biological properties of the nanoparticles but also corresponds with the increasing focus on sustainable and environmentally friendly methods in nanotechnology [23, 24]. Figure 1 presents a visual synopsis of the ethnopharmacological applications and their corresponding bioactive components, emphasizing the plant's potential for extensive medicinal uses and its appropriateness for green nanoparticle manufacturing. Notwithstanding its established pharmacological attributes, the capacity of *F. platyphylla* for nanoparticle formation remains predominantly unexamined.

This study presents the novel green production of selenium nanoparticles utilizing the leaf and bark extracts of *F. platyphylla*. The synthesized SeNPs were assessed for antibacterial activity against MRSA and *Klebsiella oxytoca* via the agar well diffusion method, and for antifungal efficacy against *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium* species using both the agar well diffusion and poisoned plate methods. Furthermore, their antioxidant activities were evaluated by DPPH radical scavenging and hydrogen peroxide (H₂O₂) tests. This research presents a sustainable method for nanoparticle synthesis, illustrating the efficacy of SeNPs as multifunctional agents against multidrug-resistant infections and oxidative stress, thereby advancing plant-based nanotechnology in biomedical research.

Materials and methods

2.1 Materials

The chemicals and reagents utilized in this investigation were obtained from K-Prime Medics Integrated Limited, Nigeria with all reagents being of analytical grade and possessing a minimum purity of 99%.

2.2. Plant materials for the preparation of extracts

The leaf (FPL) and bark (FPB) of *F. platyphylla* were obtained from a plant in Niger State, Nigeria, and validated (UILH/001/1825/2024) at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The dried materials, produced via air-drying at ambient temperature and milling, were extracted with water at 60°C for 1 hour using a 1% (wt/vol) solution. Subsequently, filtration was conducted utilizing Whatman filter paper No. 1, following by centrifugation at 4000 rpm for 20 minutes to acquire the diverse extracts, which were employed without additional purification [25].

2.3 Phytosynthesis and Characterization of Selenium NanoParticles

SeNPs were produced by combining 1 ml of each extract with 9 ml of a 10 mM Na₂SeO₃ (Sigma-Aldrich, USA) solution at 37°C for 24 hours. The alteration in color due to the bioformation of particles was observed visually, followed by the determination of the absorption pattern through scanning in the 250–800 nm range with a UV-Vis spectrophotometer (Specord 200 Plus). The produced SeNPs were then characterized utilizing Fourier transform infrared spectroscopy (Nicolet iS10 FTIR Spectrometer), transmission electron microscopy (JEM-ARM200F-G TEM JOEL-JSM 7600 FJ), energy-dispersive X-ray spectroscopy, and X-ray diffraction.

2.4 Antimicrobial efficacy of the biosynthesized *Ficus platyphylla* leaf SeNPs (FPLS) and *Ficus platyphylla* bark SeNPs (FPBS)

The agar well diffusion method was employed to evaluate the antibacterial efficacy of FPLS and FPBS against MRSA and a multidrug-resistant strain of *K. oxytoca* obtained from the University of Ilorin Teaching Hospital, Ilorin, Nigeria. Bacterial suspensions, standardized to a 0.5 McFarland standard (1×10^6 cfu/ml), were uniformly disseminated on Mueller Hinton agar plates. Sterile cork borers were employed to create wells in agar plates, which were subsequently filled with 50–200 µg/ml of the biosynthesized FPLS and FPBS. The plates were incubated at 37°C for 18 to 24 hours, and the inhibition zones surrounding each well were measured in millimeters. The antifungal efficacy of the nanoparticles against certain fungi of medical importance was assessed using the methodologies established by Yusuf-Saliyu et al. (2025). Concentrations of FPLS and FPBS (50 and 100 mg/ml) were mixed into potato dextrose agar (PDA) plates, onto which 6 mm agar plugs from 48-hour cultures of *Aspergillus flavus*, *Penicillium* sp., and *Aspergillus niger* were put. PDA plates inoculated with fungal agar plugs devoid of nanoparticles served as the control group. All plates were incubated at $28 \pm 2^\circ\text{C}$ for 72 hours. The diameters (D) of the fungal growth were measured in millimeters, and the percentage of growth inhibition was calculated accordingly.

$$\% \text{ Fungal growth inhibition} = \frac{D \text{ Control} - D \text{ Test}}{D \text{ Control}} \times 100$$

2.5 Anticoagulant activities of the biosynthesized FPLS and FPBS

In this experiment, 1 ml of FPLS and FPBS at a concentration of 100 µg/ml was uniformly mixed with blood from a healthy donor in a sterile Eppendorf tube. Blood was collected in an EDTA vial for the positive control, while the negative controls comprised just blood samples obtained in a sterile Eppendorf tube. The samples were permitted to rest for up to one hour, during which visual evaluations were performed to identify any blood coagulation. In order to confirm the results, each Eppendorf tube was inverted to evaluate the presence of anticoagulation. The tube's contents were subsequently placed on a slide and examined under an optical microscope to identify the red blood cells, in accordance with the methodology described by Yusuf-Saliyu et al. (2025).

2.6 Antioxidant activities of the biosynthesized FPLS and FPBS

The antioxidant activity of the biosynthesized FPLS and FPBS was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) test and the H₂O₂ (hydrogen peroxide scavenging) assay, adhering to the established methodology [25].

Various concentrations (1, 5, 10, 20, 30, 40 and 100 µg/ml) of FPLS and FPBS were evaluated in the DPPH assay. One milliliter of nanoparticles was combined with four milliliters of a 1 mM DPPH solution. The combination was maintained in a dark setting for 30 minutes to enable the nanoparticles to sequester the DPPH free radicals. Ascorbic acid functioned as the standard reference compound for evaluating the activity of the extracts. Following the incubation period, the solution's absorbance was assessed at 517 nm utilizing a spectrophotometer (Spectro UV-2505). The experiment was conducted in triplicate, and the DPPH scavenging activity was evaluated as follows:

$$\%DPPH \text{ scavenging effect} = \frac{AB - AS}{AB} \times 100$$

Where: AB = Blank's absorbance and AS = Sample's absorbance.

A 40 mM H₂O₂ solution was prepared with a phosphate buffer solution at pH 7.4 for the hydrogen peroxide scavenging experiment. Various concentrations of FPLS and FPBS (1, 5, 10, 20, 30, 40 and 50 µg/ml) were amalgamated with exactly 0.6 ml of a 40 mM H₂O₂ solution in 4 ml of distilled water. A spectrophotometer (Spectro UV-2505) was employed to quantify the absorbance of H₂O₂ at 610 nm following a 20-minute incubation period. The results were juxtaposed with a control solution consisting of phosphate buffer and H₂O₂. The reference chemical employed to evaluate the activity of the extracts was ascorbic acid. The H₂O₂ scavenging assay was determined through triplicate execution of the experiment using the formula provided below:

$$\% \text{ H}_2\text{O}_2 \text{ scavenging effect} = \frac{AC - AS}{AC} \times 100$$

Where AC represents the absorbance of the negative control, and AS denotes the absorbance of the sample.

