RESEARCH PAPER

Biogenic Synthesis, Comprehensive Physicochemical Characterization, and Antioxidant Potential of *Ficus racemosa* Leaf Extract-Mediated Selenium Nanoparticles

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ABSTRACT

The present study demonstrates an eco-friendly and sustainable green synthesis approach for selenium nanoparticles (F-SeNPs) utilizing aqueous leaf extract of Ficus racemosa and evaluates their biological potential. The biosynthesized F-SeNPs were comprehensively characterized using ultraviolet-visible (UV-Vis) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, dynamic light scattering and zeta potential analysis, field-emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). These analytical techniques validated the successful reduction and stabilization of F-SeNPs, detailing their surface functional groups, negative surface charge, amorphous nature, elemental purity, and spherical nanoscale morphology. The antioxidant efficacy was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The biogenic F-SeNPs exhibited significantly superior free-radical scavenging activity and reducing power compared to the crude extract, highlighting their promising potential as effective nanotherapeutic and drug delivery agents.

Keywords: Ficus racemosa, Green synthesis, Selenium nanoparticles, Biogenic synthesis, Antioxidant activity.

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1. Introduction

Biogenically synthesized selenium nanoparticles from standardized plant extracts can offer a multi-target approach that can couple antioxidant and anti-inflammatory actions with antimicrobial support. It is an eco-friendly and economical approach to hazardous chemical and physical methods [1]. Selenium is an essential trace element for humans and is incorporated into selenoproteins that play key biological roles like protecting cells from free radicals, improving thyroid hormone metabolism, regulating immune function, aiding DNA synthesis, and maintaining reproductive health [2]. The plant's many bioactive substances, including phenolics, flavonoids, tannins, terpenoids, and other antioxidants, present in *Ficus racemosa* leaf extract used as reducing and stabilizing agent to help reduce selenium ions to elemental selenium nanoparticles. Research has shown the effectiveness of *Ficus racemosa* extracts in the environmentally friendly synthesis of different metal nanoparticles, including zinc oxide and silver, emphasizing their capacity to produce nanoparticles with notable biological activity, controlled morphology, and excellent stability [3,4]. Selenium nanoparticles (SeNPs) offer distinct advantages over conventional metallic nanoparticles due to their low toxicity, high bioavailability, and superior biocompatibility. Selenium is an essential trace element naturally present in the human body; SeNPs are likely to exhibit a favorable pharmacokinetic profile. SeNPs demonstrate potent antioxidant activity and broad-spectrum therapeutic potential, including anticancer, anti-inflammatory, antidiabetic, and antimicrobial effects. The use of eco-friendly green synthesis approaches further enhances nanoparticle stability while reducing environmental burden [5,6,7]. It rapidly reduces selenium ions to elemental selenium, as indicated by a color change (Brick Red), and also prevents nanoparticle aggregation, ensuring its stability and dimensions [8,9]. It also enhances the antioxidant, anti-inflammatory, and antimicrobial properties of SeNPs, making them safe, stable, and effective for biomedical applications [10]. Recent studies have shown that *Ficus racemosa* extracts are rich in bioactive compounds with strong antidiabetic potential [11]. When combined with metallic

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nanoparticles, they act synergistically to enhance antibacterial, antioxidant, antidiabetic, and anti-inflammatory effects, improving stability, bioavailability, and therapeutic efficacy for different disorders and diseases [3,4,12,13,14,15]. Several in vivo assessments of streptozotocin-induced diabetic rats have also revealed that the aqueous and methanolic extracts of *Ficus racemosa* dramatically lowered fasting blood glucose and increased glucose tolerance and insulin sensitivity. These effects are mostly due to the regeneration of pancreatic β-cells and regulation of critical carbohydrate metabolism enzymes [16]. Advances in nanotechnology have further expanded the therapeutic potential of *Ficus* racemosa through the biosynthesis of metal nanoparticles such as silver and zinc oxide using plant extracts. These biogenically synthesized nanoparticles exhibit better bioavailability and enhanced cellular uptake than crude extracts, leading to more effective reductions in blood glucose levels and oxidative stress markers [17]. Mechanistic investigations have shown that these nanoparticles not only enhance insulin secretion, but also provide protection against pancreatic tissue injury and oxidative stress, which are the key drivers of diabetes evolution [18,19,20,21]. Although *Ficus racemosa* has been widely used for nanoparticle synthesis, to the best of our knowledge, there are no reports on green synthesis of selenium nanoparticles from leaf extracts. This is a significant gap in the research that combining Ficus racemosa phytochemicals with Selenium nanoparticles offers an eco-friendly and innovative approach that could be useful for future medical applications.

