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1.0 Introduction

A critical frontier in the future of medicine lies in the application of nanotechnology. The convergence of nano-synthesis and biotechnology presents a promising solution to this challenge through environmentally sustainable methods for producing nanomaterials using plant or microbial extracts (Chaudhary et al., 2016; Li & Xu, 2020). Among the wide array of nanoparticles developed to date, selenium nanoparticles (SeNPs) have garnered particular attention due to their unique physicochemical properties and potential therapeutic applications, including anticancer (Yaqoob et al., 2020), antioxidant (Valgimigli et al., 2018), antimicrobial (Jamdagni et al., 2018) , and anti-inflammatory effects (Lv et al., 2020). However, conventional chemical and physical methods for synthesizing SeNPs often rely on toxic chemicals, demand high energy inputs, and generate hazardous byproducts, making them environmentally unsustainable. Consequently, green nanotechnology, which leverages biological processes for nanoparticle synthesis, has emerged as an eco-friendly and sustainable alternative.

Nanomaterials are not just a "miniaturization" of macroscopic materials; they have been highly tapped for their unique bioactive properties because of their high surface area, nanoscale sizes, and significant structural orientation, which can also affect how they interact with other materials and biological systems (Kerativitayanan et al., 2015; S. Liu et al., 2023; W. Zhou et al., 2019). The fundamental concept of nanotechnology involves manipulating and managing matter at the nanometer scale to develop, produce, and apply materials for innovative purposes. The Greek word "Nanos" is the origin of the term "Nano" and it signifies extremely small or dwarf. There are multiple definitions for nanotechnology, but mainly, it is described as "the interdisciplinary field that encompasses the synthesis, design, characterization, and application of innovative nanomaterials that are at least in one dimension in the nanometre scale" (Chattopadhyay & Banerjee, 2009). New material development in nanotechnology occurs at scales ranging from a few nanometres to individual atoms or molecules. Nanotechnology frequently involves the integration of several nanostructures into larger nanosystems, such as the creation of multifunctional nanoparticles for healthcare purposes. Nanotechnology and nanoscience are demonstrating extensive applicability in scientific study.

A plethora of methods exist for the synthesis of various forms of nanomaterials, such as colloids, clusters, powders, tubes, rods, wires, thin films, etc. Conventional procedures for

synthesizing various materials have been refined to produce unique nanomaterials, and new approaches have also been devised. It is evident that nanotechnology is a multidisciplinary field. Consequently, a variety of physical, chemical, biological, and hybrid methods exist for the synthesis of nanomaterials. Nanotechnology and nanoscience are dynamic fields of study that consistently yield novel, enhanced, and more potent technologies, supplanting their predecessors. The highly structured nanoscale arrangement of atoms or molecules produces immense functional entities in both the natural and artificial worlds. Hierarchical structures that produce complex features are the result of such organization. These systems are made up of parts that may be biologically or physically derived and have similar dimensions, suggesting that there are universal laws governing how they are put together and interact.

To comprehend the logical image of nanoforms seen in the physical and biological world, the nanoscale entities of nanotechnology and nanoscience are contrasted with the nanoscale functional units of biology (Fig 1.1). Examples of this include bacterial cells, proteins, viruses, quantum dots, carbon nanotubes, and eukaryotic cell ribosomes. Human hair typically has a diameter of 50,000 nm, whereas the water molecule is less than 1 nm. It's intriguing to observe from this comparison that the majority of animal and microbial species get their energy from metabolic processes from a molecule that is 1 nm in size, which is 50,000 times smaller than hair. At the nanoscale, the biological and nanoworld are structured according to the same principles, and these discoveries provide researchers with the opportunity to investigate how they interact. (Baptista et al., 2018).

1.1 Nanobiotechnology

A subfield of nanotechnology known as "nanobiotechnology" examines the biological and biochemical applications of nanoparticles. Biotechnology and nanotechnology provided the majority of the foundations for nanobiotechnology. The phrase nanobiotechnology, which refers to the use and self-assembly of biologically directed molecules towards various technological applications, is formed by the prefix "nano" and the word "biotechnology." It focuses on minuscule particles that range in size from 1 to 100 nm. Nanoparticles have several advantageous special characteristics that provide them with a competitive edge in the biological field. For instance, Increased surface area to volume ratio makes the nanoparticles interact with biological molecules more reactive and facile. Moreover, materials at the nanoscale can have enhanced mechanical qualities, such as greater durability and flexibility, as well as altered quantum mechanical properties that affect magnetic, optical, and electrical behaviours. The two

primary focuses of nanobiotechnology research are the utilization of pre-existing biological molecules, such as DNA, proteins, enzymes, and ion channels, and the application of processed nanomaterials, including metal, metal oxide nanoparticles, magnetic nanoparticles, and quantum dot particles (QDs) (Chattopadhyay and Banerjee, 2009). Hence, nanobiotechnology has been a potential resource of applications in various domains.

In Agriculture, nanobiotechnology has been exploited in different manners for the development of nano-fertilizers (R. Liu and Lal, 2015), nano-pesticides (J. L. de Oliveira et al., 2014) and nano-sensors (Beegum and Das, 2022). The development of more nutritious and productive seeds, enhanced plant germination, increased plant growth and yield, and better storage and preservation techniques are just a few of the ways that nanomaterials have been used to advance agricultural methods. Jindal et al. studied the impact of eco-friendly synthesized zinc oxide nanoparticles from *Achillea millefolium,* which showed a significant application as a nematocidal and plant growth promotion in tomatoes, i.e., *Lycopersicon esculentum* (Jindal et al., 2025). Subsequently, Yin et al. studied the molecular mechanism and a nano-fertilizer approach of selenium-doped carbon dots for the growth promotion of tomato plants and also observed improved fruit quality (Yin et al., 2024).

Furthermore, nanobiotechnology has become a crucial field for several biomedical applications among emerging technologies. Nanobiotechnology's most significant contributions are in the fields of drug delivery, drug development, and drug discovery. Another term that can be used as an aggregate term for these pharmaceutical-based applications is “Nano-pharmaceuticals” (Allen and Cullis, 2004). Progress in nanomedical engineering and nanomedicine has resulted in the development of nanomedicines, or pharmaceuticals, characterized by their nanoparticle size (diameter ranging from 1 to 100 nm), commonly known as nano-carriers (NCs) (Karahmet Sher et al., 2024). These NCs function as transport agents, facilitating the delivery of active substances to the target structures where their action is essential. NCs safeguard the active agents (small molecules, peptides, proteins, or nucleic acids), facilitating their entrance to target structures in enough quantities and permitting regulated spatial and temporal release of these agents. Such application is highly advantageous from hydrophobic drugs and thus can be solution towards the management of rejection rate via pharmaceutical companies. Thus, the interphase of biotechnology and nanotechnology has facilitated the birth of novel insights.

1.1.1 Role of nanoparticles in biomedical applications

The remarkably extensive surface area of nanoparticles results in significant adsorption of various biomolecules on their surface, hence diminishing the nanoparticles' surface energy. The surface chemistry, composition, topography, roughness, thermodynamics, and toxicological consequences dictate the unique biological application of nanomaterials. Furthermore, biomolecules can be affixed to the nanoparticle surface physically or chemically. Biomolecules characterized by specific biological interactions, such as antibody-antigen, receptor-ligand, and DNA-DNA or DNA-RNA hybridization, were employed for the surface functionalization of nanoparticles. Using biomedically active nanoparticles in conjunction with various polymers, manifested as thin films, nano-capsules, or alternative forms, is likely to provide novel materials with altered physical, chemical, and mechanical capabilities (Rezić, 2022). Owing to their facile, adaptable size, shape, composition, and substantial surface area-to-volume ratios, nanoparticles are becoming prevalent in biological applications. Recently, there has been an increasing interest among researchers in using these nanomaterials for numerous biological applications, including targeted administration of drugs, biosensors, and bioimaging. Ideally, nanoparticles designed for biological purposes should exhibit minimal cytotoxicity, prolonged colloidal stability, and substantial loading capacity. The efficacy of nanoparticles in drug delivery applications relies on their ability to traverse the cellular barrier further, enter the plasma membrane, and be internalized by target cells after systemic delivery. In therapeutic interventions, notable instances include gold nanoparticles utilized in cancer therapy, imaging, and biosensing, attributed to their biocompatibility and optical characteristics (Hossain et al., 2024; Sharma et al., 2012). In continuation to the latter, silver nanoparticles have displayed significant application in wound healing capacity and antimicrobial potency (Shankar et al., 2020; Skomorokhova et al., 2020), and iron-oxide nanoparticles display a valuable utilization capability in magnetic resonance imaging (MRI) and hyperthermia treatment (Rahman, 2023) Furthermore, the duality of selenium nanoparticles acting as an antioxidant and as a pro-oxidant beyond critical dose has also been known to provide applications in cancer therapy, immune modulation and neuroprotection (An et al., 2023 ; Bisht et al., 2022). In addition to these notable benefits, the hurdles related to the commercialization of these applications include long-term safety, possible toxicity, scalability, and consistency in reproduction at a large scale, as well as the demanding regulatory approvals necessary for clinical translation.