2.7 Statistical analysis

All experimental results were subjected to statistical analysis utilizing SPSS Version 27.0 software. Data were presented as mean ± standard error (SE) and were obtained from three independent experiments completed in triplicate, dependent on the assay. Substantial differences among experimental groups were assessed using one-way analysis of variance (ANOVA), succeeded by Duncan's post-hoc test to compare means and emphasize significant changes among samples. A p-value less than 0.05 was deemed statistically significant.

Results and Discussion

3.1 Phytosynthesis and Characterization of Biosynthesized Selenium nanoparticles

The production of SeNPs utilizing extracts from *F. platyphylla* (FPL) leaves and bark (FPB) exhibits a significant color change, signifying the successful generation of these nanoparticles (Fig. 2B). In the instance of FPL, the initial yellow hue of the reaction mixture shifts to a ruby red upon the stabilization of the biosynthesized FPL SeNPs (FPLS). Likewise, for FPB, the initial light wine red hue intensifies to a deep wine red with hints of ruby red following the stabilization of the biosynthesized FPB SeNPs (FPLB). The alteration in hue is a prevalent occurrence noted in the production of SeNPs, commonly ascribed to the surface plasmon resonance (SPR) effect, a defining trait of metallic nanoparticles, such as selenium [28, 29].

The UV-Vis spectral analysis of *F. platyphylla* leaf and bark extracts (FPL and FPB), their selenium nanoparticles (FPLS and FPBS), and selenium salt (SE) offers substantial insights into the interactions between bioactive compounds and selenium, as well as the synthesis of SeNPs (Fig. 2). The measured maximum absorbance wavelengths (λ_{max}) of the FPL and FPB extracts at 272 nm and 288 nm, respectively, suggest the existence of phytochemicals that can interact with selenium ions. The ensuing red shifts to 338 nm for FPLS and 327 nm for FPBS indicate that these phytochemicals promote the reduction of selenium ions, resulting in the creation of stable nanoparticles. This effect corresponds with the surface plasmon resonance (SPR) property of nanoparticles, validating the successful synthesis of SeNPs [22, 30].

The absorbance readings further corroborate these observations. The initial absorbance of the leaf extract at 3.3925 dropped to 1.0031 during SeNP production, but the bark extract's absorbance reduced from 3.4858 to 1.2093. The decrease in absorbance signifies the active involvement of bioactive compounds in the manufacturing process, wherein these phytochemicals function as both reducing and capping agents [31, 32]. Conversely, the selenium salt had a λ_{max} at 250 nm with a notably reduced absorbance of 0.4803, underscoring the supplementary roles of phytochemicals in the extracts that augment the stability and optical characteristics of the SeNPs [28, 33].

The literature indicates that the plant-mediated production of nanoparticles frequently alters spectrum characteristics due to interactions between metal ions and phytochemicals. Research indicates that the fabrication of SeNPs utilizing plant extracts results in notable alterations in λ_{max} and variations in absorbance values, signifying efficient mediation by the extracts [34, 35]. The decrease in absorbance after nanoparticle formation indicates the utilization of bioactive compounds during the reduction and stabilization processes, so supporting the involvement of *F. platyphylla* extracts in the synthesis of SeNPs [36, 37]. The different functional groups revealed in the FTIR results for both the extracts and the nanoparticles confirm this.

The FTIR spectral analysis demonstrated the presence of numerous chemical linkages and vibrations associated with the biomolecules responsible for the production and stability of the SeNPs. FTIR analysis of FPB and FPBS revealed peaks indicative of hydrogen-bonded O-H stretching associated with alcohols, phenols, and carboxylic acids (2361–3479 cm⁻¹), N-H bending of primary amines (1594–1641 cm⁻¹), C-N stretching of aliphatic amines (1027–1083 cm⁻¹), and = C-H bending of alkenes (674–804 cm⁻¹) (Fig. 2). The FTIR analysis for FPL and FPLS (Fig. 3) reveals peaks corresponding to the following functional groups: O-H stretch of alcohols and phenols (3391–3489 cm⁻¹), C-H stretch of alkanes (2925 cm⁻¹), O-H stretch of carboxylic acids (2359 cm⁻¹), C = O stretch (1743 cm⁻¹), N-H bend of primary amines (1647–1652 cm⁻¹), C-N stretch of aliphatic amines (1082 cm⁻¹), and = C-H bend of alkenes (710–871 cm⁻¹). The obtained peaks indicate that the proteinaceous components in the plant extracts are responsible for the formation and biostabilization of the SeNPs [38].

Furthermore, the transmission electron micrographs of the biosynthesized SeNPs indicated that FPBS comprises polydispersed spherical, oval, and square-like nanoparticles ranging from 6.28 to 17.99 nm, whereas FPLS consists of fairly monodispersed oval nanoparticles measuring between 20.07 and 33.91 nm. The EDX spectra indicated that selenium was the predominant element in both biosynthesized nanoparticles, while the XRD patterns demonstrated their crystalline structure, characterized by distinct Bragg peaks observed (Fig. 4).

3.2 Antimicrobial activities

The antibacterial efficacy of FPLS and FPBS against *Klebsiella oxytoca* and methicillin-resistant *Staphylococcus aureus* (MRSA) exhibits a notable dose-dependent relationship. The findings demonstrate that a rise in SeNP concentration correlates with a larger zone of inhibition for both bacterial strains, with FPLS exhibiting enhanced antibacterial efficacy relative to FPBS at elevated concentrations (Fig. 6). At a dose of 200 µg/ml, FPLS demonstrated a zone of inhibition measuring 33.3 ± 0.88 mm against MRSA, whereas FPBS displayed a maximal inhibition of 32.3 ± 0.33 mm at the identical concentration (Table 1). This indicates that the leaf extract may possess more potent phytochemicals, hence augmenting its antibacterial efficacy [39, 40].

The documented antibacterial effects correspond with prior research indicating the effectiveness of selenium nanoparticles against multiple bacterial strains, including *Staphylococcus aureus*, due to their capacity to produce reactive oxygen species (ROS) that compromise bacterial cell membranes [36, 41]. The antibacterial efficacy of SeNPs is affected by their size, morphology, and synthesis process, which can influence their interaction with bacterial cells [40]. The synthesis of SeNPs utilizing plant extracts, such as those from *F. platyphylla*, offers a biocompatible and environmentally sustainable method while augmenting the antibacterial efficacy of the nanoparticles due to the presence of supplementary bioactive compounds in the extracts [42, 43]. The findings of this work support previous studies indicating that selenium nanoparticles might impede biofilm formation and display cytotoxic properties against pathogenic bacteria [41, 44]. These results are comparable to the findings of [45], where DM-SeNPs produced by another approach exhibited significant inhibition zones against *Staphylococcus aureus* (34.16 mm). These data further highlight the diversity in SeNP performance contingent upon

synthesis methodologies and target pathogens. The synthesized FPLS and FPBS had significant antibacterial efficacy against *Klebsiella oxytoca* and MRSA, with FPL-SeNPs exhibiting greater effectiveness at elevated doses. The findings underscore the potential of plant-derived selenium nanoparticles as a natural antibacterial agent, warranting further investigation for therapeutic uses in the treatment of bacterial infections.

