RESEARCH PAPER

Biofabricated Sinapis nigra-Derived Selenium Nanoparticles: Physicochemical Characterization, Enhanced Antibacterial Activity, and Prospective Therapeutic Applications in Female Reproductive Health

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ABSTRACT
The combination of biofabricated selenium nanoparticles (SeNPs) with conventional antibiotics has emerged as a promising strategy for enhancing antimicrobial efficacy against multidrug resistant bacterial pathogens by exploiting the complementary antibacterial properties of nanomaterials and antibiotics. In the present study, Sinapis nigra derived selenium nanoparticles (MS-SeNPs) were successfully biofabricated through an environmentally sustainable green synthesis approach using Sinapis nigra seed phytoconstituents as natural reducing, capping, and stabilizing agents. Successful nanoparticle formation was confirmed by characteristic UV–Visible absorption peaks between 250-265 nm, while Fourier Transform Infrared Spectroscopy (FTIR) revealed theinvolvement of hydroxyl, carbonyl, phenolic, and amine functional groups in selenium ion reduction and nanoparticle stabilization. X-ray diffraction analysis demonstrated the nanocrystalline nature of the synthesized SeNPs with crystallite sizes ranging from 28–35nm, while the highly negative zeta potential (−70 mV) indicated excellent colloidal stability. Furthermore, SEM and HRTEM analyses revealed predominantly spherical to quasi spherical nanoparticles with nanocrystalline architecture, while EDAX, EDS, elemental mapping, and SAED collectively confirmed elemental selenium, homogeneous elemental distribution, phytochemical surface functionalization, and polycrystalline structural organization. Collectively, these complementary physicochemical investigations confirmed the successful synthesis of highly stable, crystalline, and biofunctionalized selenium nanoparticles with characteristics favorable for biomedical applications.
The antibacterial activity of MS-SeNPs and antibiotic-combined MS-SeNPs was evaluated against representative Gram-positive (Staphylococcus aureus and Bacillus megaterium) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacterial pathogens. MS-SeNPs exhibited significant concentration dependent antibacterial activity, producing maximum zones of inhibition of 22.67 ±1.76 mm,25.33 ± 2.67 mm,23.33 ±1.76 mm,and 27.00 ± 0.58 mm against E. coli, P. aeruginosa, S. aureus, and B. megaterium, respectively, at 30 µg. The combination of MS-SeNPs with amoxicillin further enhanced antibacterial efficacy, producing inhibitionzonesof29.00±0.58 mm, 24.67±0.67mm, 27.33±0.67mm, and 21.00 ± 0.58 mm, respectively, demonstrating a clear synergistic enhancement in antibacterial activity. The superior antimicrobial performance can be attributed to the combined effects of the nanoparticles' nanocrystalline structure, excellent colloidal stability, phytochemical surface capping, increased interaction with bacterial cell membranes, and reactive oxygen species-mediated oxidative damage, which collectively promote efficient bacterial growth inhibition.
These findings demonstrate that Sinapisnigra derived selenium nanoparticles constitute an effective green antimicrobial nanoplatform with enhanced antibacterial potential when combined with conventional antibiotics. Owing to their excellent physicochemical stability, broad-spectrum antimicrobial activity, and environmentally sustainable synthesis, these biofabricated MS-SeNPs represent promising therapeutic candidates for combating multidrug resistant bacterial infections and hold considerable potential for future applications in antimicrobial therapy, targeted drug delivery and the prevention

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and management of infections associated with female reproductive health. However, further invitro/invivo investigations and clinical validation is required before therapeutic translation....

Keywords: Sinapisnigra, Biofabrication, Selenium nanoparticles, Greensynthesis, Physicochemical characterization, Antimicrobial activity..

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INTRODUCTION

Nanotechnology has emerged as a transformative and rapidly advancing discipline that has profoundly reshaped research across multiple scientific domains, particularly within biomedical and healthcare sciences (Kordeet al.2020). By enabling the manipulation and engineering of materials at the nanoscale, typically ranging between 1 and 100 nm, nanotechnology offers unprecedented opportunities to develop novel materials with physicochemical properties that differ fundamentally from their bulk counterparts (Dreadenet al.2012). At this scale, materials exhibit altered optical, mechanical, electrical, and biologicalcharacteristicsthat can be strategicallyharnessed for medicalandtherapeutic applications (Singh M. et al.2019). Nanoparticles, as one of the most extensively investigated nanostructures, possess distinctive features such as enhanced surface-to-volume ratio, tunable morphology, improved solubility, and superior biological interactions (Khurana A. et al.2019). These attributes have collectively driven their integration into modern medicine, including applications in diagnostics, molecular imaging, targeted drug delivery, biosensing, tissue engineering, and therapeutic interventions (Grasso et al. 2019).

The increasing adoption of nanoparticle based systems in healthcare reflects their capacity to address critical limitations associated with conventional medical strategies (Geoffrionet al.2020). Traditional therapeutic modalities, particularly in oncology, often suffer from challenges such as poor target specificity, nonselective cytotoxicity, multidrug resistance, dose limiting toxicity, and undesirable systemic side effects (Vyas and Rana (2017). Nanoparticles offer innovative solutions by enabling controlled drug release, enhanced bioavailability, and targeted delivery mechanisms that improve therapeutic precision (Vera et al. 2016). Their nanoscale dimensions facilitate efficient cellular internalization and preferential accumulation in pathological tissues via mechanisms such as the enhanced permeabilityand retention(EPR) effect (Javed and Mashwaniet al.2020). This phenomenon allows nanoparticles to selectively localize within tumor microenvironments, thereby improving therapeutic efficacy while minimizing off-target damage. Consequently, nanotechnology has become a focal point in the development of next generation biomedical systems designed to optimize treatment outcomes and reduce adverse effects (Farokhzadand Langer, 2009).

Among various bioactive elements explored within nanomedicine, selenium has attracted considerable scientific interest due to its essential physiological roles and broad therapeutic relevance (Kong et al.2014). Selenium is a vital trace element required for maintaining cellular homeostasis, immune competence, antioxidant defense, and endocrine regulation. Its biological significance is primarily mediated through its incorporation into selenoproteins, a specialized class of selenium containing proteins that regulate diverse metabolic and protective functions. These selenoproteins play central roles in redox regulation, detoxification of reactive oxygen species (ROS), modulation of inflammatory responses, and maintenance of genomic stability. Selenium dependent enzymes such as glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs) serve as critical components of the cellular antioxidant defense network, protecting cells from oxidative injury and preserving physiological balance (Barmanetal.2013).

Despite the indispensable biological importance of selenium, the clinical application of elemental selenium remains constrained by poor bioavailability, limited therapeutic efficacy, and potential toxicity at higher concentrations. Biofabricated selenium nanoparticles (SeNPs) have gained considerable attention as next generation selenium formulations owing to their enhanced bioavailability, reduced toxicity, improved biological performance, and excellent colloidal stability. In addition, phytochemical mediated green synthesis provides a naturally functionalized nanoparticle surface, rendering biofabricated SeNPs attractive candidates for diverse biomedical and therapeutic applications (Samanchi et al, 2026)

The importance of selenium in human health is underscored by the pathological consequences associated with its dysregulation (Sanvices and Marco, 2008). Selenium deficiency has been linked to impaired immuneresponses, endocrine dysfunction, increased oxidative stress, and heightened susceptibility to chronic diseases (Srivastava and Mukhopaddhyay, 2015). Conversely, excessive selenium intake may lead to toxicity, emphasizing the necessity of maintaining selenium levels within physiologically optimal limits (Ibrahim et al.2021). This delicate balance highlights selenium's dual significance as both an essential micronutrient and a biologically active therapeutic agent.