1.2 Selenium's role in biology and human health

Selenium (Se) is an essential micronutrient, as mentioned earlier. It was first discovered in 1818 by Swedish scientist Jöns Jacob Berzelius, who identified selenium in the residues of sulfuric acid production. Initially considered a toxic element, selenium's biological importance was not recognized until 1957, when Schwarz and Foltz identified it as an essential trace element for both plants and animals (Schwarz and Foltz, 1957). Selenium plays a vital role in numerous biological processes. It is a key component of selenoproteins, which are involved in antioxidant defense, metabolic regulation, immune function, and cellular homeostasis. Selenium also serves as an essential element in critical antioxidant enzymes, such as glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs), which neutralize reactive oxygen species (ROS), thereby mitigating cellular damage and contributing to the prevention of age-related diseases.

Selenium exists in nature primarily in three forms: elemental selenium, inorganic selenium, and organic selenium. Elemental selenium is difficult for organisms to acquire and use. Inorganic selenium with low bioavailability exists as selenide (Se^{2+}), selenite (Se^{4+}), or selenate (Se^{6+}). Selenium in biology mostly occurs as selenocysteine and seleno-methionine residues, exhibiting specific physiological actions associated with metabolism and the structural algorithms of these compounds (Banuelos, Lin, & Yin, 2013). The organic selenium found in organisms primarily comprises two categories: the first includes selenium-containing amino acids, such as seleno-cysteine (SeCys) and seleno-methionine (SeMet); the second encompasses selenium-containing proteins, predominantly featuring selenium in the form of selenocysteine (Kryukov et al., 2003; Werkneh et al., 2023). Selenium is a vital micronutrient for both humans and animals, but it may be harmful in large amounts. That is, following the findings reported by the WHO. It is essential to consume 55-65 µg of selenium daily to fulfil the recommended dose for healthy people. In supplementation, 400 µg/day of selenium is the maximum amount that people should tolerate. Foods with selenium concentrations below 0.1 µg/g can cause illnesses related to selenium deficiency, whereas foods with selenium concentrations above 1 µg/g can cause toxicity . Selenium in food is majorly resourced from soil content (Zhe et al., 2017), it is obtained from rock erosion, where the accumulative selenium in rocks amounts to 40% of its composition (Zhe et al., 2017). Although selenium is a vital element, the ability of humans to absorb selenium intake is contingent upon several conditions. Age is a primary regulating element. In adolescents and children, selenium consumption is elevated to ensure optimal growth and development, while in adults, it is essential for regulating immune system function and enhancing the body's antioxidant defences. In advanced age, its intake or absorption diminishes due to diminished digestive functions

(Fordyce, 2007). Selenate resources are converted into adenosine-5'-selenophosphate and then non-enzymatically converted into selenite viaz glutathione (GSH) (Fig 1.2). Furthermore, Selenite can be readily transformed into hydrogen selenide (H_2Se) by the action of thioredoxin reductase (TrxR) or sequentially changed into GSSeGS and GSSeH via the action of GSH and GPx, ultimately resulting in H_2Se.

Another important source of selenium is through diet containing organoselenium moieties such as selenomethionine (SeMet) and selenocysteine (SeCys) (Fig 1.2) (Takahashi et al., 2020). SeMet may be non-specifically transformed into methylselenol (CH_3SeH) by cystathionine γ-lyase, which is then demethylated to yield H_2Se. On the other hand, SeMet can also be trans-sulfurated into an inter-mediatory product, i.e., SeCys (Tobe and Mihara, 2018). Both the SeMet and SeCys are either demethylated or decomposed into H_2Se. This inter-terminal product can follow two pathways, i.e., H_2Se can be converted into selenophosphate ($HSePO_4^{3-}$), which can be further utilized for selenoproteins generation (Tobe & Mihara, 2018) or it can be converted into seleno-sugars, methyl-selenide (CH_3SeH), dimethyl-selenide $(CH_3)_2Se$, trimethyl-selenonium ($(CH_3)_3Se^+$), which are normally excreted via defecation process, respiration or perspiration. (Mistry et al., 2012).

Despite the absence of definitive evidence from epidemiological studies regarding the role of selenium in certain diseases among populations with low selenium status, there has been a growing interest in investigating the antioxidative effects of GPxs and TrxRs in myocardial ischemia-reperfusion (I/R) injury (Jekell et al., 2004). In male patients with I/R damage, elevated TrxR levels are reported as a response to oxidative stress. (Tanguy et al., 2003). A corrective strategy for this issue is that a selenium-rich diet can mitigate and diminish the consequences of reperfusion. Lymbury et al. demonstrated that sodium selenite, at specific doses, serves as a reperfusion solution and facilitates heart recovery after ischemia/reperfusion damage (Lymbury et al., 2006). Due to the potential of Se-based compounds reverting heart injury conditions, considerable attention has been given to the synthesis of organo-Se compounds for their biological activity. One of the most commercialized Se-based compounds to revert I/R injury is "Ebselen" i.e., 2-phenyl-1,2-benzisoselenzol-3(2H)-one (Sakurai et al., 2006).

Another prominent selenium's role is its vital contribution to the synthesis and metabolism of thyroid hormones. The thyroid gland itself stores high concentrations of selenium, highlighting

its importance in thyroid function. A class of selenoenzymes known as iodothyronine deiodinases (DIOs) plays a crucial role in converting thyroxine (T4) into its active form, tri-iodothyronine (T3) (Roy et al., 2005). Adequate selenium levels support normal thyroid function and help prevent disorders such as autoimmune thyroid disease and hypothyroidism.

It has been suggested that selenium's potential role in reducing thyroid autoimmunity may involve the activity of selenoenzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), which act as antioxidant defense systems. These enzymes help neutralize oxidative stress caused by hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) generated by thyrocytes during thyroid hormone production (Imai et al., 2009).

Efficient selenoprotein synthesis is essential for proper thyroid hormone metabolism. Variations in the selenocysteine insertion sequence (SECIS)-binding protein 2 (SBP2) can impair deiodinase function, disrupting thyroid hormone regulation.

Given selenium's diverse biological roles including antioxidant defence, immune regulation, thyroid hormone metabolism, and disease prevention it is evident that selenium is an essential trace element necessary for maintaining overall health.

In toxicity profiling, selenium insufficiency is uncommon in well-nourished populations but may occur in individuals with specific risk factors, such as those with impaired intestinal function or individuals reliant on parenteral nutrition lacking adequate selenium supplementation. Selenium deficiency can lead to Keshan disease, a rare form of cardiomyopathy, which may progress to heart failure in severe cases (Werkneh et al., 2023). Another deficiency-related condition is Kashin-Beck disease, a bone disorder characterized by osteoarthropathy, resulting in the degeneration of cartilage tissue.