Table 1
Antibacterial activities of FPLS and FPBS.

Samples	Zone of inhibition (mm)							
	K. oxytoca				S. aureus			
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
FPLS	0 ± 0	13.67 ± 0.88	16.33 ± 0.33	20 ± 1.53	21.33 ± 0.88	23.33 ± 1.20	30.33 ± 0.33	33.33 ± 0.88
FPBS	0 ± 0	12.33 ± 0.33	13.33 ± 0.33	19.33 ± 1.20	0 ± 0	26 ± 1.15	29.67 ± 0.33	32.33 ± 0.33

Data are presented as mean ± SE from three independent experiments performed in triplicate.

The antifungal tests with FPLS and FPBS demonstrate substantial suppression of three fungal species: *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium* species. The nanoparticles were evaluated using the poison plate method, and the results indicate their potent antifungal activities (Fig. 7). Both FPLS and FPBS completely inhibited *Aspergillus niger*, suppressing 100% of the fungal growth. The findings demonstrate that selenium nanoparticles derived from both the leaf and bark of *F. platyphylla* exhibit significant efficacy against this fungus species, highlighting their robust antifungal properties. Likewise, both varieties of selenium nanoparticles entirely suppressed the growth of *Penicillium* species, achieving 100% inhibition. The uniform outcome among several fungus species underscores the extensive antifungal effectiveness of the nanoparticles. The inhibition of *Aspergillus flavus* was nearly complete, with a documented inhibition rate of 97.217% for both FPLS and FPBS. *A. flavus* exhibited slightly greater resistance than the other two fungal species; nonetheless, the inhibition % still reflects a robust antifungal effect of the selenium nanoparticles against this species.

The results indicate that selenium nanoparticles derived from *F. platyphylla* possess significant potential as antifungal agents. The total suppression of *A. niger* and *Penicillium* species, along with the nearly total inhibition of *A. flavus*, highlights their efficacy. The similarity in results from both leaf and bark-derived nanoparticles further suggests that the source material does not substantially affect the antifungal activity of these nanoparticles. This study may facilitate the creation of novel antifungal therapies utilizing selenium nanoparticles, specifically targeting fungal diseases such as *A. niger* and *Penicillium* species [46].

3.3 Anticoagulant activities

The anticoagulant efficacy of SeNPs derived from FPLS and FPBS exhibited substantial suppression of blood coagulation, as illustrated in Fig. 8. Both FPLS and FPBS yielded results analogous to the activity of EDTA, utilized as the positive control, but the negative control, consisting of fresh human blood taken in an Eppendorf tube, exhibited complete coagulation. Microscopic analysis of the blood treated with nanoparticles demonstrated the integrity of red blood cell morphology, suggesting that the SeNPs successfully inhibited coagulation without inducing cellular harm. These results corroborate earlier research on the anticoagulant characteristics of biogenic selenium nanoparticles, underscoring their promise as efficacious anticoagulants [47–49].

Preventing blood coagulation is essential for preserving hemostasis, especially in patients with coagulation disorders. The development of blood clots may result in serious problems, including embolism, stroke, and sepsis if the clots become infected. Recently, nanoparticles have surfaced as prospective anticoagulants owing to their remarkable efficacy in inhibiting blood coagulation. Biogenic nanoparticles provide benefits including improved biocompatibility, sustained effectiveness at minimal dosages, and combined therapeutic and diagnostic functions [2, 49, 50]. In contrast to traditional anticoagulants such as heparin and aspirin, nanoparticles can enhance the anticoagulant efficacy of these medications synergistically. This study's findings further substantiate the anticoagulant efficacy of FPLS and FPBS, underscoring their potential utility in nanomedicine for the management of blood coagulation disorders.

3.4 Antioxidant activities

A systematic assessment utilizing the DPPH radical scavenging assay reveals a significant difference in the antioxidant properties of *F. platyphylla* leaf-synthesized SeNPs (FPLS) compared to bark-synthesized SeNPs (FPBS). FPLS demonstrated markedly enhanced activity, particularly at elevated concentrations, underscoring the vital influence of phytochemical composition on the antioxidant characteristics of plant-derived nanoparticles [22, 51, 52]. The increase in radical scavenging activity for both FPLS and FPBS is significant and depending on concentration. FPLS exhibited scavenging activity from 39.74% at 1 µg/ml to 79.99% at 50 µg/ml, whereas FPBS demonstrated a range from 37.76% at 1 µg/ml to 63.12% at 50 µg/ml (Fig. 9a). Conversely, ascorbic acid, a conventional antioxidant, demonstrated percent inhibition values ranging from 78.66% at 1 µg/ml to 93.71% at 50 µg/ml, highlighting the competitive efficacy of FPLS as powerful antioxidants [51, 53]. This concentration-dependent response indicates that FPLS possesses an enhanced ability to neutralize free radicals, which is crucial in high-stress oxidative conditions, such as those found in pharmaceuticals and nutraceuticals [22, 51, 52].

The observed concentration-dependent scavenging activity corresponds with accepted concepts in antioxidant research, wherein higher concentrations of antioxidants generally correlate with improved scavenging efficacy. This behavior can be ascribed to the enhanced availability of reactive sites on the nanoparticles, promoting their interaction with DPPH radicals. Prior research has indicated that selenium nanoparticles possess significant free radical scavenging capabilities, underscoring the idea that their antioxidant efficacy is contingent upon their concentration and structural attributes [54, 30, 55]. The enhanced antioxidant activity of FPLS relative to FPBS may be ascribed to the distinctive phytochemical

makeup of the leaf extract utilized in their manufacture. Specific phytochemicals in plant extracts might augment the antioxidant capabilities of produced nanoparticles, as these compounds may boost the reactivity and efficacy of the nanoparticles in scavenging free radicals [44, 56]. The antioxidant activity of selenium nanoparticles derived from several plant sources has been recorded, emphasizing the influence of the source material on the antioxidant capacity of the resultant nanoparticles [28, 55]. The results of this study have substantial significance for the creation of new antioxidant medicines targeting disorders associated with oxidative stress. The enhanced DPPH radical scavenging activity of FPLS indicates that these nanoparticles may serve as prospective therapeutic agents for the prevention and treatment of oxidative damage-related conditions, including neurodegenerative diseases, cardiovascular disorders, and cancer [57, 49, 58]. The capacity of these nanoparticles to efficiently eliminate free radicals designates them as attractive subjects for more research and clinical use.