In the context of female reproductive biology, selenium assumes particular importance due to its involvement in ovarian physiology and reproductive function (Ahmedet al.2017). The ovary represents a metabolically dynamic organ characterized by continuous processes of follicular growth, oocyte maturation, steroidogenesis, and cyclic

tissue remodeling. These processes inherently involve elevated metabolic activity and increased production of reactive oxygenspecies, rendering ovariantissues particularly vulnerableto oxidative stress (Toubhanset al2020).Excessive oxidative damage within the ovarian microenvironment can compromise follicular integrity, impair oocyte quality, disrupt hormonal regulation, and ultimately affect reproductive outcomes. Selenium-dependent selenoproteins play a pivotal protective role by mitigating oxidative stress and preserving cellular homeostasis (Patel et al.2024).

Glutathione peroxidases and thioredoxin reductases, two major classes of selenoproteins, function as key regulators of redox balance within ovarian tissues. These enzymesneutralize reactive oxygen species, prevent lipid peroxidation, and protect cellular components fromoxidative injury. Their activity is criticalfor maintaining oocyte viability, supporting follicular development, and ensuring proper endocrine signalling (Hou et al,2024). Beyond their antioxidant functions, selenoproteins are involved in diverse physiological pathways, including regulation of cellular proliferation, apoptosis, immune modulation, andgenomicstability. These processes are fundamental to ovarian function and reproductive competence (Sieja and Talerczyk, 2004).

Proper selenium status has been associated with improved follicular development, enhanced oocyte maturation, and optimal reproductive outcomes. In contrast, selenium insufficiency may disrupt ovarian physiology by impairing antioxidant capacity, promoting oxidative injury, and altering endocrine balance (Razaviet al.2016). Clinical and experimental studies have suggested associations between altered selenium homeostasis and reproductive disorders, including ovulatory dysfunction and polycystic ovary syndrome (PCOS) (Hajizadeh-Sharafabad et al,2019). Conversely, adequate selenium levels have been correlated with improved reproductive efficiency and cellular protection, highlighting selenium's regulatory and protective significance in female health (Mojadadiet al.2021).

Dietary intake remains the principal source of selenium for human physiology. Among plant-based dietary sources, mustard seeds, especially those derived from *Sinapis nigra*, have gained recognition as nutritionally relevant selenium rich materials (Senet al, 2025). Mustard plants exhibit the capacity to bioaccumulate selenium from soil, contributing to their micronutrient composition. A typical 20-gram serving of mustard seeds has been reported to contain approximately 40–42 µg of selenium, representing a substantial contribution toward recommended dietary requirements (Shah, 2022).Established nutritional guidelines recommend a daily selenium intake of approximately 55 µg/day for adult women, while tolerable upper intake levels are set at 400 µg/day to prevent toxicity. Maintaining selenium intake within these physiological limits is essential, as both deficiency and excessive consumption may produce adverse biological consequences (EFSA Panel, 2014). Selenium deficiency may impair antioxidant defenses and metabolic processes, whereas chronic overexposure may lead to selenosis, characterized bysymptoms including gastrointestinal disturbances, hair loss, nail brittleness, and neurological complications (FanandKizer,1990). Therefore, selenium rich dietary sources such as mustard seeds represent valuable nutritional components when consumed appropriately. Their ability to bioaccumulate selenium further enhances their relevance not only in nutritional sciences but also in biomedicaland nanotechnological research (Grygier, 2023).

Recent advancements in nanomedicine have emphasized the therapeutic advantages of selenium nanoparticles (SeNPs) (Samanchi et al, 2026). Compared to conventionalselenium forms, including inorganic salts and bulk elemental selenium, selenium nanoparticles exhibit superior bioavailability, enhanced biological activity, and reduced toxicity. These nanoparticles possess unique surface properties that enable improved interactions with biological systems (Zamboninoet al.2023). Selenium nanoparticles have demonstrated a wide spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Their nanoscale dimensions facilitate efficient cellular uptake, modulation of oxidative stress pathways, and regulation of intracellular signaling mechanisms (Bai et al.2025).

In oncologyresearch, selenium nanoparticles have attracted significant interest due to their ability to selectively target malignant cells while minimizing damage to normal tissues. Their capacity to modulate redox balance is particularly relevant, as cancer progression is closely associated with oxidative stress and redox dysregulation. Selenium nanoparticlescan induce controlled oxidative stress within tumor cells, triggering apoptosis, inhibiting cellular proliferation, and disrupting survival pathways (Vahidiet al.2020). Additionally, SeNPs have been shown to influence molecular pathways associated with cell cycle regulation, mitochondrial dysfunction, and programmed cell death. Their biocompatibility and functional versatility further enable their integration into targeted drug delivery systems, combination therapies, and advanced therapeutic platforms (Jayakodiet al.2025).

Ovarian cancer remains one of the most aggressive and clinically challenging gynecological malignancies, characterized by high mortality rates, late stage diagnosis, and the frequent development of chemoresistance (Libertoet al.2022). Conventional treatment modalities, including surgery, chemotherapy, and radiation therapy, although indispensable, are often associated with systemic toxicity, non-specific tissue damage, and limited long term efficacy. The emergence of therapeutic resistance and treatment-associated complications underscores the urgent need for innovative therapeutic strategies capable of enhancing treatment precision while reducing adverse effects (Riturajet al.2025).

Nanoparticle based therapeutic systems offer a promising direction in this regard. Their ability to facilitate targeted delivery, enhance drug retention, improve pharmacokinetics, and modulate tumor specific pathways positions them as valuable tools in cancer therapeutics. Seleniumnanoparticles, inparticular, present

abiologicallyrelevant candidate for ovarian cancer research, given selenium's intrinsic role in ovarian physiology, antioxidant defense, and cellular regulation (Xin et al.2017). The integration of selenium's therapeutic properties with nanoscale advantages provides opportunities to develop safer, more selective, and potentially more effective treatment strategies (Shinde, 2023).

Moreover, the biofabrication of selenium nanoparticles using selenium rich botanical sources such as *Sinapis nigra* introduces an environmentally sustainable and biologically compatible synthesis pathway (Rahimzadehet al.2024). Plant-mediated nanoparticle synthesis leverages naturally occurring phytochemicals that function as reducing, stabilizing, and capping agents, thereby eliminating the need for toxic reagents and harsh reaction conditions. This approach not only enhances environmental safety but may also improve nanoparticle stability and biological functionality (Shekhar and Yadav,2026).

Collectively, the convergence of nanotechnology, selenium biology, dietary relevance, and ovarian health provides a compelling scientific rationale for investigating biofabricated selenium nanoparticles in ovarian cancer therapeutics. Such strategies hold significant promise for advancing precision nanomedicine, improving therapeutic outcomes, and mitigating treatment-associated toxicity (Kesharwwaniet al.2025).

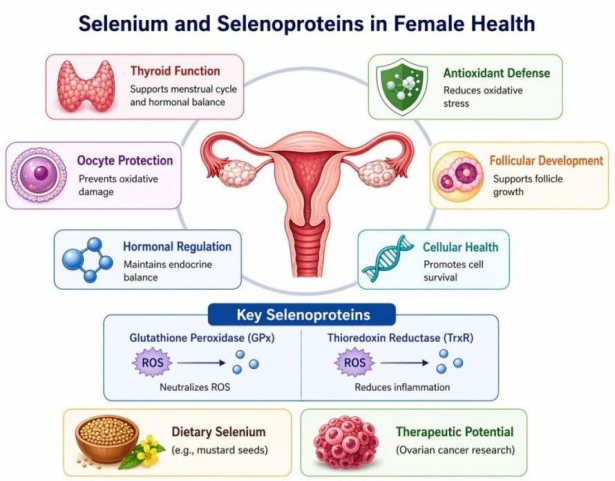


Figure1:Role of selenium and selenoproteins in female reproductive health.
Schematic illustration depicting the biological functions of selenium in maintaining female reproductive physiology. Source: Generated using ChatGPT Plus

Recent advances in nanomedicine have highlighted the therapeutic advantages of selenium nanoparticles (SeNPs).Compared to inorganic or bulk selenium forms, SeNPs exhibit improved bioavailability, enhanced biological activity, and reduced toxicity. These nanoparticles have demonstrated diverse biomedical properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Zhang et al.2023). Their nanoscale dimensions enable efficient cellular interactions, modulation of oxidative stress pathways, and selective biological effects. Importantly, SeNPs have shown promising anticancer potential through mechanisms involving redox regulation, apoptosis induction, and inhibition of tumor progression (Anjumet al.2025).