2. Materials and Methods

2.1 Collection and Authentication of the plant: *Ficus racemosa* leaves were sourced from Chandwad, Nashik, Maharashtra, and their identity was confirmed by the Botany Department of SNJB K.K.H.A. Arts, S.M.G. Lodha Commerce, and S.P.H. Jain Science College, Chandwad, Nashik, Maharashtra, India. Freshly collected leaves were thoroughly washed with distilled and deionized water, shade-dried at room temperature for 7 days to preserve phytoconstituents, and then finely powdered. To prepare the extract, 50 g of the leaf powder was added to 500 mL of double-distilled water and heated at 60°C for 2 h with continuous stirring using a magnetic stirrer. The mixture was then cooled and filtered through a Whatman No. 1 filter paper. The resulting clear extract was used as a natural reducing and stabilizing agent for the biosynthesis of Se nanoparticles.

2.2 Qualitative analysis: Qualitative analysis of Ficus racemosa leaf extract was one of the important steps to identify key phytochemical groups, mainly polyphenolic compounds, which play an important role in the biosynthesis of F-SeNPs, acting as natural reducing agents that help the conversion of selenium ions into nanoparticles, while stabilizing them to prevent aggregation. Qualitative identification of polyphenolic compounds was performed using Ferric chloride, Lead acetate, Alkaline reagent, and Shinoda tests, which supports the scientific understanding of phytochemicals [22,23].

2.3 Quantitative analysis: Total Phenolic Content (TPC) Estimation: TPC was measured using the Folin-Ciocalteu method, which is based on a redox reaction between phenolic compounds and the Folin-Ciocalteu reagent consists of a mixture of phosphomolybdic and phosphotungstic acids. For the assay, a calibration curve was prepared using gallic acid (20–100 µg/mL), and plant extracts were prepared at 1 mg/ml. Each 1 mL sample was combined with 0.25 mL of Folin-Ciocalteu reagent and 1.25 mL of 20% sodium carbonate solution. After incubating the mixture for 40 min at room temperature, absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Schemadzu-1800) (24).

2.4 Biogenic Synthesis of Selenium Nanoparticles utilizing Ficus racemosa leaves extract: Fresh *Ficus racemosa* leaves were collected, washed thoroughly, rinsed with distilled water to remove surface impurities, and air-dried at room temperature for 7 days in the shade (summer). The dried leaves were ground into a fine powder, and 10 g of the powder was extracted in 100 mL of distilled water by heating at 60–70°C for 60 min. The cooled mixture was filtered through Whatman No. 1 filter paper to obtain a clear extract. For the biogenic synthesis of selenium nanoparticles, 100 mL of 10 mM selenious acid was mixed with 10 mL of leaf extract and 3 mL of 100 mM ascorbic acid, and a visible color change from pale yellow to reddish-brown, as shown in Figure 2, indicated nanoparticle formation, which was further confirmed by UV-visible spectroscopy [25, 26, 27, 28].

2.5 Characterization:

2.5.1 Color: - The color change from yellow to brick-red serves as a visual confirmation of selenium nanoparticle formation, indicating successful reduction and stabilization during the synthesis process [29].

2.5.2 UV- Visible spectrophotometer: The biogenically synthesized F-SeNPs using *Ficus*

racemosa leaf extract were characterized using a UV-visible spectrophotometer (Shimadzu 1800) with a wavelength range of 200-800 nm and a resolution of 2 nm for the detection of surface plasmon resonance (SPR), which occurs because of the collective oscillation of electrons on the selenium nanoparticle surface when exposed to light, indicating their formation and stability [30].

2.5.3 Fourier Transform Infrared (FTIR) spectroscopy: The analysis was performed using a Jasco 4100 ATR PRO ONE with a zinc selenide crystal model to investigate the chemical interactions between plant phytoconstituents and selenium nanoparticles. FTIR spectroscopy was used to identify vibrational transitions from infrared absorption, revealing the functional groups responsible for nanoparticle reduction and stabilization. A small amount of dried selenium nanoparticle sample was placed directly onto the diamond ATR crystal, and the spectrum was recorded in the range of 4000-400 cm-1 using the software Spectra Manager [31].

2.5.4 Field Emission Scanning Electron Microscopy: The surface morphology and size of selenium nanoparticles synthesized using plant extracts were analyzed using Field Emission Scanning Electron Microscopy (FESEM). The analysis was performed using a Carl Zeiss Model Supra 55 (Germany). A small amount of dried F-SeNPs was deposited on conductive carbon tape mounted on an aluminum stub and coated with a thin layer of gold using a sputter coater to improve conductivity. The prepared specimen was then examined under high-vacuum conditions, and high-resolution micrographs were obtained at varying magnifications to evaluate the morphology, size distribution, aggregation state, and surface topography of the F-SeNPs [32].