Conversely, excessive selenium intake can lead to toxicity, known as selenosis. This condition may arise from the overconsumption of selenium-rich foods, dietary supplements, or occupational exposure (Takahashi et al., 2020; Werkneh et al., 2023). Adverse health effects associated with elevated selenium levels include gastrointestinal disturbances, hair loss (alopecia), nail brittleness, irritability, fatigue, and, in more severe cases, respiratory issues such as pulmonary edema and bronchitis (Qiao et al., 2022; Takahashi et al., 2020; H. Wang et al., 2024).

1.3 Selenium bulk versus nano-state

Although selenium is essential for biological activities, bulk selenium is reported to possess notable drawbacks, including reduced bioavailability, toxicity at elevated dosages, and restricted cellular absorption. These demerits can be overcome by the selenium nanoparticles, which are a more effective and safer alternative. Bulk selenium has low bioavailability, resulting in suboptimal use and requiring increased dosages for therapeutic efficacy. This inefficiency might decrease its efficacy in disease prevention and therapy (Hatfield et al., 2014). Excessive selenium in its bulk form can lead to selenosis, characterized by neurological damage, alopecia, and gastrointestinal issues. The limited therapeutic spectrum of bulk selenium compounds limits dosage control (Hatfield et al., 2014). Bulk selenium compounds, like sodium selenite and selenate, possess a limited therapeutic window, characterized by a narrow margin between efficacious and hazardous dosages.

Selenium, like other materials, exhibits size and shape effects when reduced to the nanoscale. As the size of the material, in this case selenium, diminishes, fewer energy levels are needed to accommodate all the electrons of each selenium atom compared to the bulk state. As the energy levels are divided between the ground state and the Fermi level, a reduction in the number of needed energy levels results in a rise in the energy gap, δ, between succeeding energy levels. If the level spacing, δ, exceeds the thermal energy, kT, quantum confinement can be detected. Selenium is a semiconductor with an energy bandgap of 1.79 eV at the bulk level. Nevertheless, it demonstrates a significantly greater value at the nanoscale, contingent upon the size of the nanoparticle (Chaudhary et al., 2016).

Most pollutants consist of dyes utilized in the textile, cosmetic, culinary, and pharmaceutical industries. In the photocatalytic degradation of various dyes, when light irradiates a solution containing SeNPs and dye, the energy is absorbed by the SeNPs (Barani et al., 2023; Chaudhary et al., 2016). Consequently, energy absorption facilitates the transition of an electron (e−) from the valence band (VB) to the conduction band (CB), concurrently generating holes (h+) in the valence band to facilitate the reduction or oxidation of organic molecules. Both produced electrons (e−) and holes (h+) contribute to the photocatalytic process (Barani et al., 2023).

Green synthesized SeNPs with *Moringa olifera* extract showed a bandgap energy exceeding that of bulk α-Se (2.0 eV) and commercial Se powder (1.8 eV), attributable to the quantum size effect (Hassanien et al., 2019). SeNPs can degrade SY dye under both UV and sun exposure (Hassanien et al., 2019). Rohdamine (RhB) is a cationic dye characterized by its great solubility in water. It is very carcinogenic, mutagenic, and neurotoxic. RhB was shown to be degraded

viz., biogenic SeNPs, where the nanoparticles acted as a photocatalyst along with H_2O_2 (Che et al., 2017). Fuchsin dye is a cationic dye classified under the triarylmethane category. It is combustible and utilized in the dyeing of fabrics, leather, cotton, and similar materials. Fuchsin, similar to the aforementioned dyes, has excellent stability and is non-biodegradable.

Apart from the photocatalytic application, there is another significant distinction between bulk selenium and SeNPs, which lies in their immunomodulatory action. Selenium is recognized for its impact on immune function, including T-cell activation, cytokine synthesis, and the augmentation of natural killer (NK) cell activity. Recent investigations indicate that SeNPs demonstrate more powerful immunomodulatory effects compared to bulk selenium (Chen et al., 2022). Selenium nanoparticles have been demonstrated to augment vaccination effectiveness by stimulating antibody production and T-cell responses (Chen et al., 2022). Besides their anticancer and immunomodulatory uses, SeNPs have significant antibacterial efficacy. Although bulk selenium demonstrates restricted antibacterial properties, selenium nanoparticles may effectively suppress the proliferation of several bacterial, fungal, and viral pathogens. Their antimicrobial methods including the destruction of microbial membranes, disruption with DNA replication, and the development of oxidative stress. The elevated surface reactivity of SeNPs facilitates direct interaction with microbial structures, resulting in rapid microbial cell death (Wadhwani et al., 2017). This feature facilitates novel applications of SeNPs in wound healing, medical device coatings, and infection prevention.

Since SeNPs offer a credible solution to the narrow therapeutic window shown by bulk selenium. Engineering functionalized SeNPs for drug delivery to specific sites helps to improve treatment results by lowering off-target effects or systemic toxicity (Li & Xu, 2020). Their capacity for conjugating with biomolecules guarantees exact aimed of cancerous cells, therefore improving the effectiveness of chemotherapy and reducing adverse effects (Li & Xu, 2020). Selenium nanoparticles represent a significant advancement in human health applications compared to bulk selenium. Their superior bioavailability, reduced toxicity, enhanced antioxidant properties, and targeted delivery mechanisms provide them ideal candidates for various therapeutic interventions. (Bisht et al., 2022).

Therefore, despite bulk selenium being an essential mineral, its limitations necessitate the synthesis and application of its selenium nanoparticles to achieve optimal therapeutic efficacy. A thorough exploration of the comprehensive potential of SeNPs in therapeutic settings and their long-term safety is essential.

2.0 Review of literature

2.1 Selenium as an element

Selenium (Se) is a trace element vital for both humans and animals. It is extensively present in diverse tissues and organs of both. Selenium is intricately linked to human health and plays a role in regulating several physiological activities. Selenium was initially identified by the Swedish scientist Berzelius in 1817 inside the leftovers of sulfuric acid production and was formerly regarded as a harmful element. In 1957, Schwarz and Foltz established that selenium is an essential element for mammals (Schwarz and Foltz, 1957). Selenium is a chalcogenide of group 17 in the periodic table which is a microelement as mentioned critical of human vital functions. It is crucial for maintaining several physiological processes; hence, interest in Se research has increased significantly in numerous domains aimed at enhancing public health over the past several decades. The biological role of selenium (Se) pertains to its integration into proteins essential for metabolism via selenocysteine (SeCys). Although being one of the most critical elements, Se cannot be extracted due to its limited availability in the earth's crust. Its crustal abundance is 0.130 ppm. Thus, selenium is predominantly extracted as a by-product of copper refining. The predominant minerals that contain selenium are copper selenides, including Klockmannite (CuSe), Berzelianite (Cu_2Se), Bellidoite (Cu_2Se), Umangite (Cu_3Se_2), and Athabascaite (Cu_5Se_4). The unequal distribution of copper ores globally results in limited availability to selenium from only a few nations.

Selenium is a solid at ambient temperature but transitions to a liquid state over 221°C. It is chemically stable and does not undergo oxidation at ambient temperature. Indeed, selenium has a lower affinity for oxygen in comparison to sulfur. There are only two recognized kinds of selenium oxides: selenium dioxide (SeO_2) and selenium trioxide (SeO_3). Selenium dioxide is produced from the burning of selenium in the presence of air. This molecule exhibits chemical stability yet dissolves in water, yielding selenous acid (H_2SeO_3). Under standard temperature and pressure, selenium manifests in two forms: amorphous (orange, red) and trigonal (grey). Selenium is a direct bandgap semiconductor with an energy bandgap in the visible spectrum (3.2–1.6 eV), hence demonstrating photovoltaic properties, enabling the direct conversion of

light into power. Furthermore, selenium exhibits photoconductive properties, since its electrical resistance diminishes with heightened light.

2.2 Selenium in its nanoform

75 Selenium (Se) exists in both inorganic forms (selenite and selenate) and organic forms (selenomethionine and selenocysteine), contributing to its chemical ambiguity. According to Zhu et al. , naturally occurring selenium can be classified based on its physical properties into crystalline and amorphous forms (Zhu et al., 2019).