Given the critical role ofselenium in ovarian physiology and cellular protection, selenium nanoparticles represent an emerging area of interest in ovarian cancer research. Ovarian cancer remains one ofthe most challenging gynecological malignancies, characterized by late-stage diagnosis, therapeutic resistance, and poor clinical outcomes. Nanoparticlebased therapeutic strategies offer opportunities to enhance treatment precision, improve drug delivery, and mitigate systemic toxicity (Golaraet al.2023). Based on these facts, biofabricated selenium nanoparticles, particularly those synthesized using selenium rich botanical sources such as *Sinapis nigra*, may provide a biologically relevant and therapeutically promising platform for ovarian cancer investigations.

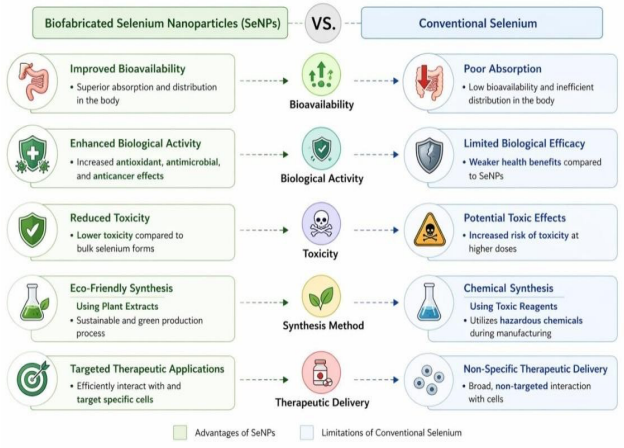


Figure2.Comparative overview of biofabricated selenium nanoparticles(SeNPs)and conventional selenium. Presentation of a side-by-side comparison of biofabricated selenium nanoparticles (SeNPs) and conventional selenium, emphasizing key physicochemical and biological differences relevant to biomedical applications.
Source: Generated using ChatGPTPlus

Material and methods:

:Collection of plant material

Seeds of *Sinapis nigra* were collected from local fields of Tirupati, Andhra Pradesh, India. The following chemicals and glass ware were used as follows: Sodium Selenate (Sigma–Aldrich, Munich, Germany), Nutrient agar, Nutrient broth (Himedia, Mumbai, India), Ascorbic acid (Sigma–Aldrich), Antibiotic discs (Himedia), glassware (Borosil, Mumbai, India), and Whatman No. 1 filter paper.

:Synthesis of Mustard derived selenium nanoparticles

Sodium selenite was employed as the selenium precursor for nanoparticle synthesis. The prepared *Sinapis nigra* seed extract was introduced dropwise into 10mL of10mM sodium selenite solution under continuous magnetic stirring to promote homogeneous mixing and initiate nanoparticle formation. The reaction mixture was subsequently incubated under dark conditions at 27 ± 2°C and agitated at 120 rpm using an orbital shaker for 24 hours. The progression of the reduction process was monitored visually by observing the characteristic color transition of the reaction medium. Ascorbic acid was added as a reducing facilitator to enhance the conversion of selenium ions into elemental selenium. Following completion of the reaction, the synthesized selenium nanoparticles were separated by high-speed centrifugation. The resulting pellet was washed repeatedly with distilled water and appropriate organic solvents to eliminate residual reactants and impurities as shown in figure 3. The final Mustard derived Selenium Nanoparticles (MS-SeNP) pellets were dissolved in sterile MilliQ water and used for further studies like spectral characterization and biomedical applications like antibacterial, antioxidant and anticancer studies.

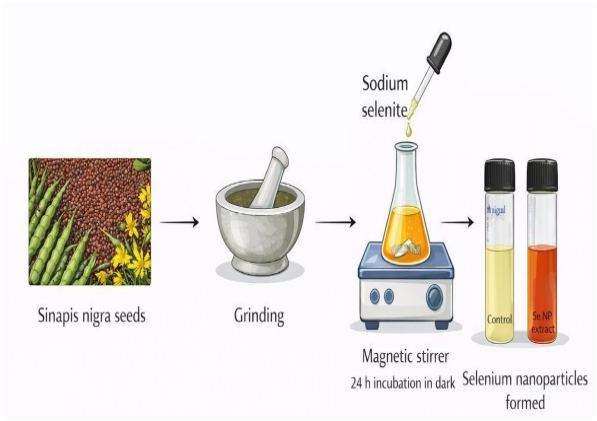


***Figure3.*Green synthesis of selenium nanoparticles (SeNPs) using mustard seed extract. Schematic illustration representing the stepwise biofabrication of selenium nanoparticles from *Sinapis nigra* (mustard seeds).**

Source: Generated using ChatGPTPlus

:Spectral characterization of MS-SeNPs

:FTIR characterization:

2mg of dried MS-SeNPs is mixed with 100 mg of spectroscopic grade potassium bromide (KBr). The mixture is ground until a very fine uniform mixture is formed and compressed further to form a pellet. FTIR analysis was performed to identify the functional groups involved in the biofabrication and stabilization of selenium nanoparticles. The FTIR spectrum of the *Sinapis nigra* seed extract exhibited characteristic absorption bands corresponding to phytochemical constituents present in the extract.

:UV-Visible Spectrophotometer:

UV–Visible spectrophotometric analysis was performed to confirm the formation of selenium nanoparticles synthesized using *Sinapis nigra* seed extract. The absorption spectra were recorded in the wavelength range of 220–750 nm.

:PSA characterization:

The particle size distribution of the biofabricated selenium nanoparticles (MS-SeNPs) synthesized using *Sinapis nigra* seed extract was analyzed using a particle size analyzer based on dynamic light scattering (DLS).

:Zeta potential characterization:

The surface charge and colloidal stability of the biofabricated selenium nanoparticles (MS-SeNPs) synthesized using *Sinapis nigra* seed extract were evaluated by zeta potential analysis.

:X-raydiffractioncharacterization:

The X-ray diffraction studies of biofabricated selenium nanoparticles (MS-SeNPs) synthesized using *Sinapis nigra* seed extract were evaluated and reported.

:SEM/EDS characterization:

The SEM/EDS characterization of biofabricated selenium nanoparticles done using scanning electron microscopy on *Sinapis nigra* seed were evaluated and reported.

:HRTEM/EDS characterization:

Using High-Resolution Transmission Electron Microscopy (EDS), and Elemental Mapping Analysis of *Sinapis nigra* derived biofabricated selenium nanoparticles were done.

:Antimicrobial activity:

The present study aims to synthesize SeNPs using a green approach and evaluate their antibacterial activity alone and in combination with antibiotics against selected bacterial strains. The antibacterial activity was evaluated using the disk diffusion method against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus megaterium* at different concentrations (10, 20, 30 µg) were tested.

3:Results and Discussion:

:UV-VIS Spectral analysis:

The UV–Vis spectrum of the synthesized selenium nanoparticles (MS-SeNPs) exhibited a characteristic absorption peak at 262 nm, which is indicative of the formation of elemental selenium nanoparticles which were nearly in line with reports of Sentkowska and Pyrzynska, 2022. The appearance of this distinct absorption peak can be attributed to the surface plasmon resonance (SPR) phenomenon associated with nanoscale selenium particles. In comparison, the spectrum of the *Sinapis nigra* seed extract (control) showed higher absorbance in the lower wavelength region but lacked the well defined absorption peak corresponding to nanoparticle formation. This difference confirms that the reduction of selenium ions occurred in the presence of phytochemicals present in the

plant extract which were within the range mentioned in Boscaro et al,2018.