2.5.5 Transmission Electron Microscopy (TEM): The internal morphology, spatial distribution, and particle size of the F-SeNPs were evaluated using TEM. The FEI Tecnai T20 instrument was used and operated at an accelerating voltage of 200 keV. For the TEM analysis, a drop of the diluted F-SeNPs suspension was deposited onto a carbon-coated copper grid and dried at room temperature to permit solvent evaporation [33].

2.5.6 Zeta potential: The surface charge and colloidal stability of F-SeNPs were evaluated using a zeta potential analyzer (Anton Paar). The suspension of F-SeNPs was diluted appropriately with deionized water and transferred to a disposable cuvette for measurement. Zeta potential values were recorded at room temperature, indicating the electrostatic stability of the nanoparticle dispersion [34].

2.5.7 Energy Dispersive X-ray Spectroscopy (EDX): The elemental composition of the F-SeNPs was determined using EDX with FESEM on a Carl Zeiss Supra-55 instrument from Germany. A small amount of dried F-SeNPs powder was evenly spread onto a carbon-coated aluminum stub to ensure conductivity and reduce charging effects during imaging. The sample was then placed into the FESEM chamber and analyzed under high-vacuum conditions to improve detector sensitivity and achieve precise elemental detection. The EDX detector identified X-ray emission peaks for selenium and other elements, providing qualitative and semi-quantitative data on the elemental composition of F-SeNPs [35].

2.5.8 X-ray Diffraction (XRD): This is a non-destructive technique used to analyse the crystallographic or amorphous structure of F-SeNPs. The analysis was performed using an XRD instrument (Malvern Panalytical). The dried nanoparticle powder was placed on a clean glass sample holder, and the diffraction patterns were recorded over a 2θ range of 10°–80° using Cu-Kα radiation (λ = 1.5406 Å). Sharp diffraction peaks in the XRD pattern indicate a crystalline structure with well-ordered Se atoms, whereas broad peaks suggest an amorphous structure with a disordered atomic arrangement. Instrument parameters such as voltage, current, and scan rate were set according to standard operating conditions [36].

2.6 Biological activities

2.6.1 Antioxidant Potential of Selenium Nanoparticles

2.6.1.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) **Free Radical Scavenging Assay:** A 100 µg/mL stock solution of ascorbic acid was prepared by dissolving 10 mg in 100 mL methanol, from which working standards were obtained by diluting aliquots of 20–100 µL to 3 mL with methanol. A100 µg/mL stock of F-SeNPs was prepared, and the same dilutions were prepared for both the plant extract and F-SeNPs. The 0.1 mM DPPH solution was freshly prepared by dissolving 3.9 mg in methanol (100 mL) and protected from light due to its sensitivity to light. For the assay, 1 mL DPPH reagent was added to 3 mL of each test solution and incubated in the dark at room temperature for 10 min. The absorbance was measured at 517 nm using a Shimadzu UV-1800 spectrophotometer. The controls included solvents without SeNPs and ascorbic acid as a positive

standard to assess antioxidant activity by comparing DPPH radical scavenging across samples (37).

2.6.1.2 FRAP (Ferric Reducing Antioxidant Power) Assay: The reducing capability of the biosynthesized F-SeNPs and crude *Ficus racemosa* leaf extract was evaluated following the method with minor modifications. The working FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM in a 10:1:1 (v/v/v) ratio, followed by pre-warming at 37 °C for 10 min. Aliquots of varying sample concentrations (20, 40, 60, 80, and 100) were mixed with 2.85 mL of working FRAP reagent and incubated in the dark at room temperature for 30 min. The reduction of the ferric-tripyridyltriazine complex to the ferrous form was monitored spectrophotometrically by recording the absorbance at 593 nm against an appropriate blank using a UV-Vis spectrophotometer (Shimadzu UV-1800). Ascorbic acid was evaluated concurrently under identical conditions as the reference antioxidant standard. All determinations were performed in triplicate (n=3)[38].

3. RESULTS AND DISCUSSION:

3.1 Qualitative analysis:

Table 1. Qualitative phytochemical screening of aqueous *Ficus racemosa* leaf extract

Phytochemicals	Test Name(s)	Observation (Color/Ppt)	Present
Alkaloids	Dragendorff's Wagner's	Brown ppt	++
Flavonoids	Shinoda Lead acetate	Yellow ppt	+++
Phenols	Ferric Chloride Folin-Ciocalteu	Blue color Blue color	+++
Tannins	Ferric Chloride Lead acetate	Blue color white ppt	+
Saponins	Foam Test	Persistent froth	+
Glycosides	Keller-Killiani Legal's	Pink Orange	+
Terpenoids	Salkowski	Reddish-brown ring	+

(+++) Strongly present; (++) Moderately present; (+) Mildly present; (–) Absent.