Crystalline selenium includes *monoclinic selenium* (m-Se) and *trigonal selenium* (t-Se). The monoclinic form consists of Se_8 rings and appears in three allotropes: alpha (α), beta (β), and gamma (γ), each varying in structural stability. Among these, trigonal selenium (t-Se) is the most thermodynamically stable. Tutihasi et al. described t-Se as comprising infinite helical chains of selenium atoms. Strong covalent bonds hold these chains together, while weak van der Waals forces connect adjacent chains, resulting in its characteristic black colour, high electrical conductivity, and remarkable photoconductive properties traits that make it highly suitable for photovoltaic applications (Tutihasi and Chen, 1967).

In contrast, amorphous selenium (a-Se) lacks a defined crystalline structure. It exists in red, black, and vitreous forms, characterized by a globular, non-crystalline morphology. As Oliveira et al. noted, this structure exhibits lower cytotoxicity compared to crystalline selenium, making a-Se a more biocompatible option for medical and biological applications, such as X-ray photodetectors, due to its excellent optical properties (Oliveira et al., 2025).

The emergence of nano-selenium (Nano-Se) has further expanded selenium's functional potential. In its nanoform, selenium predominantly exists in its zero-oxidation state (Se^0), which exhibits lower toxicity and higher bioavailability compared to its oxidized counterparts (Se^{4+} and Se^{6+}). Selenium nanoparticles (SeNPs) demonstrate a range of advantageous properties, including enhanced biological activity, improved dispersibility, high surface area, and superior bioavailability. These features distinguish them significantly from bulk selenium, spurring growing interest in nanotechnology-driven selenium research.

However, SeNPs are inherently unstable and prone to transition into inactive states (Abadi et al., 2023; J. L. de Oliveira et al., 2014). To address this, researchers have developed encapsulation strategies using suitable capping agents to improve nanoparticle stability.

Biologically, SeNPs have shown promising antioxidative potential. Kojouri et al. reported that SeNPs improve cellular respiratory activity more effectively than sodium selenite, highlighting their superior antioxidant capabilities at the cellular level (Kojouri & Sharifi, 2013). In the monograph *Nanotoxicology: From In Vitro and In Vivo Research Models to Health Risks* (2009), Jinsong Zhang underscored SeNPs as the safest modern selenium compound, despite general safety concerns surrounding nanomaterials (J. Zhang et al., 2005).

Chemically, SeNPs are low-reactive, zero-valent selenium, and are often efficiently excreted from the body after ingestion (Y. Huang et al., 2021). Their dual characteristics, which share both organic and inorganic selenium benefits, enable them to support immunomodulation, anti-aging, and overall metabolic function.

In a comparative toxicity study, Zhang et al. found that selenite compromised liver catalase and superoxide dismutase activity in mice, whereas SeNPs exhibited no such adverse effects, reinforcing their biocompatibility and reduced toxicity (J. Zhang et al., 2005)

2.3 Synthesis routes for SeNPs

Nanoparticles play an important role in modern therapeutics by reducing toxicity, enhancing bioactivity, and enabling controlled and targeted release of active compounds. Among inorganic nanoparticles, selenium nanoparticles (SeNPs) have attracted considerable attention due to their unique bioactive properties at the nanoscale. This makes their synthesis particularly important for biomedical applications. SeNPs can be synthesized through a variety of methods, including chemical, physical, and biological approaches, each offering specific advantages with respect to nanoparticle stability, morphology, size control, and functionalization.

2.3.1 Chemical route for SeNP synthesis

Chemical synthesis of selenium nanoparticles (SeNPs) primarily involves reduction reactions that convert selenium salts into zero-valent selenium (Se^0), typically stabilized by various capping agents. This approach encompasses several techniques, including chemical reduction with inorganic and organic reducing agents, physicochemical methods, electrochemical processes, and stabilization using diverse compounds (Bisht et al., 2022; Ijaz et al., 2020; Tyagi et al., 2016)

One of the earliest reported methods utilized a wet chemical process, where sodium selenosulfate served as the selenium precursor and polyvinyl alcohol (PVA) acted as the stabilizer, yielding spherical SeNPs ranging from 76 to 150 nm in size (Menazea et al., 2020).

Advancements in chemical reduction introduced glucose as both a reducing and capping agent, enabling the synthesis of highly uniform SeNPs at elevated temperatures (~115 °C). These glucose-capped nanoparticles were later evaluated for their pro-apoptotic effects in cancer cells (Nie et al., 2016).

To enhance biocompatibility and minimize toxicity, natural reducing agents such as glucomannan and chitosan have been employed, demonstrating lower cytotoxicity compared to synthetic stabilizers (Biswas et al., 2018; X. Cheng et al., 2014). Similarly, ascorbic acid has been used in solution-phase synthesis, yielding stable, monodispersed colloidal SeNPs when stabilized with polysaccharide-based capping agents, including chitosan, konjac glucomannan, acacia gum, and carboxymethyl cellulose (Song et al., 2022). The resulting nanoparticles have shown excellent stability, making them promising candidates for biomedical applications.

Other notable precursors, such as selenium dioxide, have also been utilized to synthesize spherical SeNPs (~13 nm) in ice-cold conditions, with polyvinylpyrrolidone (PVP) as the stabilizer. These PVP-coated SeNPs have demonstrated anti-inflammatory and analgesic properties in irradiated animal models (El-Ghazaly et al., 2016).

Further innovations include the use of glutathione as a reducing agent under alkaline conditions (pH 8.48), with bovine serum albumin (BSA) as a capping agent to prevent nanoparticle aggregation. These BSA-stabilized SeNPs exhibited selective cytotoxicity toward cancer cells, sparing normal cells, and thus represent a promising tool for targeted cancer therapy(Adam-Dima et al., 2024).

Incorporation of green chemistry principles into chemical synthesis has led to the use of starch derivatives as natural stabilizing agents. These "green-capped" SeNPs displayed outstanding colloidal stability at room temperature, with no signs of aggregation over time (Baluken et al., 2024; Ying et al., 2022).

While most chemical synthesis methods yield amorphous SeNPs, specific biomedical and material science applications require crystalline forms. For example, trigonal selenium clusters have been synthesized via solvothermal methods, wherein initially amorphous nanoparticles undergo structural rearrangement along the c-axis, forming hexagonal rods. This unique butterfly-like morphology is attributed to steric hindrance and divergent lateral edge growth patterns during transformation (K. Zeng et al., 2013).

In general, sodium selenite remains one of the most commonly used selenium salts in chemical reduction methods. Nanoparticle size and morphology are typically regulated using surfactants or growth-inhibiting agents such as polyvinylpyrrolidone and folic acid, allowing for greater control over particle functionality and application specificity.

2.3.2 Physical route for SeNP synthesis

Physical synthesis methods provide an alternative to chemical approaches by utilizing mechanical or energy-based processes to produce selenium nanoparticles (SeNPs) without the need for chemical reducing agents. These methods are especially valued for generating high-purity nanoparticles, thereby eliminating the risk of chemical contamination and residual toxicity. Key physical synthesis techniques include laser ablation, microwave irradiation, hydrothermal processing, and ultrasonication.

Among these, laser ablation is one of the most extensively studied techniques. It involves directing a high-energy laser beam at a solid selenium target submerged in a liquid medium (typically water). The intense energy input vaporizes the selenium, which then condenses into nanoparticles upon cooling. Parameters such as laser wavelength, pulse duration, power density, and ambient gas conditions can be fine-tuned to control the size, stability, and morphology of the resulting nanoparticles (Baimler et al., 2024).

Studies have shown that exposing selenium pellets to a high-repetition-rate laser beam for specific time intervals leads to the formation of stable colloidal SeNP solutions (Guisbiers et al., 2016). Further refinements, such as the use of conical cuvettes, have been employed to reduce water consumption and prevent solvent evaporation during irradiation, improving process efficiency.