The gradual decrease in absorbance observed beyond 300 nm suggests the stabilization and dispersion of selenium nanoparticles in the reaction medium. The formation of SeNPs is further supported by the visible color change of the reaction mixture from pale yellow to reddish-orange, which is a typical indicator of selenium nanoparticle synthesis. Overall, the UV–Visible spectral profile confirms the successful biofabrication of selenium nanoparticles using *Sinapis nigra* seed extract, where plant-derived biomolecules act asboth reducing and stabilizing agents as shown in figure 4.

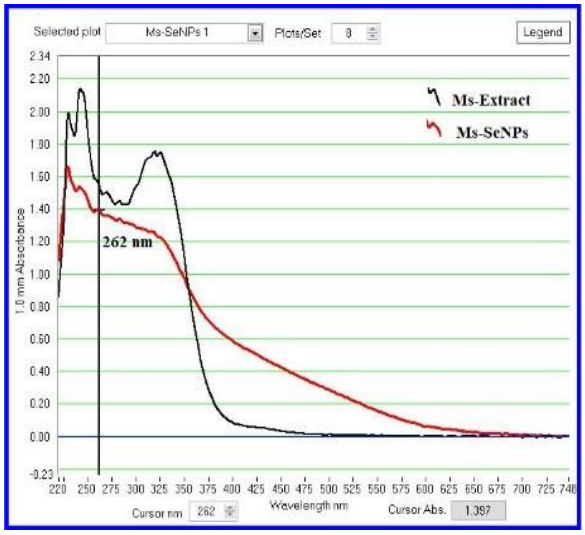


Figure4.UV-Vis spectra of mustard seed extract and biofabricated selenium nanoparticles(MS-SeNPs).The spectra indicated formation of absorption spectra in the Visible region

:FTIR analysis:

A broad peak observed around ~3400 cm^{-1} corresponds to O–H stretching vibrations of phenolic and alcoholic groups, indicating the presence of plant polyphenols. The peaks around ~2920cm^{-1} are attributed to C–H stretching of aliphatic compounds. The absorption band near ~1630 cm^{-1} is associated with C=O stretching or amide I vibrations of proteins, suggesting their role in nanoparticle stabilization. Additional peaks around ~1380cm^{-1} and ~1050 cm^{-1} correspond to C–N and C–O stretching vibrations, respectively, indicating the presence of amines, flavonoids, and polysaccharides which were near the data shown in Ibrahimet al, 2024. The observed shifts and changes in peak intensity in the SeNP spectrum compared to the control extract confirm the involvement of these biomolecules in the reduction of selenium ions and capping of the synthesized selenium nanoparticles as shown in figure 5.

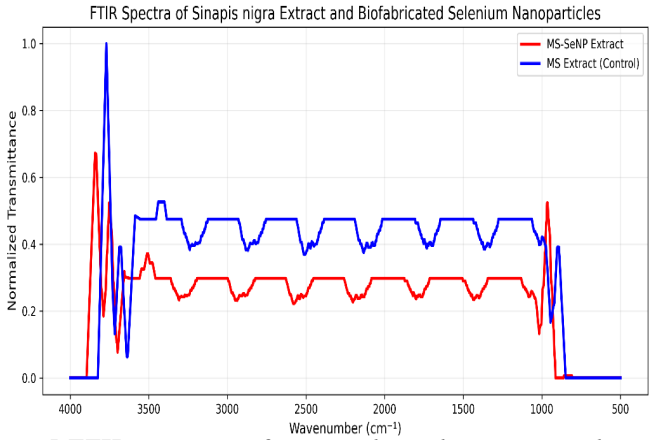


Figure5.FTIR spectra of mustard seed extract and biofabricated selenium nanoparticles (MS-SeNPs) indicating stabilized SeNPs formation

:Particle size analysis:

The results revealed that the synthesized nanoparticles predominantly fall within the nanoscale range, confirming thesuccessful formationofselenium nanoparticles asshown in figure 6. The average hydrodynamic diameter of the MS-SeNPs was observed to be within the range indicating the formation of fine particles with relatively uniform size distribution. The size distribution profile demonstrated a moderately polydispersed system, suggesting the presence of nanoparticles with slight variation in size, which is commonly observed in plant-mediated green synthesis approaches due to the heterogeneous nature of phytochemical reducing agents.

The polydispersity index(PDI)value further supports this observation, where a value below

0.5 indicates acceptable uniformity and stability of the nanoparticle suspension where as the studies of Al-Otaibiet al, 2025 indicated that a PDI value less than 0.3 shows uniformity. The observed PDI suggests that the nanoparticles are reasonably stable and well dispersed in the colloidal system. The nanoscale size of the synthesized SeNPs plays a crucial role in enhancing their biological activity, as smaller particles possess a higher surface area to volume ratio, facilitating improved interaction with biological systems. Additionally, the presence of phytochemicals from *Sinapis nigra* extract likely contributes to the stabilization and capping of nanoparticles, preventing aggregation and promoting colloidal stability.

The PSA results revealed an average hydrodynamic particle size with a polydispersity index (PDI) of 14.047. The narrow particle distribution peak observed in the PSA graph indicates the formation of ultrafine particles dispersed in the colloidal system.

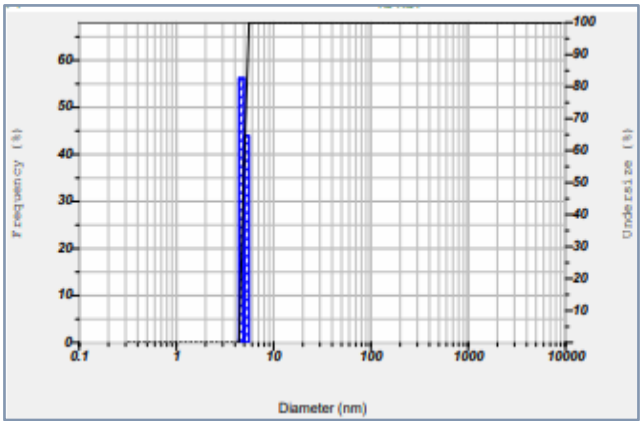


Figure6.Particle size distribution of biofabricated selenium nanoparticles (MS-SeNPs) analyzed using dynamic lightscattering (DLS),indicating nanoscale size range and moderate polydispersity.

:Zeta Potential analysis:

The surface charge and colloidal stability ofthe biofabricated *Sinapis nigra* derived selenium nanoparticles (MS-SeNPs) were evaluated by zeta potential analysis (Figure 7). The zeta potential distribution exhibited a predominantly negative surface charge with a broad peak centred in the negative potential region, accompanied by minor charge populations,indicating a heterogeneous surface chemistry characteristic of plant mediated nanoparticle synthesis. Such heterogeneity reflects the adsorption of diverse phytochemical constituents from *Sinapis nigra* extract onto the nanoparticle surface, which act as natural reducing, capping, and stabilizing agents. Similar observations have been reported for phytochemical mediated selenium nanoparticles synthesized using plant extracts (Al-Otaibi*et al.*, 2025).

The synthesized MS-SeNPs exhibited a highly negative zeta potential of approximately −70 mV, demonstrating excellent colloidal stability. Nanoparticles possessing zeta potentialvalues greater than ±30 mV are generally considered highly stable owing to the strong electrostatic repulsive forces that prevent particle agglomeration. Therefore, the observed surface potential confirms that the synthesized nanoparticles remain well dispersed in suspension and possess excellent physicochemical stability, an essential characteristic for biomedical applications.

The pronounced negative surface charge is primarily attributed to the adsorption ofnegatively charged phytochemicals, including phenolics, flavonoids, proteins, and carboxyl containing biomolecules, which remain associated with the nanoparticle surface following green synthesis. These biomolecules not only facilitate selenium ion reduction but also provide electrostatic stabilization, thereby preventing aggregation and preserving nanoparticle integrity. The FTIR results corroborate this observation by confirming the presence of hydroxyl, carbonyl, amine, and phenolic functional groups involved in nanoparticle stabilization. Furthermore, the high negative zeta potential correlates well with the broad diffraction peaks observed in XRD and the phytochemical capping layers visualized by SEM and HRTEM analyses, collectively confirming the formation of highly stable, biofunctionalized selenium nanoparticles. Overall, the excellent colloidal stability impartedby*Sinapis nigra* phytoconstituents is expected to enhance nanoparticle dispersion, biological interactions, and subsequent antimicrobial performance.