Phytochemical analysis of Ficus racemosa leaf extract confirmed the presence of several secondary metabolites. Alkaloids, flavonoids, phenols, and tannins were detected, along with saponins, glycosides, and terpenoids, as shown in Table 1. These findings indicated that the extract is rich in bioactive compounds, which contribute to its therapeutic potential.

3.2 Quantitative Analysis:

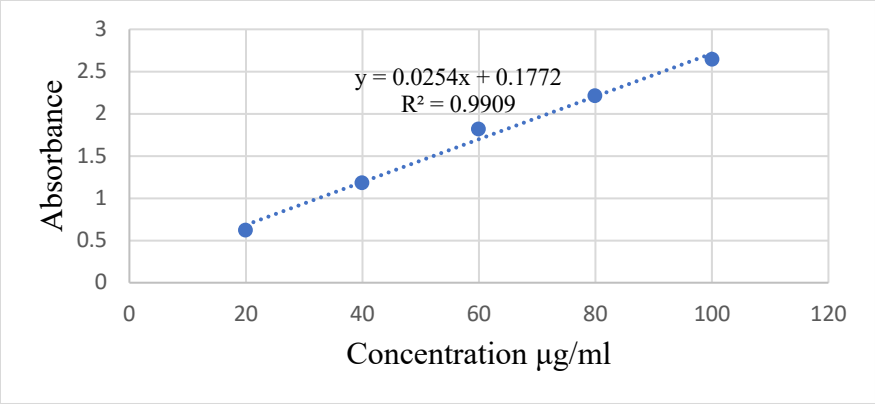


Figure 1. Calibration curve of Gallic acid

The total phenolic content (TPC) of the Ficus racemosa leaf extract was evaluated using the Folin-Ciocalteu method. Based on the calibration curve as shown in Figure 1, the TPC was determined to be 63.90 ± **0.6** mg of gallic acid equivalent (GAE) per gram.

3.3 Characterization

3.3.1 Color

The formation of a brick-red color of F-SeNPs confirms the reduction of $Se4^{+}$ to elemental selenium (Seo). Surface plasmon resonance (SPR) color shift is attributed to surface plasmon resonance (SPR) and confirms successful nanoparticle synthesis, with plant phytochemicals acting as natural reducing and stabilizing agents.

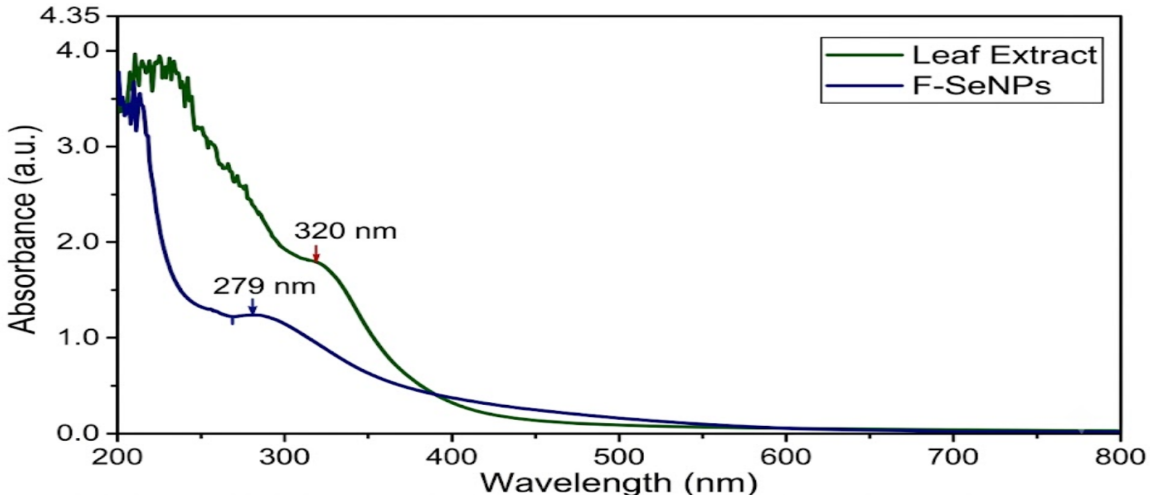


Figure 2. UV-Visible spectra of the *Ficus racemosa* leaf extract and mediated F-SeNPs

3.3.2 UV-Visible spectrophotometry

The extract showed maximum absorbance (λmax) at 320 nm (Figure 2), a signature commonly attributed to the π→π* and n→π* electronic transitions present in polyphenolic and flavonoid compounds. These phytochemicals, characterized by aromatic rings and hydroxyl functional groups, strongly absorb in the UV region and function as reducing and capping agents during nanoparticle formation. After reaction with selenious acid and ascorbic acid, the spectrum of the F-SeNPs revealed a distinct absorbance peak at 279 nm, indicative of the formation of elemental selenium (Se0) nanoparticles. The observed blue shift in λmax from 320 nm for leaf extract to 279 nm for F-SeNPs reflects significant transitions in the electronic environment as well as the presence of surface plasmon resonance (SPR).