The assessment of hydrogen peroxide (H_2O_2) scavenging capacity in FPLS and FPBS has demonstrated notable disparities in their antioxidant efficacy. The results demonstrate that FPLS display much more scavenging activity than FPBS at all evaluated concentrations. The scavenging activity of FPLS varied from 50.75% at 1 $\mu\text{g/ml}$ to 77.47% at 50 $\mu\text{g/ml}$, whereas FPBS exhibited a more modest increase from 17.32% at 1 $\mu\text{g/ml}$ to 64.84% at 50 $\mu\text{g/ml}$ (Fig. 9b). The concentration-dependent enhancement in scavenging activity corresponds with prior research that has recorded the antioxidant properties of selenium nanoparticles derived from diverse plant sources, highlighting the contribution of phytochemicals to their effectiveness [59, 60]. The comparison analysis with ascorbic acid, a recognized antioxidant, emphasizes the potential of FPLS as effective agents in alleviating oxidative stress. Ascorbic acid exhibited exceptional scavenging efficiency, varying from 78.66% at 1 $\mu\text{g/ml}$ to 93.71% at 50 $\mu\text{g/ml}$, establishing a standard for assessing the antioxidant activity of other substances [28, 49]. Despite FPBS demonstrating reduced activity compared to FPLS, its efficacy enhanced with elevated concentrations, achieving roughly 64% inhibition at 50 $\mu\text{g/ml}$. This observation indicates that although FPLS are more efficacious, FPBS retain considerable antioxidant potential that could be utilized in diverse applications [44, 61].

The concentration-dependent scavenging activity noted in both nanoparticle forms corresponds with the accepted principles of antioxidant processes, wherein elevated antioxidant concentrations often correlate with improved scavenging efficacy. This behavior can be ascribed to the enhanced availability of reactive sites on the nanoparticles, which promotes interaction with H_2O_2 and other reactive oxygen species (ROS). The results align with prior research indicating the involvement of diverse nanoparticles in reactive oxygen species (ROS) scavenging, highlighting the significance of nanoparticle composition and surface characteristics in influencing antioxidant effectiveness [62–64]. Furthermore, the findings indicate that the manufacturing method and the parent material of the nanoparticles substantially affect their antioxidant characteristics. The nanoparticles generated from *F. platyphylla* leaves FPLS shown superior efficacy in H_2O_2 scavenging relative to those obtained from the bark FPBS. This observation may be associated with the intrinsic phytochemical makeup of the leaf extract, which could augment the antioxidant activity of the resultant nanoparticles. Prior studies have demonstrated that plant extracts

abundant in phenolic compounds and flavonoids can augment the antioxidant capabilities of produced nanoparticles, thus enhancing their effectiveness in scavenging reactive oxygen species [65, 66, 63].

Overall, this finding underscores the considerable potential of *F. platyphylla* leaf selenium nanoparticles (FPLS) in the formulation of antioxidant pharmaceuticals for both biological and industrial uses. FPLS demonstrate increased efficacy, rendering them appropriate for application in pharmaceuticals, nutraceuticals, and cosmetics to mitigate oxidative stress. Their significant antioxidant activity at elevated concentrations indicates possible use in food preservation and the mitigation of oxidative damage in diverse formulations. Furthermore, FPLS demonstrate potential as efficient H₂O₂ scavengers, with prospective uses in pharmaceuticals, nutraceuticals, cosmetics, industrial, and environmental domains. Their capacity to scavenge reactive oxygen species makes them promising candidates for therapeutic strategies to reduce oxidative damage in biological systems (Sentkowska and Pyrzyńska, 2022; Ghasemi, 2024).

Conclusion

This study effectively illustrates the green synthesis of SeNPs utilizing hot water extracts from *F. platyphylla* leaves (FPL) and bark (FPB), representing the inaugural report of this biosynthetic method for SeNPs with this particular plant. The produced SeNPs were comprehensively studied, demonstrating unique structural and optical features, with FPL-derived SeNPs exhibiting enhanced antibacterial activity. The SeNPs demonstrated considerable antibacterial efficacy against multi-drug-resistant microorganisms, such as MRSA and *Klebsiella oxytoca*, as well as strong antifungal properties against *Aspergillus* and *Penicillium* species. The SeNPs exhibited strong antioxidant qualities, as indicated by their DPPH scavenging activities, and revealed effective anticoagulant characteristics, comparable to traditional anticoagulants. The results indicate that SeNPs derived from *F. platyphylla* may be viable candidates for diverse medicinal applications, tackling the increasing issue of antibiotic resistance and disorders associated with oxidative stress. This study highlights the potential of *F. platyphylla* in sustainable nanoparticle synthesis and establishes a basis for further investigation of plant-based nanotechnology in biomedical research.

Declarations

Author Contributions SAA conceived, supervised, interpreted the results, carried out all the laboratory investigations, wrote part of the manuscript and edited the manuscript; BOY interpreted part of the results, wrote part of the manuscript, collected and analyzed the data; JAE interpreted the results, wrote part of the manuscript; AL Validation and review.

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Availability of Data and Material Data for the work are available with the authors.

Code Availability Not applicable.

Declarations Conflict of interest Authors declare that there is no conflict of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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Figures