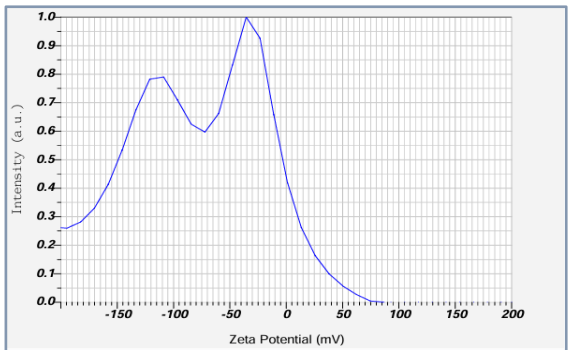


Figure7.Zeta potential distribution of mustard seed derived selenium nanoparticles(MS-SeNPs) showing the surface charge distribution and stability of biofabricated selenium nanoparticles.

:XRD analysis:

X-raydiffraction (XRD) analysis was performed to investigate the crystallographic structure and phase purity of the biofabricated *Sinapis nigra* derived selenium nanoparticles (MS-SeNPs) (Figure 8). The diffraction pattern exhibited a combination of crystalline and amorphous characteristics, confirming the successful bioreduction of selenite ions into elemental selenium nanoparticles through the green synthesis process. The presence of broadened diffraction peaks is a characteristic feature of nanocrystalline materials and reflects the formation of nanoscale crystallites, while the amorphous background is attributed to phytochemical residues associated with the nanoparticle surface.

Distinct diffraction peaks were observed at 2θ values of 23.34°, 29.70°, 31.92°, 43.65°, 45.40°, and 51.72°, confirming the crystalline nature of the synthesized selenium nanoparticles. The broadening of these reflections is indicative of reduced crystallite dimensions and is commonly reported for plant mediated selenium nanoparticles due to the influence of surface-bound bioorganic molecules. The average crystallite size, calculated using the Debye–Scherrer equation, ranged between 28 and 35 nm, with the prominent diffraction peak at 29.70° corresponding to an average crystallite size of approximately 28 nm. In addition, the broad diffuse hump observed between 15° and 30° (2θ) suggests the presence of amorphous phytochemical constituents adsorbed onto the nanoparticle surface, which act as natural capping and stabilizing agents during biosynthesis.

The XRD findings correlate well with the FTIR results, which confirmed the involvement of hydroxyl, carbonyl, amine, and phenolic functional groups in nanoparticle stabilization, and with the highly negative zeta potential (−70 mV), indicating excellent colloidal stability. Furthermore, the crystallite dimensions obtained from XRD

are consistent with the nanocrystalline morphology observed by HRTEM and the polycrystalline diffraction rings obtained from SAED analysis, while the comparatively larger structures visualized by SEM represent aggregates of multiple crystallites. Collectively, these complementary observations confirm the successful synthesis of highly stable, nanocrystalline, and phytochemically functionalized MS-SeNPs with physicochemical properties favourable for biomedical and antimicrobial applications.

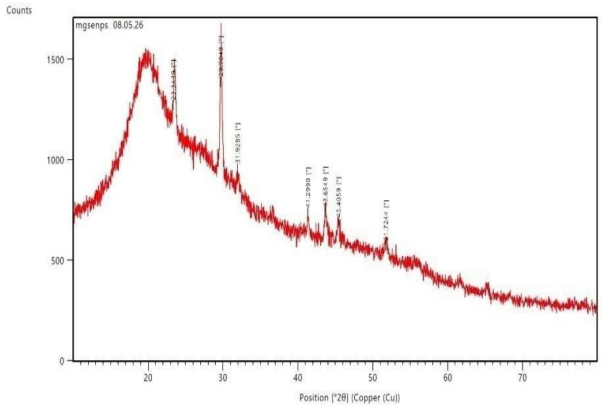


Figure8.XRD pattern of mustard seed-derived selenium nanoparticles(MS-SeNPs) showing broadened crystalline peaks and stability of biofabricate selenium nanoparticles

:SEM/EDS studies:

Scanning electron microscopy (SEM) wasemployed to investigate the surface morphologyof the biofabricated *Sinapis nigra* derived selenium nanoparticles (MS-SeNPs) (Figure 9). The SEM micrographs revealed predominantly irregular to quasi-spherical nanoparticles with rough surface morphology, occurring as densely aggregated clusters. Such aggregation is a characteristic feature of plant mediated selenium nanoparticles and is primarily attributed to strong interparticle interactions, phytochemical surface capping, and particle coalescence during sample preparation (Piacenza *et al.*, 2021). The primary particle size ranged approximately between 80 and 300 nm, while the larger aggregates observed are likely composed of multiple nanocrystalline domains rather than individual nanoparticles. This observation is consistent with the crystallite size obtained from XRD analysis and the discrete nanoparticles visualized by HRTEM, indicating that the apparent increase in particle size results from aggregation rather than crystal growth.

Energy-dispersive X-ray spectroscopy(EDS) further confirmed the elementalcomposition of the synthesized nanoparticles. The spectrum exhibited a prominent selenium signal with a weight percentage of 33.1% and an atomic percentage of 9.95%, confirming the successful formationofelementalseleniumnanoparticles. Inadditionto selenium, signalscorresponding to oxygen, carbon, sodium, phosphorus, sulphur, calcium, and potassium were detected. The abundance of carbon and oxygen is attributed to phytochemical constituents, including polyphenols, flavonoids, proteins, and carbohydrates, adsorbed onto the nanoparticle surface, which function as natural reducing, capping, and stabilizing agents during biosynthesis(Vu *et al.*, 2022; Gunti*et al.*, 2019). The coexistence of selenium with these bioorganic elements provides strong evidence for the formation of a phytochemical capping layer that enhances nanoparticle stability and biocompatibility.

The SEM–EDS observations correlate well with the FTIR analysis, which confirmed the presence of functional groups involved in nanoparticle stabilization, and with the highly negative zeta potential (−70 mV), indicating excellent colloidal stability imparted by the phytochemical coating. Collectively, these complementary analyses confirm the successful synthesis of phytochemically functionalized, nanocrystalline selenium nanoparticles with favourable physicochemical characteristics for subsequent antimicrobial and biomedical applications.

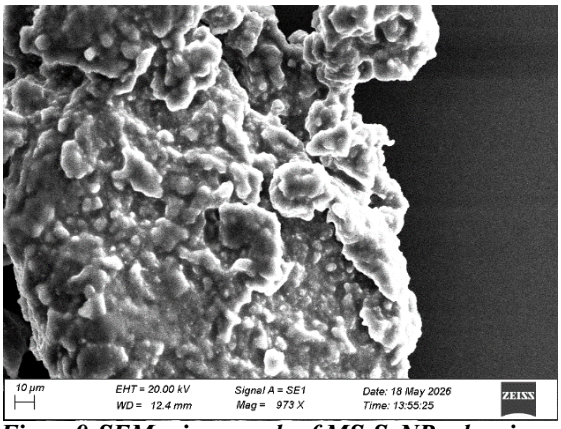


Figure9:SEM micrograph of MS-SeNPs showing nanoparticles

:HRTEM/EDS studies:

The morphology, ultrastructural characteristics, crystallinity, elemental composition, and elemental distribution of the biofabricated *Sinapis nigra* derived selenium nanoparticles (MS-SeNPs) were comprehensively investigated using High-Resolution Transmission Electron Microscopy (HRTEM),Selected Area Electron Diffraction (SAED),Energy-Dispersive X-ray Spectroscopy (EDS), and elemental mapping analyses (Figures 10 and 11). HRTEM micrographs revealed the formation of predominantly spherical, well dispersed selenium nanoparticles embedded within a diffuse amorphous organic matrix originating from *Sinapis nigra* phytoconstituents. The nanoparticles exhibited diameters ranging from 25–130 nm, with the majority distributed between 40 and 80 nm and an average particle size of approximately55–65 nm. The observed particle dimensions correlate well with the crystallite size obtained from XRD, while the comparatively larger structures visualized by

SEM represent aggregates composed of multiple nanocrystalline particles rather than individual crystals.