3.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

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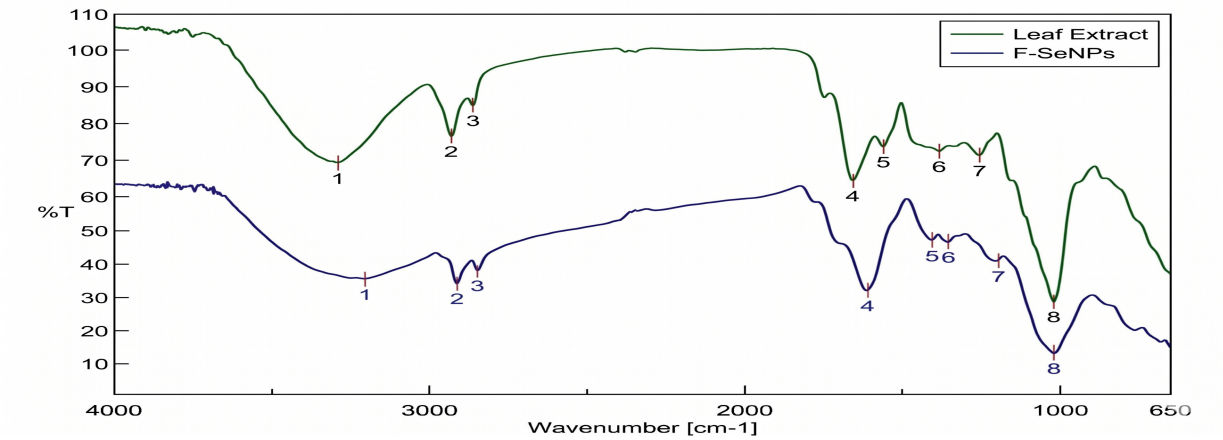


Figure 3. FT-IR Spectra of *Ficus racemosa* leaf extract and its mediated F-SeNPs

FTIR analysis of Ficus racemosa leaf extract and F-SeNPs showed characteristic peaks for hydroxyl (3282 cm-^{1}), C-H (2923, 2851 cm-^{1}), amide/C=C (1639, 1541 cm-^{1}), and C-O stretching (1241 cm-^{1}), confirming the presence of polyphenols, flavonoids, proteins, and carbohydrates. Similar peaks with notable shifts and reduced intensities were observed for the F-SeNPs at 3202, 2913, 1611, and 1020 cm-^{1}as shown in Figure 3. These changes indicate the active participation of -OH, C=O, and C-O functional groups in the reduction and capping of selenium ions, confirming the successful green synthesis.