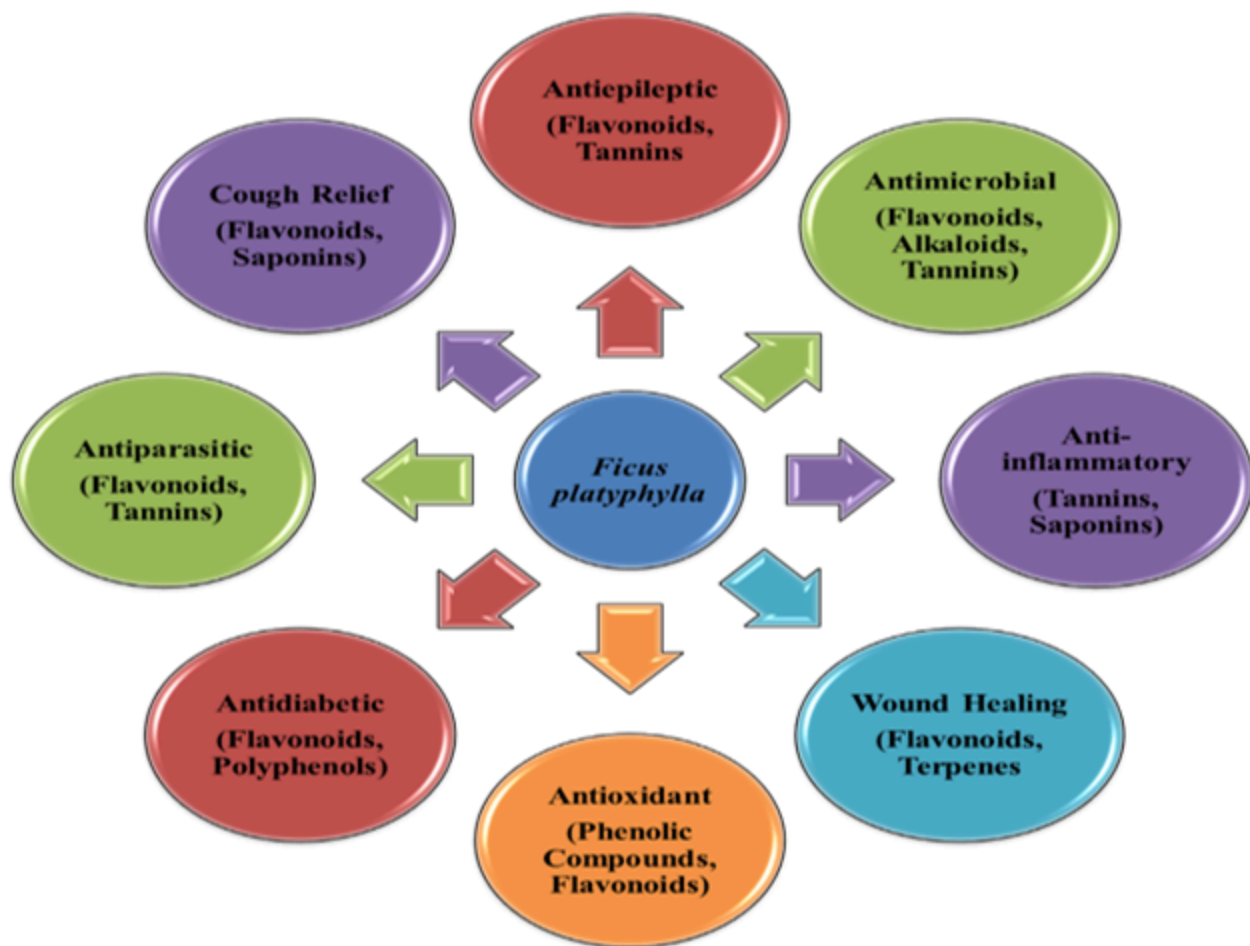


Figure 1

Ethnopharmacological Uses of *F. platyphylla* and Associated Bioactive Compounds

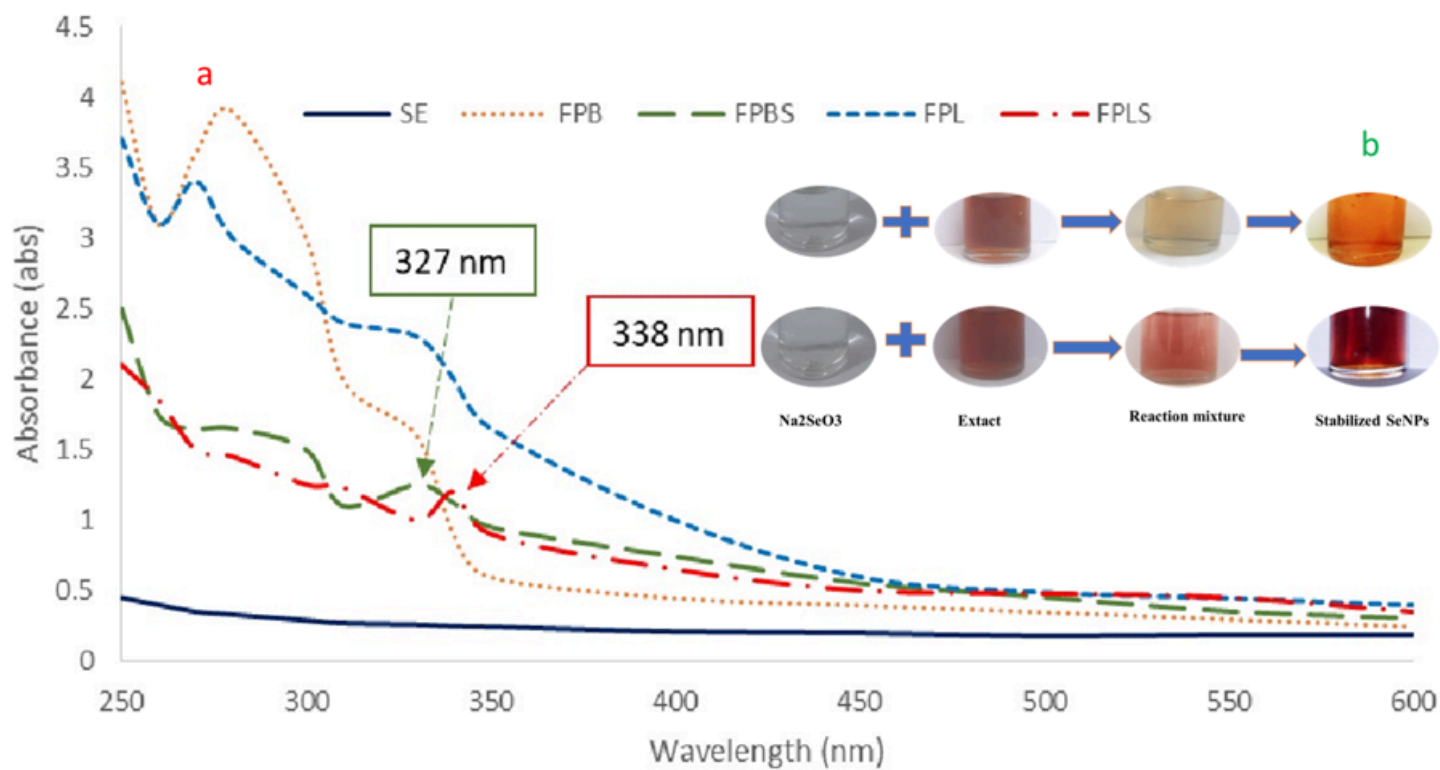


Figure 2

course of synthesis of SeNPs (A) and UV-Vis spectroscopic analysis of biosynthesized SeNPs (FPBS and FPLS), Plant extracts (FPB and FPL) and selenium solution (SE) (B)