The laser ablation technique has also been applied in the fabrication of selenium-doped nanocomposites, where SeNPs are embedded within polyvinyl alcohol-chitosan matrices to enhance antibacterial activity. Notably, solvent selection significantly influences the final morphology of SeNPs: non-polar solvents tend to yield elongated crystalline nanorods, whereas polar solvents favor the formation of spherical amorphous nanoparticles (Baimler et al., 2024)

Another promising method is microwave irradiation, which offers rapid and uniform heating, resulting in faster synthesis compared to conventional thermal methods. In this approach, selenous acid is reduced in the presence of amino acid-based capping agents, producing a range of nanostructures such as nanoballs, nanotubes, and multi-armed nanorods. The size and shape

of the synthesized SeNPs are largely dependent on the precursor concentration, type of capping agent, and microwave exposure time (Zambonino et al., 2021).

Hydrothermal synthesis and ultrasonication have also been effectively employed to produce SeNPs with controlled morphology. Hydrothermal methods involve high-temperature and high-pressure conditions to facilitate nucleation and directional growth. For instance, using glucose as a stabilizing agent, selenium nanorods with diameters of 200–300 nm and lengths up to 3 µm have been successfully synthesized (Cao et al., 2011). The integration of hydrothermal processing with microwave-assisted synthesis has further enabled the development of selenium-based hydrogels, particularly for wound healing applications.

Ultrasonication, another physical technique, employs acoustic cavitation to initiate nanoparticle formation. When combined with green extraction methods, in which plant-derived bioactive compounds serve as reducing and stabilizing agents, ultrasonication enhances both the biocompatibility and stability of SeNPs. This synergistic approach is gaining traction for environmentally sustainable nanoparticle synthesis (Ikram et al., 2021)

2.3.3 Biogenic synthesis for SeNPs

Biogenic synthesis has emerged as a sustainable and environmentally friendly alternative to traditional chemical and physical methods for producing selenium nanoparticles (SeNPs). This approach leverages biological systems including plant extracts, bacteria, fungi, and algae to synthesize SeNPs under mild, and non-toxic conditions (Ghosh et al., 2021). A key advantage of biogenic synthesis lies in its ability to yield biodegradable, cost-effective, and inherently biocompatible nanoparticles, often naturally encapsulated in organic compounds that enhance colloidal stability and functionality (Zambonino et al., 2021)

Among biogenic routes, microbial synthesis has been extensively studied. Various bacterial strains, such as *Bacillus paramycoides*, have been employed to reduce selenite (Se^{4+}) into elemental selenium nanoparticles (Se^0) under controlled pH conditions. These bio-synthesized SeNPs have exhibited potent antibacterial activity against pathogens including *Staphylococcus aureus* and *Escherichia coli*, with minimum inhibitory concentrations (MICs) typically ranging between 400 and 600 µg/mL.

Plant-mediated synthesis represents another widely researched avenue, where phytochemicals extracted from seeds, leaves, or bark act as both reducing and stabilizing agents. Leaf extracts from various medicinal plants have been successfully used to synthesize SeNPs with notable

antibacterial and anticancer properties, highlighting the therapeutic potential of this green synthesis route (Cittrarasu et al., 2021; Ikram et al., 2021)

Innovative strategies such as microbial electrochemical systems have also been developed, wherein bacteria like *Shewanella spp.* facilitate selenium reduction via bioelectrochemical reactions (Ho et al., 2021) Additionally, enzymatic pathways have been identified in bacterial species such as *Bacillus mycoides*, where thiol-based mechanisms are implicated in the reduction and nucleation of selenium nanoparticles (Lampis et al., 2014). These discoveries have deepened our understanding of microbial nanoparticle assembly, revealing the involvement of specific proteins and redox enzymes in selenium metabolism and nanoparticle formation.

Collectively, biogenic synthesis offers a green, scalable, and biocompatible platform for the production of SeNPs, with promising implications for biomedical, environmental, and industrial applications.

2.4 Bioactive Potential of Selenium Nanoparticles (SeNPs)

Selenium nanoparticles (SeNPs) possess a wide array of bioactive properties, making them highly promising in the field of biomedicine. Their ability to function as antioxidants enables them to combat oxidative damage caused by free radicals, while their anticancer activity positions them as potential agents in cancer therapy. Additionally, SeNPs exhibit broad-spectrum antimicrobial effects, effectively inhibiting the growth of various pathogens (T. Huang et al., 2019; Jamdagni et al., 2018). They have also shown potential as antidiabetic agents, improving glucose metabolism and insulin sensitivity.

Beyond therapeutic applications, SeNPs are being explored for use in nano-biosensor development, wastewater treatment, and bioremediation(Das et al., 2022; Eswayah et al., 2016). Moreover, their ability to neutralize heavy metals by antagonizing their toxic effects highlights their potential in environmental detoxification strategies.

2.4.1 Antioxidant Property of SeNPs

3 Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive molecules generated during metabolic and biochemical processes in the human body. Although 27 they play roles in cellular signalling, their excessive accumulation leads to oxidative stress, which contributes to cellular damage and the progression of chronic diseases (Rana, 2021).

Antioxidants play a vital role in neutralizing these reactive species and maintaining redox balance.

They play a significant role in scavenging free radicals generated during metabolic events, including reactive oxygen and nitrogen species, therefore safeguarding cells from oxidative stress and damage. In comparison to other selenium species, SeNPs can enhance selenoenzyme activities with comparable efficacy and less toxicity. The size and shape of selenium nanoparticles (SeNPs) significantly influence their antioxidant characteristics; specifically, smaller SeNPs have enhanced antioxidant activity (P. Liu et al., 2025).

Several studies have confirmed the antioxidant capacity of SeNPs. For instance, Battin et al. demonstrated that SeNPs significantly reduced free radical levels, thereby preventing oxidative DNA damage in both *in vivo* and *in vitro* systems (Battin et al., 2011). Their study offered mechanistic insights, suggesting that metal coordination plays a key role in determining the antioxidant efficacy of selenium compounds.

The antioxidant action of SeNPs is achieved by a twofold mechanism: direct scavenging of reactive oxygen species (ROS) and regulation of endogenous antioxidant mechanisms. *In vitro* investigations have shown that SeNPs may directly neutralize free radicals, including DPPH (2,2-diphenyl-1-picrylhydrazyl), $ABTS^{+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), superoxide anions, and hydroxyl radicals. Their elevated surface area to volume ratio markedly amplifies their interaction with free radicals, hence improving radical scavenging effectiveness relative to bulk selenium. For instance, involving carbon tetrachloride-induced hepatic injury in rats, SeNPs treatment resulted in a marked reduction of malondialdehyde (MDA) levels, a key indicator of lipid peroxidation, and simultaneously restored glutathione (GSH) levels, thereby ameliorating oxidative stress (Ebaid et al., 2021).

SeNPs are also been known to be involved in the regulation of redox-sensitive signalling pathways that influence antioxidant responses at the transcriptional level. The factor erythroid 2–related factor 2 (Nrf2) pathway is a crucial mechanism that regulates the antioxidant gene expression. Upon activation, Nrf2 translocates to the nucleus and interacts with antioxidant response elements (AREs), enhancing the expression of genes including GPx1, TrxR1, HO-1, and NQO-1. In spinal cord injury models, epigallocatechin gallate (EGCG)-functionalized selenium nanoparticles (SeNPs) markedly decreased reactive oxygen species (ROS) levels, enhanced mitochondrial activity, and activated the Nrf2 pathway, resulting in increased neural survival and functional recovery (H. Zhou et al., 2024). Further evidence was provided by Xu

et al., who examined the protective effects of biogenic SeNPs synthesized by *Lactobacillus casei* ATCC 393 on the intestinal epithelial barrier. Their findings revealed that SeNPs mitigated ROS-induced mitochondrial dysfunction via activation of the Nrf2 signalling pathway. The upregulation of key antioxidant proteins such as Nrf2, HO-1, and NQO-1 suggested that SeNPs promote intrinsic cellular defense mechanisms (Xu et al., 2019). These findings position SeNPs as promising candidates for managing oxidative stress.

SeNPs demonstrate dose-dependent redox activity. At low doses, they function as antioxidants by enhancing cellular defenses and neutralizing reactive oxygen species (ROS). At elevated concentrations or in malignant situations, they may function as pro-oxidants, inducing apoptosis through reactive oxygen species production and the activation of mitochondrial and p53 pathways (Qiao et al., 2022). This selective mechanism creates an intriguing therapeutic opportunity wherein SeNPs save normal cells from oxidative damage while specifically provoking oxidative stress in cancer cells, so functioning as both antioxidant and anticancer agents.