3.3.4 FESEM and TEM

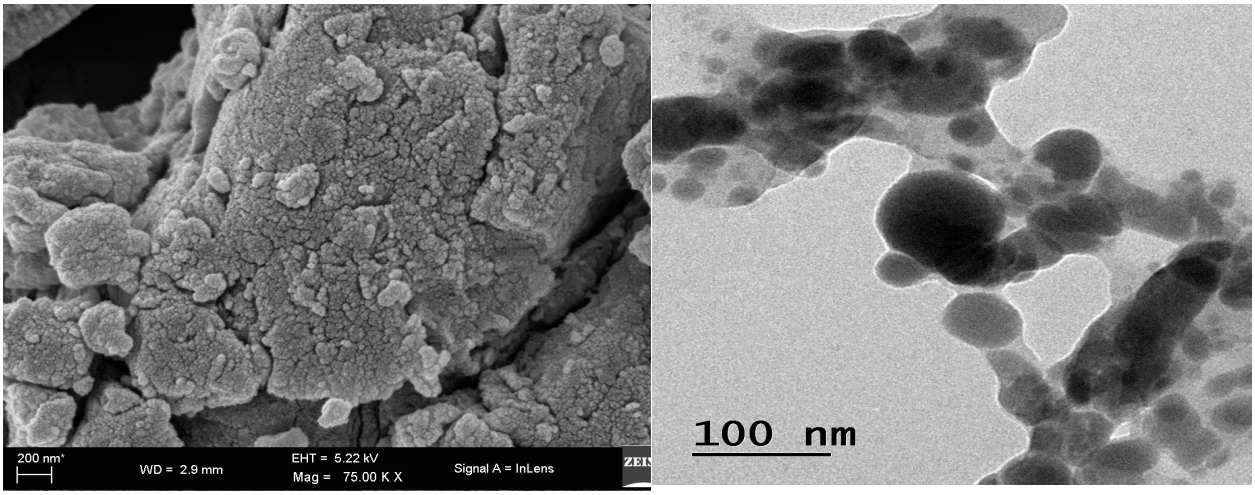


Figure 4. FESEM and TEM images for F-SeNPs

FESEM analysis of the F-SeNPs revealed aggregated, semi-spherical nanoparticles with rough surface morphology as shown in Figure 4. The particles appeared in the size range of 10-40 nm and formed dense clusters, indicating effective bioreduction and stabilization by the phytoconstituents present in the extract. The images confirm the uniform morphology of typical green-synthesized F-SeNPs. TEM analysis of the F-SeNPs revealed predominantly spherical to irregularly shaped nanoparticles with a size range below 100 nm. The particles appeared well-dispersed with slight aggregation due to the biological capping agents from the plant extract.

3.3.5 ZETA Potential

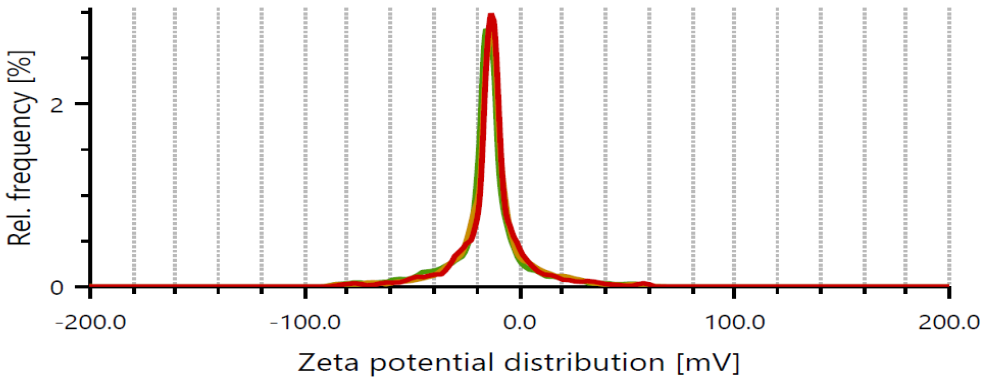


Figure 5. Zeta potential for F-SeNPs synthesized from *Ficus racemosa* leaves

Extract

Zeta potential analysis showed a value of –14.8 mV for the F-SeNPs, which indicates acceptable colloidal stability as shown in Figure 5. The negative surface charge confirms the successful capping of the nanoparticles by phytoconstituents present in the extract, which provides electrostatic repulsion. The value falls within the range considered sufficient for stable dispersion in aqueous media, supporting the effective role of the plant extract in stabilizing selenium nanoparticles for further biological activities

3.3.6 XRD

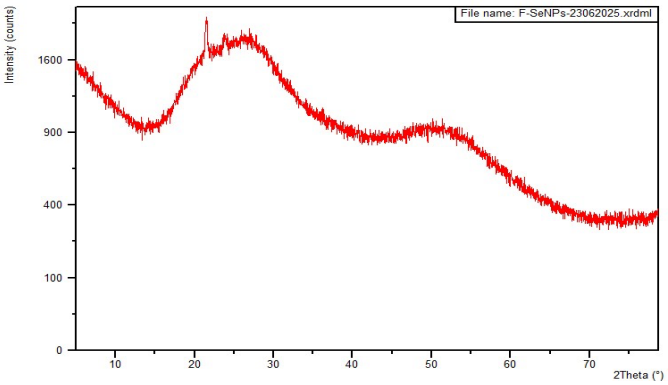


Figure 6. XRD spectra of F-SeNPs synthesized from *Ficus racemosa* leaf extract

X-ray diffraction patterns of the F-SeNPs are broad, diffuse patterns that exhibited no sharp Bragg peaks, which indicated that the nanoparticles were amorphous, as shown in Figure 6. This is frequently observed in green synthesis, where bioactive phytoconstituents present in plant extracts disrupt the ordered crystalline growth of NPs. The wide XRD halo shows a core of amorphous selenium, which is expected to increase superficial reactivity and better biomolecular interactions (favourable for biological activities) while needing proper capping and storage conditions to avoid crystallization during shelf life.