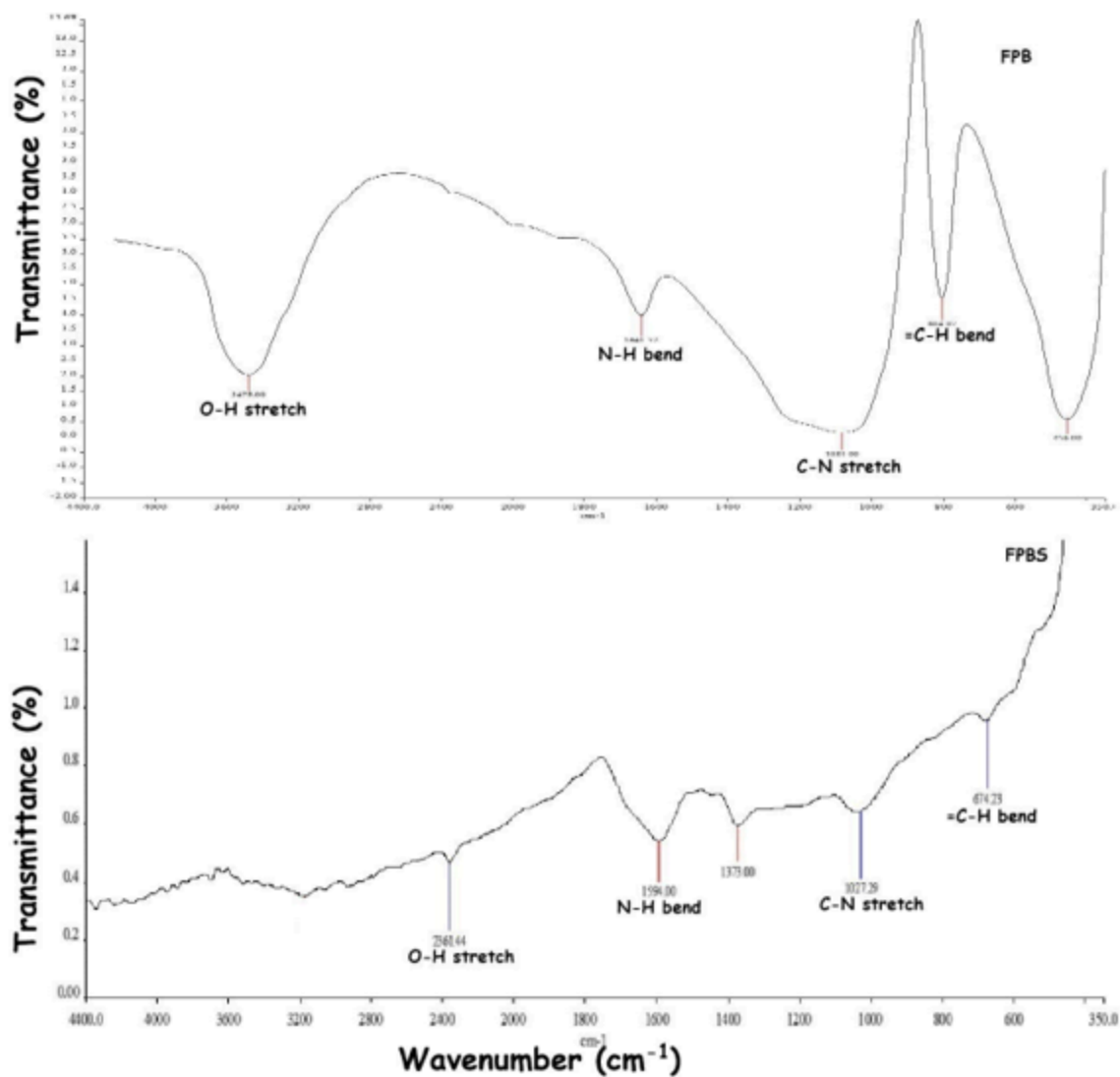


Figure 3

FTIR spectra analysis of FPB extract and biosynthesized FPBS

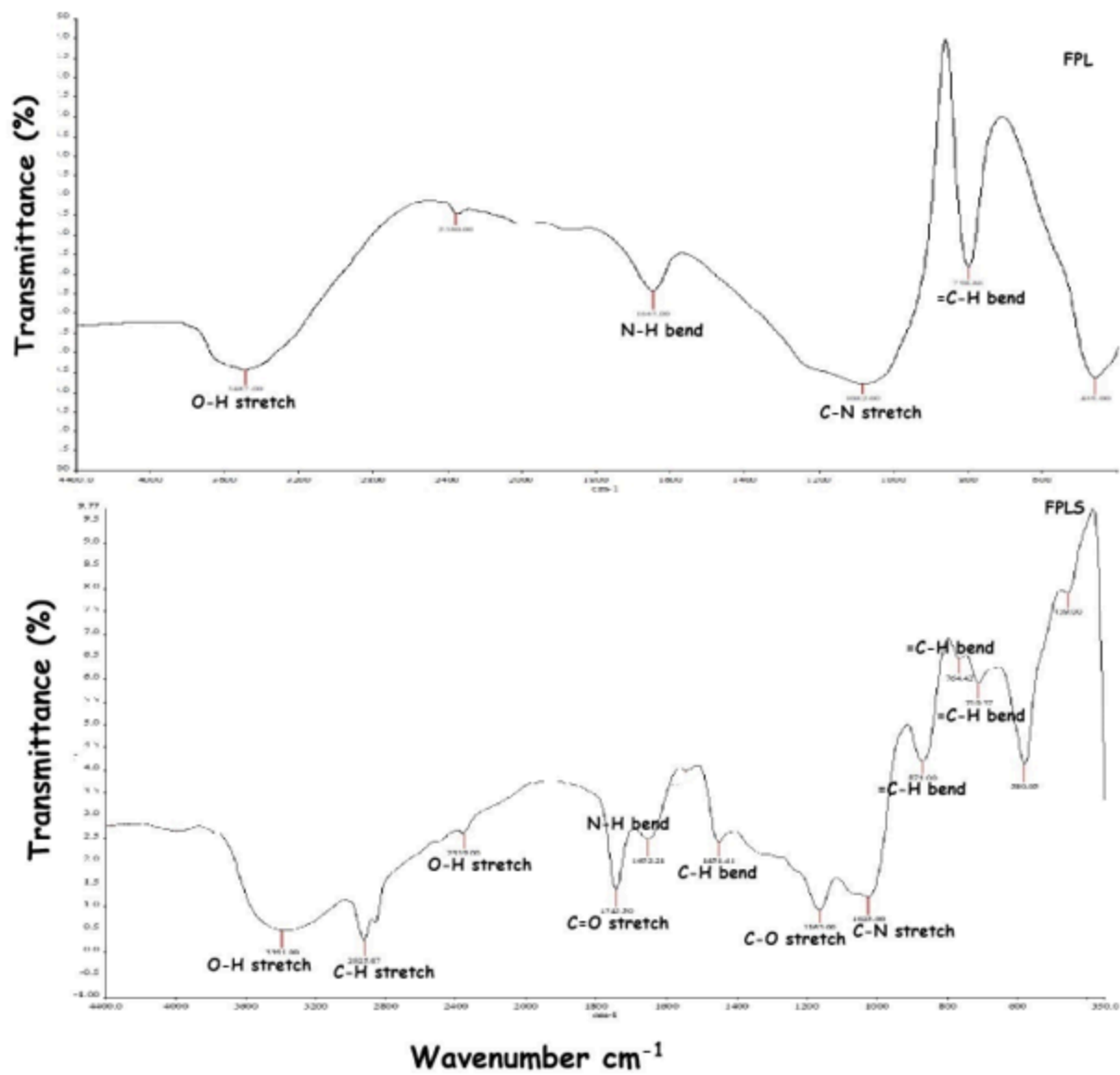


Figure 4