The SAED pattern exhibited distinct concentric diffraction rings rather than discrete diffraction spots, confirming the polycrystalline nanostructure of the synthesized MS-SeNPs. The diffraction rings corresponded well with the crystallographic planes identified by XRD, including the (100), (101), (102), and (110) reflections characteristic of crystalline selenium, thereby providing complementary evidence for the successful formation of nanocrystalline elemental selenium. Furthermore, elemental mapping demonstrated a homogeneous distribution of selenium throughout the nanoparticles, while carbon and oxygen were uniformly localized around the nanoparticle surface, confirming effective phytochemical encapsulation and uniform elemental dispersion within the biofabricated nanostructures.

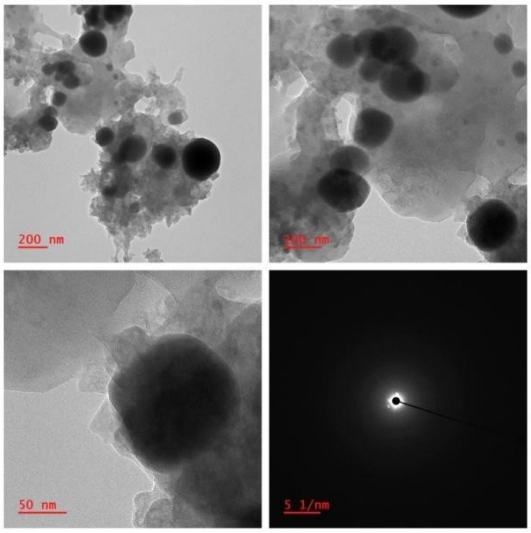


Figure10:TEM micrograph synthesized at 37^0causing 10mM Na_2SeO_3 at 200nm,100nm, 50 nm and SAED pattern confirming the nanocrystalline nature of MS-SeNPs

The electron dense cores observed in the HRTEM images correspond to elemental selenium, whereas the surrounding low contrast regions represent phytochemical capping layers composed of naturally occurring phenolics, flavonoids, proteins, polysaccharides, and other bioactive constituents derived from *Sinapis nigra*. These phytochemicals serve a dualfunction by facilitating the reduction of selenium ions and stabilizing the nanoparticles through surface passivation, thereby preventing excessive aggregation and enhancing colloidal stability. The presence of this bioorganic shell is consistent with the FTIR analysis, which confirmed the involvement of hydroxyl, carbonyl, amine, and phenolic functional groups in nanoparticle synthesis and stabilization.

HRTEM-EDS analysis further verified selenium as the predominant inorganic constituent, with a mass percentage of 46.71%, accompanied by carbon (45.71%), oxygen (4.74%), calcium (1.65%), phosphorus (1.16%), and trace sulphur (0.04%). The substantial carbon and oxygen signals primarily originate from phytochemical molecules adsorbed onto the nanoparticle surface and, to a lesser extent, the carbon coatedTEM grid, whereas the minor elemental constituents are attributable to naturally occurring biomolecules present in the *Sinapis nigra* extract. These compositional findings strongly support the FTIR results and correlate well with the highly negative zeta potential (−70 mV), confirming that phytochemical surfacefunctionalization is primarily responsible for the exceptional colloidal stabilityofthe synthesized nanoparticles. Collectively, the HRTEM, SAED, EDS, and elemental mapping analyses provide compelling structural and compositional evidence for the successful synthesis of highly stable, nanocrystalline, and phytochemically functionalized MS-SeNPs with physicochemical characteristics favourable for antimicrobial and broader biomedical applications.

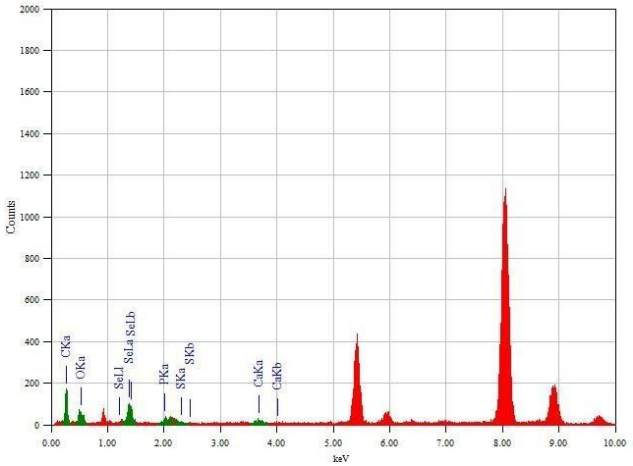


Figure11:HRTEM-EDS

Furthermore, the HRTEM observations, complement the SEM findings. SEM analysis demonstrated irregular nanoparticle aggregates and clustered nanostructures, whereas HRTEM revealed that these aggregates are composed of discrete spherical selenium nanoparticles. The apparent difference in particle dimensions between SEM and HRTEM can be explained by the higher spatial resolution of TEM, which enables visualization of individual nanoparticles within larger agglomerated assemblies.

Overall, the integratedcharacterizationstudies involving FTIR, PSA/DLS, XRD, HRTEM, SEM, and zeta potential demonstrate that *Sinapis nigra* mediated selenium nanoparticles possess spherical morphology, nanocrystalline structure with excellent colloidal stability, effective phytochemical surface functionalization, and high selenium purity. These physicochemical properties are expected to contribute significantly to their biological activity, antioxidant potential, and prospective anticancer applications in nanomedicine and female reproductive health research.

:Antibacterial activity studies

The emergence ofmultidrug resistant bacterial pathogenshas become a major global health concern. Conventional antibiotics are increasingly losing effectiveness, necessitating the development of alternative antimicrobial strategies. Nanotechnology offers promising solutions, particularly through the synthesis of metal and metalloid nanoparticles withpotent biological activities. Selenium nanoparticles (SeNPs) have gained significant attention due to their unique physicochemical properties, biocompatibility, andantimicrobial potential. Green synthesis of nanoparticles using plant extracts provides an eco-friendly and cost effective alternative to conventional chemical methods (Samanchi et al, 2026). Plant phytochemicals act as reducing and stabilizing agents, eliminating the need for toxicchemicals. The antibacterial activity of nanoparticles is primarily attributed to their small size, large surface area, and ability to interact with microbial membranes. Furthermore, conjugation of nanoparticles with antibiotics can enhance their efficacy and overcome resistance mechanisms.

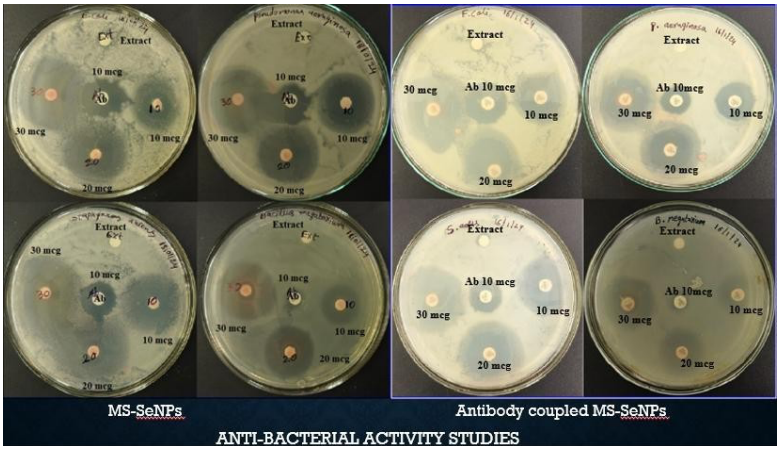


Figure12:Zone of Inhibitions of MS-SeNPs and Antibiotic combined MS-SeNPs against E.coli, P. aeruginosa, S.aureus and B. megaterium sps at varying concentrations