3.3.7 Energy-dispersive X-ray (EDX): Energy-dispersive X-ray spectroscopy (EDX) was employed to investigate the elemental composition of the synthesized selenium nanoparticles (SeNPs). The obtained spectrum exhibited a prominent characteristic peak at approximately 1.38 keV, along with additional peaks at approximately 11.22 and 12.50 keV, corresponding to the Lα, Kα, and Kβ emission lines of selenium, respectively, as shown in Figure 7. These characteristic signals confirm the presence of selenium in the analyzed sample region. The spectrum was predominantly characterized by selenium-associated signals, whereas distinct peaks corresponding to carbon and oxygen were not clearly resolved. This observation may be influenced by the limited sensitivity of EDX toward low-energy X-rays emitted by light elements, as well as sample preparation and instrumental acquisition conditions.

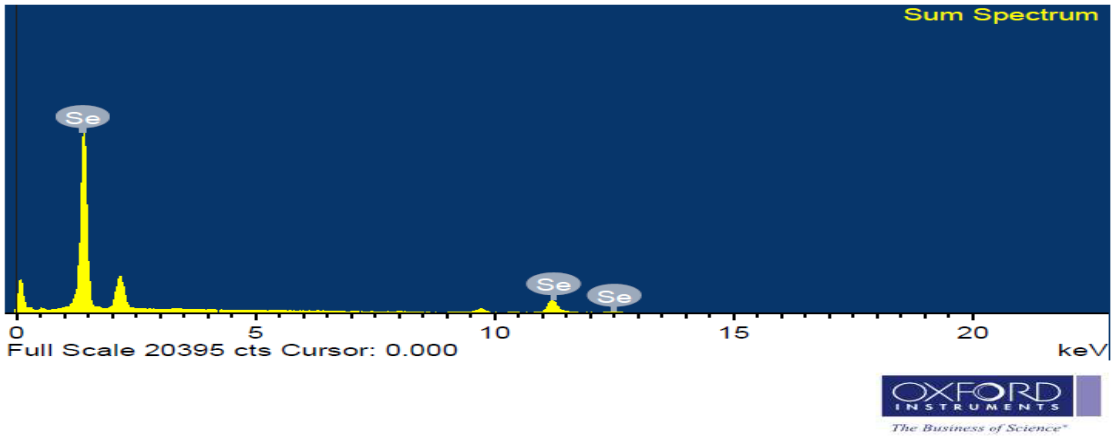


Figure 7. EDX image of F-SeNPs mediated from *Ficus racemosa* leaves extract

3.4 Biological activities: -
3.4.1 Antioxidant Potential of Selenium Nanoparticles:
3.4.1.1 DPPH Radical Scavenging Assay (RSA):

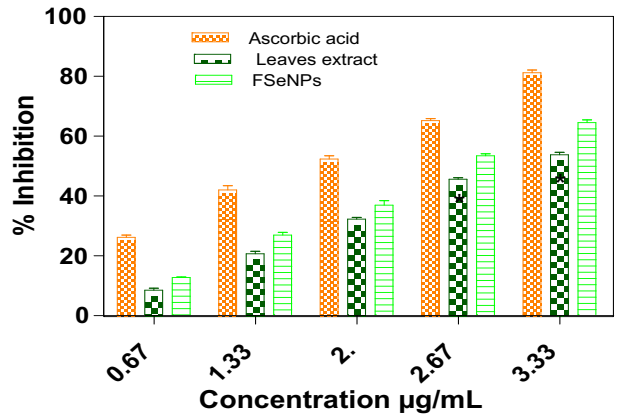


Figure 8. Antioxidant Activity of *Ficus racemosa* leaves extract and its mediated F-SeNPs measured by DPPH assay

At 0.67–3.33 µg/mL, all samples showed a clear, concentration-dependent increase in DPPH radical quenching, with activity consistently ordered as ascorbic acid > F-SeNPs > plant extract, as shown in Figure 8. Ascorbic acid reached 26.24, 42.08, 52.36, 65.25, and 81.21% inhibition; F-SeNPs achieved 12.77, 26.95, 37.00, 53.43, and 64.54%; and the plant extract produced 8.51, 20.69, 32.27, 45.63, and 53.78% at 0.67, 1.33, 2.00, 2.67, and 3.33 µg/mL, respectively. The IC_{50} values were found to be 1.85 µg/mL for ascorbic acid, 2.53 µg/mL for F-SeNPs, and 3.02 µg/mL for the plant extract**.** These results clearly demonstrate that F-SeNPs enhance the antioxidant potential of the plant extract and show their promising role as effective antioxidants.