In a related study, Krishnan et al. investigated a novel formulation involving selenium nanoparticles (Sh-SeNPs) synthesized from the medicinal plant *Spermacoce hispida*, conjugated with s-allyl glutathione (SAG), a glutathione analog known for its hepatoprotective effects. The resulting SAG-Sh-SeNPs were evaluated in a rat model of acetaminophen (APAP)-induced liver and kidney toxicity (Krishnan et al., 2019). Treatment with these nanoparticles preserved the morphological integrity of hepatic and renal tissues, significantly reducing oxidative stress and restoring metabolic balance. Notably, the synergistic interaction between SAG and SeNPs offered greater protection than SeNPs alone, suggesting enhanced therapeutic potential than the bipotent capping agent. The structural and surface properties of SeNPs affect their antioxidant efficacy. Smaller nanoparticles often demonstrate elevated surface energy and increased reactivity, hence augmenting their ROS scavenging efficacy. Furthermore, surface modifications utilizing biocompatible polymers such as chitosan, dextran, or PEG, as well as natural antioxidants like flavonoids, enhance dispersion and bioavailability while also amplifying antioxidant effects (Abadi et al., 2023).

Thus, SeNPs exhibit potent antioxidant activity through their ability to scavenge free radicals and enhance endogenous antioxidant enzyme systems. Their low toxicity, high bioavailability, and multi-targeted action make them appealing candidates for preventing and managing oxidative stress-related pathologies.

2.4.2 Anti-microbial properties of SeNPs

The rise of multidrug-resistant (MDR) bacteria and fungi represents a pressing global health challenge, necessitating the development of novel and effective antimicrobial agents. In this context, selenium nanoparticles (SeNPs) have emerged as promising candidates due to their broad-spectrum antimicrobial activity and low cytotoxicity. Their efficacy is influenced by several factors, including particle size, surface chemistry, and the synthesis method employed (Sans-Serramitjana et al., 2023). SeNPs' unique efficacy stems from a blend of direct microbial cell damage, inhibition of virulence mechanisms like biofilm formation, and synergistic interactions with conventional antibiotics. Distinct advantages of SeNPs include reduced toxicity compared to inorganic selenium salts and improved bioavailability when functionalized, leading to a surge of interest in their therapeutic and industrial applications (Filipović et al., 2021)

The primary antibacterial mechanism of SeNPs involves biofilm disruption and inhibition of microbial growth. Pathogenic microorganisms often resist antibiotics through protective barriers such as the cell wall and membrane, which limit drug penetration. SeNPs, particularly through selenium ion release, can compromise cell wall integrity and increase membrane permeability, leading to ionic imbalance, disruption of intracellular processes, and ultimately, cell death.

Although elemental selenium was once considered biologically inert, advances in nanotechnology have enabled the synthesis of SeNPs with enhanced bioactivity. Huang et al. investigated the size-dependent antibacterial effects of SeNPs, finding that particles approximately 81 nm in diameter exhibited maximum inhibitory activity against both methicillin-sensitive (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Huang et al., 2019). Mechanistic studies revealed that SeNPs induce ATP depletion, reactive oxygen species (ROS) generation, and disruption of membrane potential, ultimately leading to bacterial cell death. Importantly, these SeNPs showed low cytotoxicity in mammalian cells at concentrations up to 25 µg/mL, indicating a favourable safety profile (Huang et al., 2019).

Another antimicrobial approach attributed to SeNPs can be due to their dual pro-oxidant and antioxidant nature. SeNPs can lead to the generation of reactive oxygen species (ROS) within microbial cells. SeNPs can increase intracellular ROS levels, leading to oxidative stress that damages DNA, lipids, and proteins. The investigation, including *Providencia*-derived SeNPs

recorded elevated ROS production, which was associated with bacterial mortality (H. Zhang et al., 2021).

Another way to use nanoparticles, specifically SeNPs, is to employ them in conjugation with commercial drugs to increase the initial drug's bioactivity. SeNPs can enhance antibiotic efficacy, frequently enabling dosage reductions that mitigate adverse effects and evade resistance. The linezolid stabilized SeNP experimentation demonstrated synergistic effects against MRSA, resulting in increased protein breakdown and bacterial suppression. Functionalized SeNPs improve efficacy when utilized as coatings on medical surfaces; for example, SeNP-coated PVC outperformed Ag-coated PVC in inhibiting *S. aureus* colonization (Han et al., 2021). In a similar study, Chung et al. synthesized BSA-stabilized SeNPs measuring under 40 nm, which demonstrated potent antibacterial effects against *S. aureus*. These nanoparticles exhibited IC_{50} values significantly lower than those affecting human dermal fibroblasts, underscoring their selectivity and safety (Chung et al., 2020). To enhance target specificity, SeNPs have been conjugated with microbial targeting ligands, such as in the development of Se-MSNPs (selenium-conjugated MRSA-targeting nanoparticles). These conjugates significantly enhanced antibacterial efficacy against MRSA, compared to unmodified SeNPs, which showed limited activity against *E. coli*.

The surface chemistry of SeNPs also plays a crucial role in modulating both antimicrobial efficacy and cytocompatibility (Kaushal et al., 2023). Filipović et al. evaluated SeNPs stabilized with bovine serum albumin (BSA), chitosan, and glucose, all of which exhibited activity against *Candida albicans*. Notably, BSA-coated SeNPs showed lower cytotoxicity toward mammalian cells, highlighting the importance of surface modification strategies to optimize biocompatibility without compromising antimicrobial performance (Filipović et al., 2021). Another approach employed by SeNPs in antimicrobial effects is the disruption of quorum sensing among the bacterial cells. Selenium nanoparticles (SeNPs) have been shown to interfere with quorum sensing (QS) which is an essential bacterial chemical communication necessary for regulating virulence and biofilm development via several ways. Molecular docking experiments with *Pseudomonas aeruginosa* shown that SeNPs directly interact with essential QS regulators LasI, LasR, RhlI, RhlR, and MvfR (Gómez-Gómez et al., 2019). These interactions probably disrupt acyl-homoserine lactone (AHL) production and receptor activation, hence diminishing the transcription of virulence factors regulated by quorum sensing (QS) (Gómez-Gómez et al., 2019).

In line with environmentally sustainable practices, green synthesis methods have been explored to enhance the antimicrobial potential of SeNPs. Hernández-Díaz et al. synthesized SeNPs using *Tagetes erecta* (marigold) extracts, which demonstrated strong antibacterial activity against pathogens such as *Serratia marcescens, Enterobacter cloacae*, and *Alcaligenes faecalis* (Hernández-Díaz et al., 2021). These biogenic SeNPs also exhibited antioxidant properties, suggesting additional therapeutic value in managing oxidative stress-related infections.

2.4.3 Health related properties of SeNPs

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from insufficient insulin production or impaired insulin utilization. Over the past few decades, the global prevalence of diabetes has surged dramatically, with an estimated 463 million individuals affected as of 2019 (Borgnakke, 2019). SeNPs have been explored as a potential therapeutic intervention for diabetes due to their role in regulating blood glucose levels.

Several studies have demonstrated the efficacy of SeNPs in diabetes management. Deng et al. developed insulin-loaded selenium nanoparticles (INS-SeNPs) using ionic cross-linking and *in situ* reduction techniques to facilitate oral insulin delivery (Deng et al., 2017). The study found that these SeNPs effectively overcame the absorption barrier, improving insulin bioavailability. Similarly, Zeng et al. reported that chitosan-capped SeNPs exhibited significant antidiabetic effects in streptozotocin-induced diabetic rats, further supporting their potential as a therapeutic agent (Zeng et al., 2018). The study primarily used *in vitro* assays to assess the antidiabetic capabilities of the nanoparticles rather than their mechanistic approach. The following study provides initial comprehension but, they cannot precisely replicate the complex interactions observed in a living organism.