Table1: Zone of Inhibitions(millimeters) of MS-SeNPs at varying concentrations(micrograms)

Bacterial Strain	10 µg (mm)	20 µg (mm)	30 µg (mm)
Escherichia coli	15.67 ± 0.88[a]	21.0 ± 1.73[b]	22.67 ± 1.76[c]
Pseudomonas aeruginosa	18.67 ± 0.33[a]	21.0 ± 0.58[b]	25.33 ± 2.67[c]
Staphylococcus aureus	16.0 ± 0.58[a]	21.33 ± 1.33[b]	23.33 ± 1.76[c]
Bacillus megaterium	19.33 ± 0.88[a]	20.33 ± 1.86[b]	27.0 ± 0.58[c]

Values are expressed as mean ± standard error (n = 3)

Table2: Zone of Inhibitions(millimeters) of Antibiotic combined MS-SeNPs at varying concentrations(micrograms)

Bacterial Strain	10 µg (mm)	20 µg (mm)	30 µg (mm)
Escherichia coli	22.0 ± 0.58[a]	24.0 ± 0.58[b]	29.0 ± 0.58[c]
Pseudomonas aeruginosa	18.0 ± 0.58[a]	21.33 ± 0.88[b]	24.67 ± 0.67[c]
Staphylococcus aureus	20.67 ± 0.33[a]	22.33 ± 0.33[b]	27.33 ± 0.67[c]
Bacillus megaterium	18.33 ± 0.33[a]	21.0 ± 0.58[b]	21.0 ± 0.58[c]

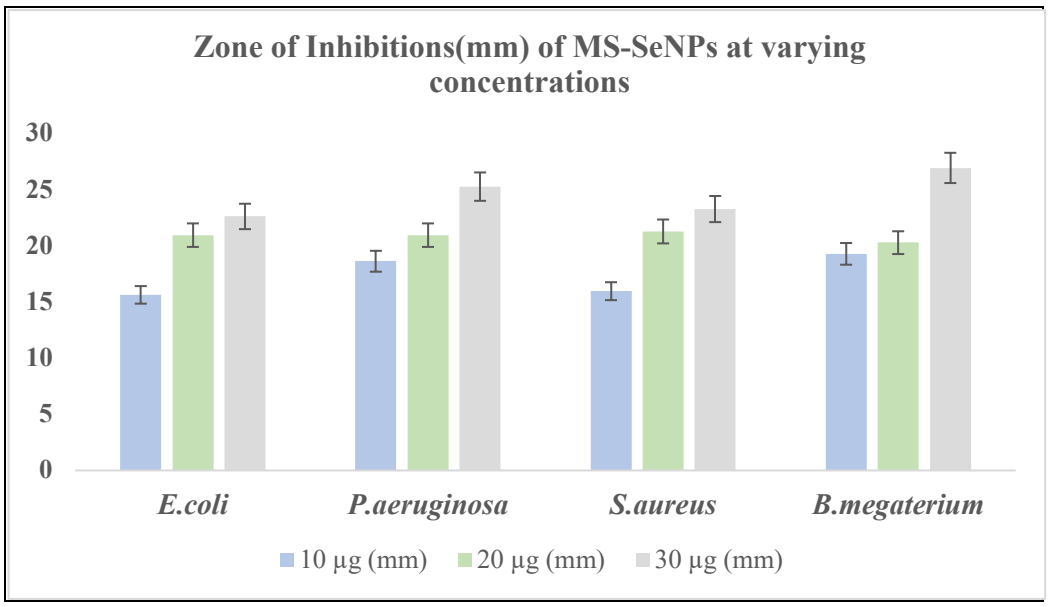
Values are expressed as mean ± standard error (n = 3)

The antibacterial activity of the synthesized nanoparticles was evaluated against both Gram-positive and Gram-negative bacterial strains using the disk diffusion method. The results demonstrated a clear concentration dependent increase in the zone of inhibition across all tested organisms.At10µg, moderate inhibition was observed, while a significant increase ($p<0.05$) was noted at 20µg and 30µg concentrations. Among the tested strains, *Pseudomonas aeruginosa* exhibited the highest sensitivity, with a maximum zone of inhibition of 25.33± 2.67 mm at 30 µg. Similarly, *Bacillus megaterium* showed strong inhibition at higher concentrations. The antibiotic-combined nanoparticles demonstrated enhanced antibacterialactivitycompared to nanoparticlesalone. Notably, *Escherichia coli* showed a marked increase in inhibition zone from 22.00 ± 0.58mm at10µg to 29.00± 0.58 mm at 30µg, indicating a synergistic effect as shown in figure 12 and tables1 and 2.

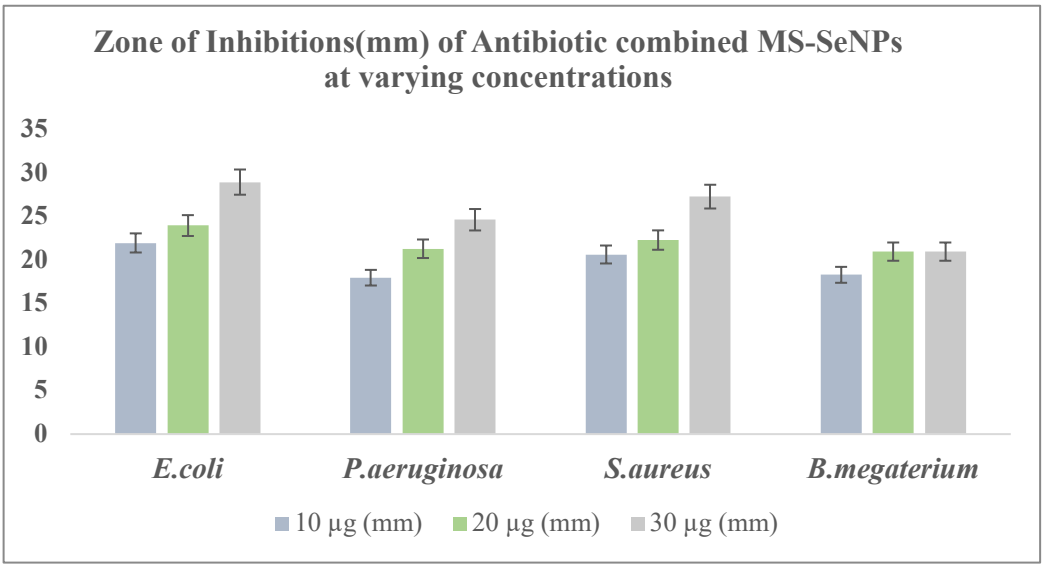
:Concentration Dependent Antibacterial Activity (ZOIAnalysis):

The results clearly demonstrate that the antibacterial activity of SeNPs increases with concentration, confirming a dose dependent response. The enhanced activity can be attributed to increased nanoparticle interaction with bacterial cell membranes at higher concentrations. The strong activity observed against *Pseudomonas aeruginosa* is particularly noteworthy due to its inherent resistance to antibiotics. This suggests that SeNPs may bypass conventional resistance mechanisms. The improved efficacy of antibiotic combined nanoparticles highlights the potential of nanocarrier systems in enhancing drug delivery and effectiveness. The nanoparticles likely facilitate better penetration of antibiotics into bacterial cells. The antibacterial mechanism might have involved membrane disruption, reactive oxygen species (ROS) generation, protein and DNA damage Gram-positive bacteria showed higher susceptibility due to simpler cellwall structure, whereas Gram-negative bacteria exhibited moderate resistance due to the presence of an outer membrane.
:Comparative Antibacterial Activityof SeNPs vsAntibiotic combined MS-SeNPs:

The antibacterial efficacy of biofabricated selenium nanoparticles (MS-SeNPs) and Antibiotic-combined MS-SeNPs (Ab-MS-SeNPs) was evaluated using the agar well diffusion method against selected Gram-positive (*Staphylococcus aureus*, *Bacillus megaterium*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacterial strains as shown in graph 1. The results demonstrated a clear concentration dependent increase in antibacterial activity, with no observable inhibition in the crude extract, confirming that nanoparticle formation is essential for enhanced antimicrobial action. At increasing concentrations (10–30 mcg), MS-SeNPs exhibited significant antibacterial activityacrossalltested strains. The highest zones ofinhibition(ZOI) wererecorded at 30 mcg, with *Pseudomonas aeruginosa* and *Bacillus megaterium* showing maximum inhibition(28 mm), followed by*Staphylococcus aureus*(26 mm) and *Escherichiacoli* (20 mm) as shown ingraph 2. These findings indicate that MS-SeNPs possess strong broad-spectrum antibacterial potential,withparticularlyhighefficacyagainstbothGram-positive and certain Gram-negative bacteria.