3.4.1.2 Ferric Reducing Antioxidant Power (FRAP) Assay:

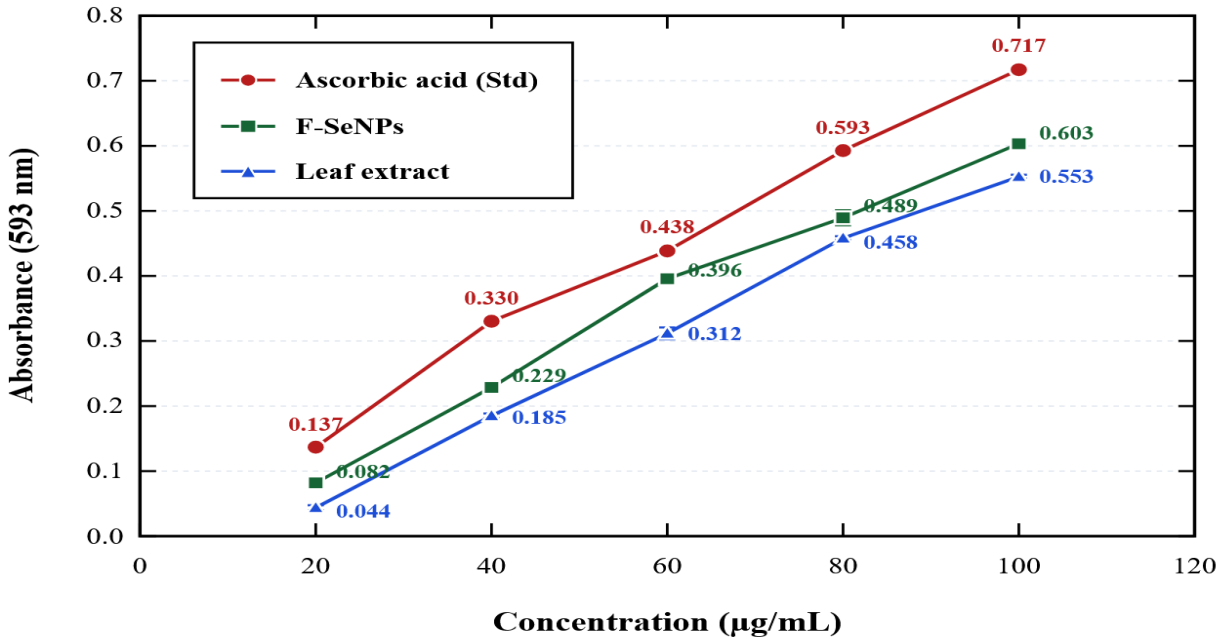


Figure 9. Ferric Reducing Antioxidant Power (FRAP) assay of biosynthesized F-SeNPs, *Ficus racemosa* leaf extract, and ascorbic acid standard. Absorbance measured at 593 nm. Values represent Mean ± SD ().

The electron-donating antioxidant potential of the green-synthesized F-SeNPs was confirmed using the FRAP assay, which measures the capability of antioxidants to reduce the colorless complex to the intense Prussian-blue chromophore. All samples demonstrated a strong, concentration-dependent increase in absorbance across the tested concentration range of 20–100, displaying robust linearity with correlation coefficients exceeding. At the maximum concentration of 100, biosynthesized F-SeNPs exhibited an absorbance of, which was significantly greater than that of the crude *Ficus racemosa* leaf extract, approaching the reducing power of standard ascorbic acid. This marked enhancement in reductive potential substantiates that biogenic stabilization by polyphenolic phytoconstituents cooperatively acts with the redox-active elemental selenium core to facilitate rapid electron transfer.

4. CONCLUSION:

In conclusion, biogenic selenium nanoparticles (F-SeNPs) were successfully synthesized via a green, eco-friendly approach utilizing an aqueous leaf extract of *Ficus racemosa* as both the reducing and stabilizing agent. Comprehensive physicochemical characterization using UV-Vis, FTIR, zeta potential, FESEM, TEM, EDX, and XRD confirmed the formation of stable, amorphous, and well-dispersed nanoparticles with average dimensions below 100 nm. In vitro antioxidant evaluations revealed that F-SeNPs exhibit potent, concentration-dependent free-radical scavenging (DPPH) and ferric-reducing antioxidant power (FRAP), substantially outperforming the crude botanical extract. This superior radical-quenching capacity highlights the synergistic efficacy between the selenium nanometal core and the surface-capping phytoconstituents of *F. racemosa*. Overall, these findings validate *F. racemosa*-mediated F-SeNPs as an effective, biocompatible nanoplatform with significant potential for countering oxidative stress-mediated cellular damage in future drug delivery and therapeutic applications.

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