In another prominent study, the mechanistic basis of SeNPs' anti-diabetic action has been extensively studied. Abdulmalek et al. demonstrated that SeNPs enhance insulin sensitivity by upregulating critical proteins involved in insulin signalling, including phosphorylated insulin receptor substrate-1 (pIRS-1), protein kinase B (pAKT), and glycogen synthase kinase-3β (pGSK-3β) in diabetic rat models. These molecular interactions enhance glucose uptake and metabolic control (Abdulmalek & Balbaa, 2019). Gutiérrez et al. further explored the antioxidant and anti-inflammatory effects of SeNPs in diabetes management, showing that luteolin- and diosmin-capped SeNPs reduce oxidative stress by increasing the activity of key antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione

peroxidase (GPx) (Gutiérrez et al., 2022). This reduction in oxidative stress is associated with decreased inflammation, improved insulin sensitivity, and enhanced pancreatic β-cell function (Gutiérrez et al., 2022).

Cancer is characterized by the uncontrolled proliferation of abnormal cells, leading to tumor formation and, in many cases, metastasis. Genetic and epigenetic alterations, including histone modifications and increased ROS levels, play a critical role in cancer progression (Kumar & Prasad, 2021). Due to their unique redox properties, SeNPs have been extensively studied for their anticancer potential.

SeNPs exert anticancer effects through multiple mechanisms, including ROS generation, disruption of mitochondrial function, caspase activation, apoptotic protein regulation, cell cycle arrest, and modulation of antioxidant defences (Ahmad et al., 2015; Luo et al., 2012; Yu et al., 2012). The induction of apoptosis is the most widely studied anticancer mechanism of SeNPs. Varlamova explored the molecular mechanisms of SeNPs in hepatocellular carcinoma, demonstrating their ability to trigger programmed cell death in tumor cells (Varlamova, 2024).

Recent research by Vafadar et al. demonstrated the fabrication of selenium and copper-based nanocomposites (Se-CuNPs) utilizing salicylic acid and hyaluronic acid (Vafadar et al., 2025). These nanocomposites had inhibitory effects on the growth of the HepG2 cell line. The research indicated that CD44 overexpression is present in hepatocellular cancer cells (Vafadar et al., 2025). Consequently, hyaluronic acid, a key component in the fabricated selenium/copper nanocomposite, exhibited effective and selective targeting of these receptors. This further enables the precise delivery of the therapeutic payload to liver cancer cells, optimizing effectiveness and reducing harm to healthy tissue. The research illustrates the cytotoxic properties of Se-CuNPs on HepG2 cells, and offers few details on the modes of action involved. Comprehending the precise mechanisms by which these nanoparticles induce cytotoxicity or impede cellular growth is essential for enhancing their design and therapeutic use. Ferro et al., further demonstrated that BSA-capped SeNPs exhibit selective cytotoxicity against cancer cells while sparing normal human dermal fibroblasts (Ferro et al., 2025). Additionally, the combination of BSA-SeNPs with a nano-vaccine led to a marked reduction in tumor size in an EO771 breast cancer murine model, highlighting their potential for cancer immunotherapy.

In addition to their antibacterial and anticancer properties, SeNPs demonstrate antiparasitic action against *Leishmania* spp. and antifungal activity against *Candida*, *Aspergillus*, and dermatophytes. Biogenic SeNPs originating from *Bacillus* species have significantly reduced

Leishmania promastigotes and amastigotes *in vitro* and demonstrated considerable scolicidal effectiveness (Beheshti et al., 2013).

The unambiguous proof of the bioactive potential of selenium nanoparticles renders them a desirable subject for investigation, particularly through cost-effective synthesis methods, especially green synthesis techniques.

2.5 Selenium Nanoparticles (SeNPs): Emerging Applications in Therapeutics and Beyond

Selenium nanoparticles (SeNPs) have attracted significant interest due to their broad-spectrum applications in medicine, particularly in drug delivery, diagnostics, and therapeutic interventions (Abdillah et al., 2021). Their lower toxicity compared to conventional selenium compounds enhances their viability as potent antioxidants and bioactive agents (Bisht et al., 2022; Mikhailova, 2023).

One of the most promising therapeutic applications of SeNPs lies in targeted drug delivery systems. For instance, Luo et al. research group developed a light-responsive drug delivery platform using selenium-infused polymers synthesized with metal-organic frameworks (MOFs) (Luo et al., 2019). They employed redox-cleavable di-(1-hydroxylundecyl) selenide to create a selenium-containing polymer, which was then integrated with porphyrin-based zirconium MOFs to form shell-core nanocomposites. Upon light irradiation, the porphyrin component generated reactive oxygen species (ROS), triggering the cleavage of the selenium polymer and releasing the chemotherapeutic drug doxorubicin (DOX). This system supports combined photodynamic and light-responsive chemotherapy, offering a precise and controlled therapeutic approach.

In medical-device applications, Li et al. engineered a multifunctional antimicrobial coating through UV-induced crosslinking of an HPA-BPA copolymer (Li et al., 2023). This coating effectively prevented protein adsorption and bacterial colonization, thereby reducing biofouling. Its one-pot synthesis and robust adhesion to substrates make it suitable for practical deployment on catheters, implants, and other medical surfaces, potentially lowering infection rates and improving clinical outcomes.

In the context of infectious diseases, SeNPs have also demonstrated diagnostic utility. Wang et al. developed a SeNP-based lateral flow immunoassay capable of simultaneously detecting anti-SARS-CoV-2 IgM and IgG antibodies (Wang et al., 2020). The test delivered clear results within 10 minutes, with detection thresholds of 20 ng/mL for IgM and 5 ng/mL for IgG.

Clinical validation involving 90 COVID-19 patients and 263 negative controls revealed excellent diagnostic performance, achieving 93.33% sensitivity and 97.34% specificity compared to RT-PCR and clinical diagnosis. Notably, the assay showed no cross-reactivity with common interfering substances and retained stability after 30 days at 37 °C, making it a promising point-of-care tool for rapid and economical COVID-19 serological testing.

Beyond diagnostics, SeNPs enhance the delivery and bioavailability of various therapeutic agents, including peptides, proteins, vaccines, and hydrophilic drugs. Their nanoscale size and tunable surface characteristics enable efficient cellular uptake and improved interaction with biological membranes, leading to higher therapeutic efficacy (Shirazi et al., 2025). Additionally, their intrinsic antioxidant properties allow them to neutralize ROS, protecting both the therapeutic cargo from oxidative degradation and mitigating oxidative stress implicated in various pathologies.

Despite these advantages, concerns around toxicity remain critical. While SeNPs are generally considered safer than other selenium forms, high dosages or prolonged exposure may still pose toxicological risks. Rigorous *in vivo* and *in vitro* assessments are essential to ensure safety before clinical translation. Furthermore, challenges related to the scalability and cost-efficiency of SeNP production must be addressed. Although biogenic synthesis methods offer environmentally friendly alternatives, optimization and standardization are crucial for large-scale manufacturing (Hernández-Díaz et al., 2021).

65 SeNPs also hold substantial promise in modern agriculture. They are known to alleviate biotic and abiotic stresses, such as salinity, drought, heavy metal toxicity, and microbial infections, by enhancing antioxidant enzyme activity and metabolic resilience in plants (Ahmad et al., 2024). As a biofortification agent, SeNPs improve crop yield, seed germination, and nutritional quality, offering a more bioavailable and less toxic form of selenium enrichment compared to inorganic counterparts.

Overall, SeNPs emerge as a versatile nano-platform across diverse domains, including agriculture, environment, and healthcare. However, to fully realize their potential, it is imperative to deepen investigations into their biological interactions, metabolic pathways, and long-term safety. Additionally, the development of cost-effective synthesis strategies will be instrumental in scaling SeNP applications. Reducing dependency on expensive reagents and simplifying production protocols will enhance accessibility and promote the adoption of SeNPs for sustainable and high-impact solutions across multiple sectors (Mikhailova., 2023).