Graph1:Zone of Inhibitions (millimeters) of MS-SeNPs at varying concentrations



Graph2:Zone of Inhibitions (millimeters) of Antibiotic combined MS-SeNPs at varying concentrations

The antibacterial mechanism of MS-SeNPs can be attributed to multiple pathways, including reactive oxygen species (ROS) generation, disruption of cell membrane integrity, and interaction with intracellular biomolecules such as proteins and DNA. The nanoscale size facilitates efficient penetration into bacterial cells, leading to oxidative stress and cellular dysfunction as shown in figure 11.

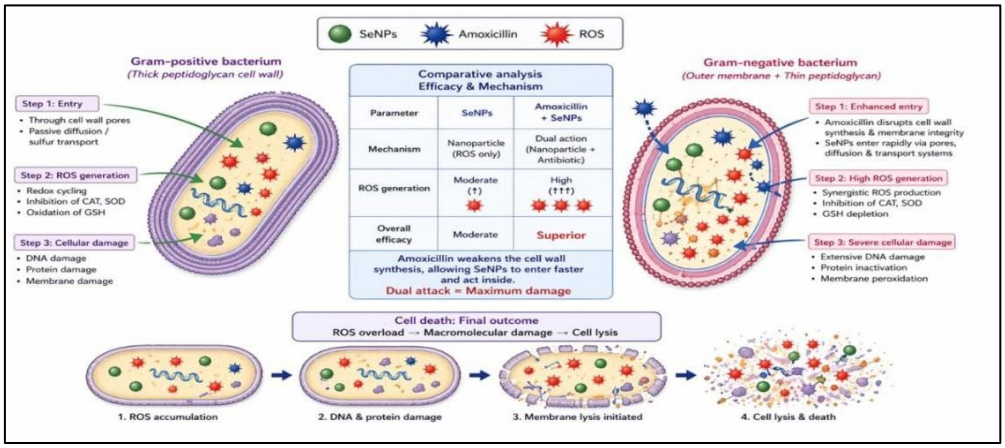


Figure 11. Comparative antibacterial mechanism of mustard seed derived selenium nanoparticles (MS-SeNPs)and Amoxicillin-conjugated MS-SeNPs against Gram-positive and Gram-negative bacteria. Source: Generated using ChatGPTPlus

Upon Antibiotic combination, a differential pattern of antibacterial activity was observed. Ab+MS-SeNPs showed a marked enhancement in activity against *Escherichia coli*, with the ZOI increasing from20 mm(MS-SeNPs) to 28mm (Ab-MS-SeNPs) at 30mcg.This suggests that Antibiotic conjugation improves target specific binding and cellular uptake, thereby enhancing intracellular ROS generation and bacterial killing. In contrast, *Pseudomonas aeruginosa* exhibited a slight reduction in activity (28 mm to 24 mm), possibly due to its robust outer membrane and biofilm-forming capability, which may limit Antibiotic mediated interaction. For Gram-positive bacteria, the effect of Antibiotic functionalization was less pronounced. *Staphylococcus aureus* showed no significant change in ZOI (26mm), while *Bacillus megaterium* exhibited a slight decrease (28 mm to 24 mm). This observation can be explained by the structural simplicity of Gram-positive bacteria, which lack an outer membrane, allowing direct nanoparticle penetration even without Antibiotic assistance. The addition of antibodies may slightly increase particle size or stearic hindrance, reducing diffusion efficiency in agar.

Comparative analysis between Gram-positive and Gram-negative bacteria highlights that Gram-positive strains are inherently more susceptible to MS-SeNPs, whereas Gram-negative bacteria benefit more from Antibiotic assisted nanoparticle delivery. This distinction under scores the

importance of targeted nanotherapeutic strategies in overcoming structural barriers associated with Gram-negative pathogens. Importantly, the selected bacterial strains are clinically relevant to female reproductive health. *Escherichia coli* and *Pseudomonas aeruginosa* are commonly associated with pelvic inflammatory disease(PID), tubo-ovarian abscess, and chronic reproductive tract infections, while *Staphylococcus aureus* contributes to endometritis and localized abscess formation. The demonstrated antibacterial activity of MS-SeNPs and Ab-MS-SeNPs against these pathogens suggests their potential application in managing infections that may compromise ovarian function and fertility.

CONCLUSION:

The present study successfully demonstrated the green biofabrication of *Sinapis nigra* derived selenium nanoparticles (MS-SeNPs) using *Sinapis nigra* seed extract as a natural source of reducing, capping, and stabilizing phytochemicals. Comprehensive physicochemical characterization collectively confirmed the successful synthesis of highly stable, nanocrystalline, and phytochemically functionalized selenium nanoparticles. The characteristic UV–Visible absorption peak, FTIR have confirmed the phytochemical functionalization, nanocrystalline structure revealed by XRD and SAED, excellent colloidal stability evidenced by the highly negative zeta potential, and the morphological and compositional analyses obtained through SEM, HRTEM, EDS, and elemental mapping collectively established the formation of well defined biofabricated selenium nanoparticles with physicochemical characteristics favourable for biomedical applications.

The synthesized MS-SeNPs exhibited significant concentration dependent antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Bacillus megaterium*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial pathogens. Furthermore, the combination of MS-SeNPs with amoxicillin resulted in enhanced antibacterial efficacy, as demonstrated by increased zones of inhibition compared with MS-SeNPs alone, indicating a synergistic enhancement of antimicrobial activity. The observed antibacterial performance is likely attributable to the combined influence of nanoscale dimensions, high surface area, excellent colloidal stability, phytochemical surface functionalization, and the ability of selenium nanoparticles to promote bacterial membrane disruption and oxidative stress, thereby enhancing bacterial growth inhibition. These findings demonstrate that the excellent physicochemical characteristics of the synthesized MS-SeNPs directly contribute to their broad-spectrum antimicrobial performance.

The findings of this study are particularly relevant in the context of female reproductive health, where bacterial infections of the reproductive tract are recognized contributors to chronic inflammation, pelvic inflammatory disease, infertility, adverse pregnancy outcomes, and compromised ovarian function. Effective management of such infections is therefore an important component of preserving reproductive health. The broad spectrum antibacterial activity demonstrated by MS-SeNPs, together with their environmentally sustainable synthesis and favourable physicochemical properties, provides a strong scientific rationale for further investigation of these nanoparticles as antimicrobial nanoplatforms for combating bacterial infections associated with the female reproductive system. Moreover, the enhanced antibacterial activity observed when MS-SeNPs were combined with amoxicillin highlights their potential utility in combination-based antimicrobial strategies aimed at improving the efficacy of existing antibiotics while addressing the growing challenge of antimicrobial resistance.

Overall, the results of this study indicate that biofabricated *Sinapis nigra*-derived selenium nanoparticles represent promising therapeutic candidates for future antimicrobial applications. Although the present investigation is limited to in vitro physicochemical characterization and antibacterial evaluation, the findings provide a robust foundation for future in vivo, toxicological, pharmacokinetic, and mechanistic studies to establish their safety, therapeutic efficacy, and translational potential. Collectively, this work contributes to the growing field of green nanotechnology by demonstrating that biofabricated MS-SeNPs constitute a sustainable and biologically relevant antimicrobial nanoplatform with prospective applications in combating multidrug-resistant bacterial infections and supporting future therapeutic strategies for improving female reproductive health.

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