FTIR spectra analysis of FPL extract and biosynthesized FPLS

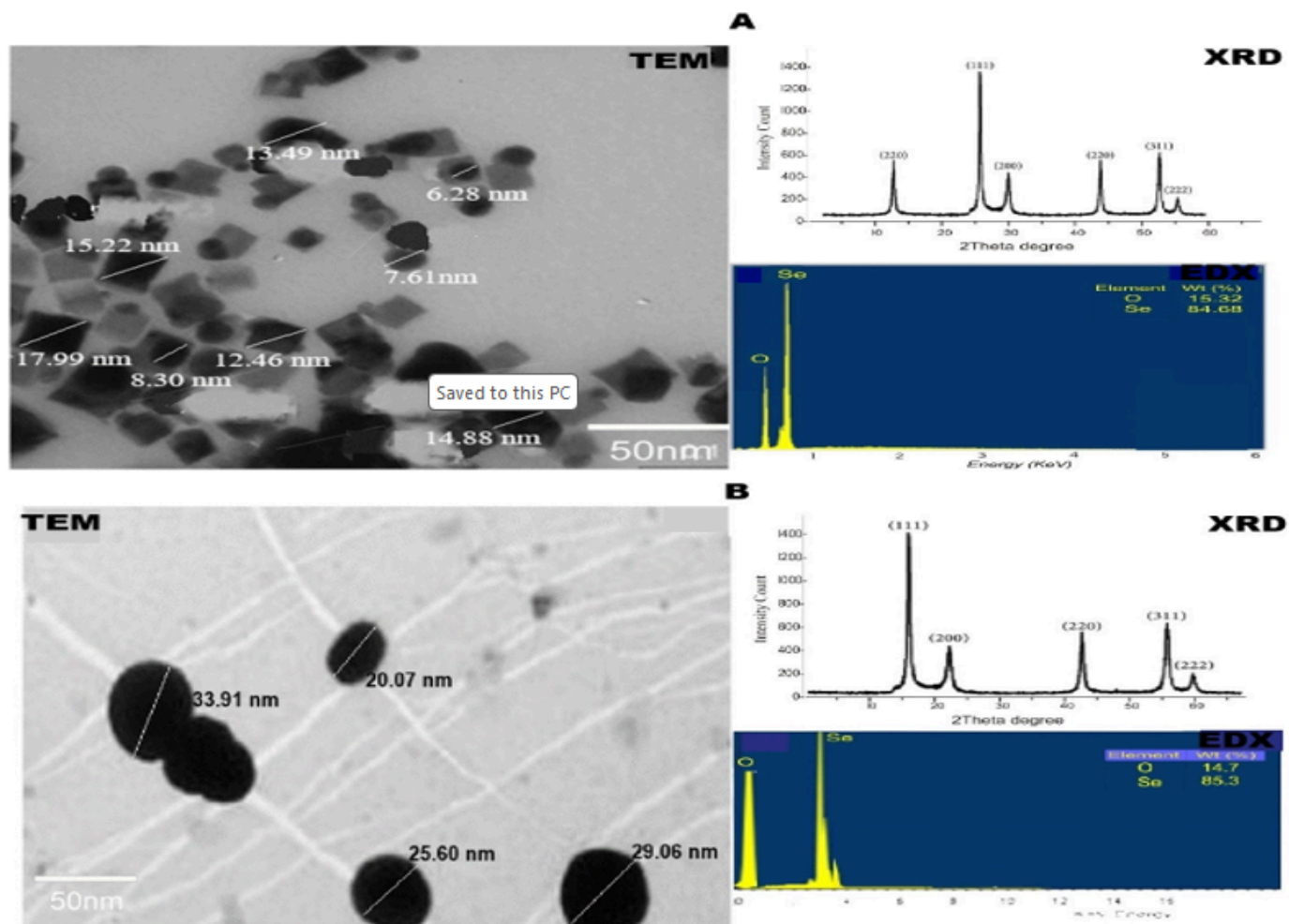


Figure 5

TEM micrograph, EDX, XRD of FPBS (A), TEM micrograph, EDX, XRD of FPLS (B)

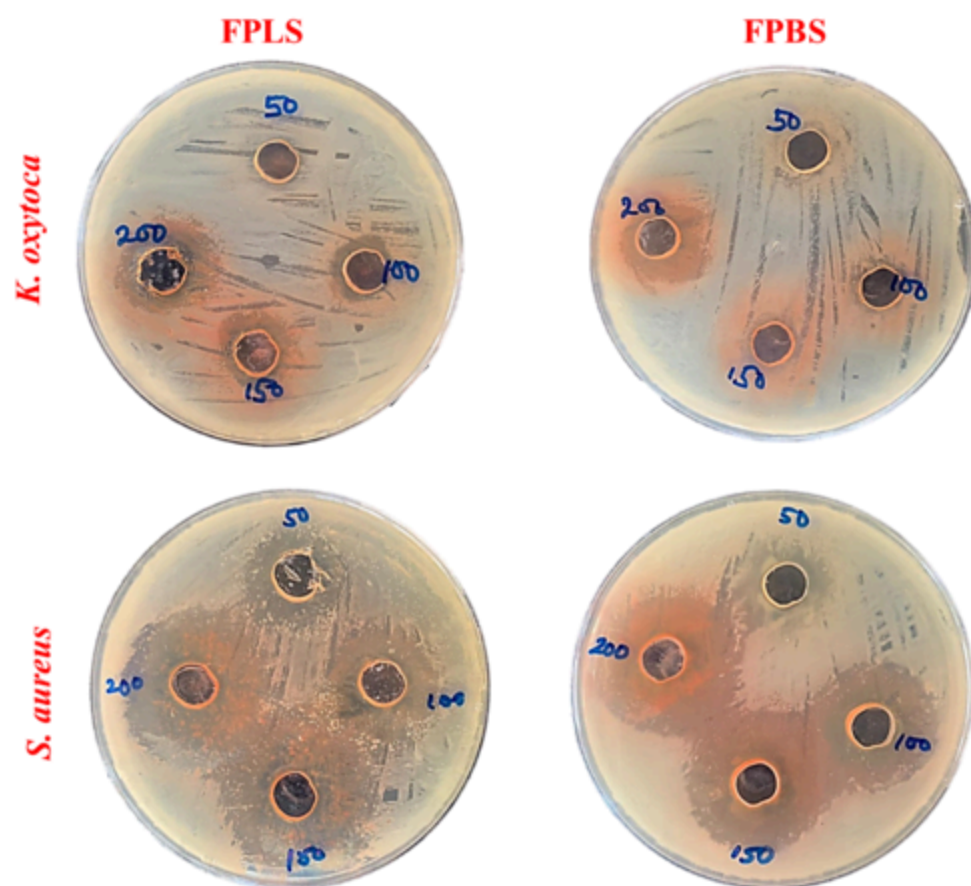


Figure 6

The antibacterial activities of biosynthesized SeNPs

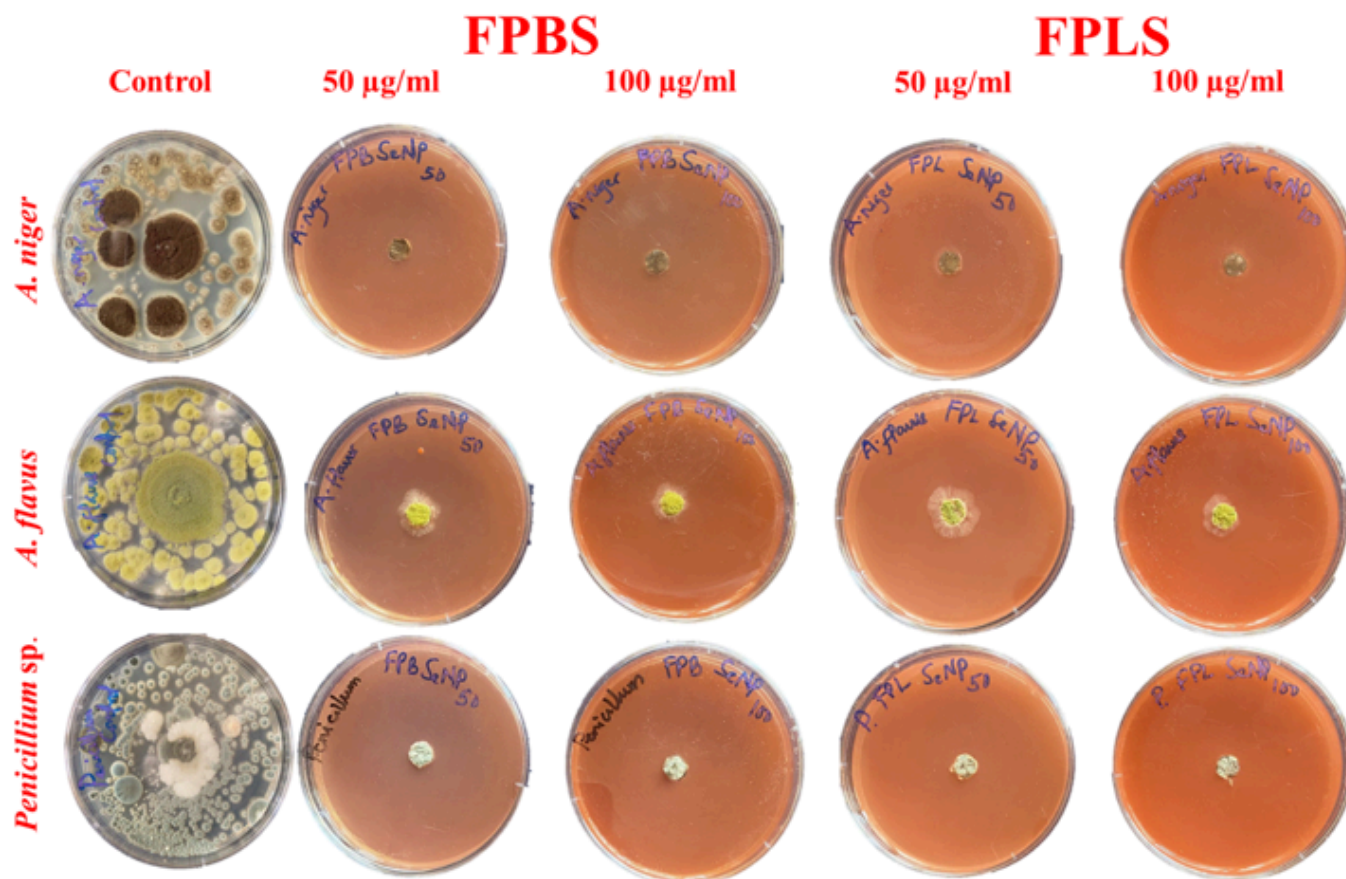


Figure 7

The antifungal activities of the biosynthesized SeNPs

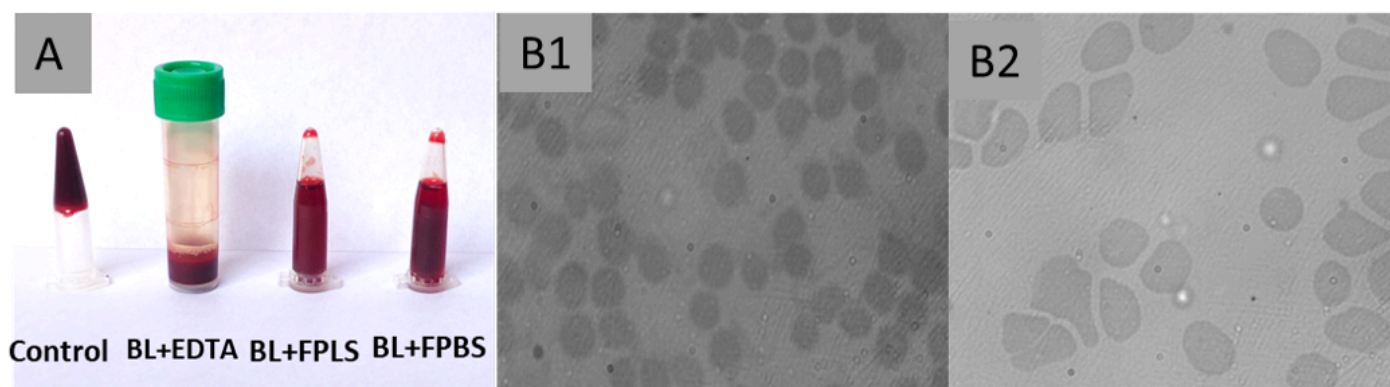


Figure 8

Anticoagulant efficacy of FPLS and FPBS: (A: clotted blood; blood in EDTA (no clotting); and blood + FPLS and FPBS (no clotting)); B1 and B2, microscopic examination of blood treated with FPLS and FPBS

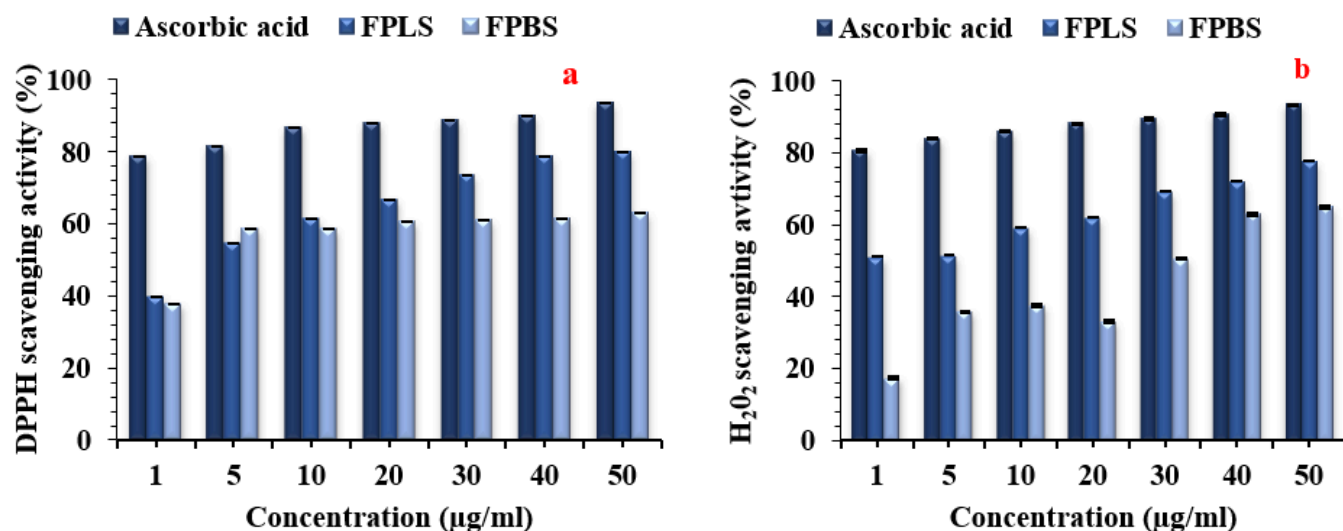


Figure 9

Antioxidant activity of *F. platyphylla* leaf and bark derived SeNPs, (A) DPPH (B) H₂O₂. Data are presented as mean ± SE from three independent experiments performed in triplicate. Statistical significance was determined by ANOVA with Duncan's test for mean comparison ($p < 0.05$). Error bars indicate the standard error of the mean