Research Gap / Lacunae

The literature review highlights the considerable therapeutic potential of selenium nanoparticles (SeNPs), with numerous studies showcasing their efficacy in drug delivery, antioxidation, antimicrobial, and anticancer activities. A variety of synthesis methods, including physical, chemical, and biological routes, have been explored, offering insights into cost-effective and scalable production techniques. Notably, green synthesis approaches are gaining momentum due to their eco-friendliness and biocompatibility.

However, a critical gap persists in the use of endophytic fungi as biogenic agents for the green synthesis of SeNPs. While several plant extracts and microbial systems have been studied, the unique biosynthetic capabilities of endophytic fungi remain largely untapped. These organisms possess diverse metabolic pathways that can produce secondary metabolites functioning as natural reducing, capping, and stabilizing agents, potentially enhancing nanoparticle stability and bioactivity more effectively than conventional agents.

Given the growing interest in optimizing SeNPs for biomedical and industrial applications, there is a pressing need to explore novel, sustainable biosynthesis routes that also offer enhanced functional properties.

The study aims to bridge this gap by addressing the following objectives:

1. **Generation of SeNPs using selected fungal strains**
2. **Stabilization of SeNPs using biological and biocompatible agents**
3. **Determination of bioactivities of SeNPs**

This doctoral study was therefore undertaken to synthesize selenium nanoparticles using biocompatible agents derived from endophytic fungi and to comprehensively evaluate their bioactive characteristics.

3.0 Experimental methodology

3.1 Sample collection and isolation

Specimens of *Nerium oleander* were collected from the horticultural section of Thapar Institute of Engineering and Technology (TIET), Patiala, India. Young leaves and bark samples were randomly sampled, placed in sterile vials, and transported to the laboratory for further processing and isolation (Fig 3.1). To remove surface debris, the leaf samples were first rinsed with double-distilled water, followed by surface sterilization using 70% ethanol for 1 minute, immersion in a 4% aqueous sodium hypochlorite solution (Spectrochem, India) for 3 minutes, and a final treatment with 70% ethanol for 10 seconds. The samples were then rinsed thoroughly with double-distilled water and dried under aseptic conditions in a biosafety cabinet. Sterilized leaf segments were placed on potato dextrose agar (PDA) plates supplemented with 1 mg/ml ampicillin (HiMedia, India), sealed with parafilm, and incubated in the dark at 25 ± 5°C until fungal colonies emerged. Hyphal tips from emerging colonies were subcultured to obtain pure isolates. The endophytic fungi were subsequently cultured in potato dextrose broth (PDB) and incubated at 25 ± 5°C in an orbital shaker for 15 to 20 days.

3.2 Molecular characterization of the fungal strain

The molecular identification and sequencing of the screened fungal strain were outsourced and conducted by Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. As per the protocol provided by them, Fungal genomic DNA was isolated using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research). Fungal biomass (50–100 mg) was suspended in phosphate-buffered saline (PBS) and subsequently transferred to a ZR BashingBead™ lysis tube with lysis buffer. Mechanical disruption was done with a bead beater followed by centrifugation at 10,000 x g. The supernatant was then collected and filtered using a spin column. and the DNA was adhered to a silica membrane utilizing the supplied binding buffer. After multiple washing processes, the DNA was eluted in elution buffer and measured using a Nanodrop spectrophotometer. The quality of DNA was assessed using 1% agarose gel electrophoresis. Furthermore, after the DNA isolation, amplification was followed using the Internal Transcribed Spacer (ITS) region of the fungal rRNA gene cluster was amplified using ITS5F (GGA AGT AAA AGT CGT AAC AAG G) and NL4R (5'GGTCCGTTTCAAGACGG 3') primers using polymerase chain reaction (PCR) according to the manufacturer's

instructions. The PCR product was further sequenced, analysed and a phylogenetic tree was done using the neighbor-joining method with a bootstrap value of 1000 hits through Mega 11.0 (Pennsylvania State University, USA).

3.3 Extraction of the secondary metabolites

Once the fungal culture broth was fully developed with mycelial growth, the mycelia were separated using Whatman filter paper. The resulting filtrate was subjected to solvent extraction as described by Sathiyaseelan et al. (Sathiyaseelan et al., 2021). Ethyl acetate was employed to extract the secondary metabolites, and the organic phase was subsequently evaporated to dryness using a rotary evaporator (Heidolph - HiVap Core, Germany). The resulting crude extract was collected as a dry residue. The obtained secondary metabolites were designated as NL(A), NL(B), NL(C), NL(D), NL(E), and NL(F), respectively.

3.4 Screening for anti-microbial potency of the fungal isolate's extracts

The bacterial test culture used in this study was procured from the Microbial Type Culture Collection (MTCC), Chandigarh, for evaluating antimicrobial efficacy. A total of eight bacterial strains and four filamentous fungal strains were employed to assess microbial susceptibility of the fungal extracts i.e., (NL(A) to NL(F)). The bacterial strains were categorized into two groups: four Gram-positive and four Gram-negative. The specific strains used for the antimicrobial efficacy assay are listed below.

- Gram positive strains
  - *Enterococcus faecalis* (MTCC No. 6845)
  - *Bacillus subtilis* (MTCC No. 441)
  - *Listeria spp.* (MTCC No. 4214)
  - *Staphylococcus aureus* (MTCC No. 902)
- Gram negative strains
  - *Escherichia coli* (MTCC No. 448)
  - *Salmonella enterica* (MTCC No. 1165)
  - *Acinetobacter calcoacetius* (MTCC No. 1948)
  - *Serratia marcescens* (MTCC No. 2645)
- Filamentous fungal strains
  - *Aspergillus niger*
  - *Fusarium laterium*

- *Alternaria alternata*
- *Aspergillus flavus*

The antimicrobial screening was conducted using the micro-broth dilution method. Bacterial strains were cultivated in Mueller-Hinton broth (HiMedia Labs, India) and incubated at 37°C until an optical density of 0.1 at 600 nm was reached. A 100 µL aliquot of the test extract was serially diluted in a microtiter plate across a concentration range of 2 to 2000 µg/mL. Subsequently, bacterial inoculum was added to each well to achieve a final volume of 150 µL. Three controls were included in parallel: a culture control (broth with bacterial inoculum), a negative control (broth only), and a positive control (ampicillin with bacterial inoculum). Plates were incubated at 37°C for 24 hours, and microbial growth was assessed by measuring absorbance at 600 nm.

The filamentous fungal strains were sub-cultured from agar slants onto Sabouraud Dextrose Agar (SDA) for fungal susceptibility testing. After 5 to 10 days of growth, spore suspensions were prepared by flooding the culture plates with phosphate-buffered saline (PBS) and gently scraping the surface with a sterile spreader. The suspension was collected in sterile falcon tubes and vortexed to disperse clumps. The suspension was treated with 1 to 2% Tween-20 to prevent spore aggregation. The final spore concentration was adjusted to 3×10^6 spores/mL in Sabouraud Dextrose Broth (SDB). For the assay, 100 µL of the serially diluted extract (2–2000 µg/mL) was added to each well, followed by 100 µL of the prepared spore suspension. The microtiter plates were incubated in the dark at 28°C for 24 to 48 hours, and absorbance was recorded at 530 nm to evaluate fungal growth inhibition.

According to the initial screening of the fungal extracts, NL(E) extract showed antibacterial potential but did not exhibit any antifungal activity, and strain NL(A) displayed no potential antibacterial activity but displayed a selective antifungal activity against *Aspergillus niger*. Whereas NL(C) was able to demonstrate decent inhibitory activity against *A. niger* and *F. laterium*, it also showed a positive result in antibacterial screening for *E. faecalis* and *S. aureus*, *E. coli,* and *S. enterica*. Furthermore, to expand the search for bipotent capping agents for the selenium nanoparticles. EPS, i.e., exopolysaccharide, was also extracted from the NL(C) isolate via chilled ethanol precipitation and dialyzed for the screening procedure. Among the six EPS extracts, only NL(C)'s EPS extract was able to show a positive result in antibacterial screening against three-gram positive strains and two-gram negative strains, for antifungal

screening. This resulted in the NL(C) isolate acting as a bioactive capping agent for the green synthesis of selenium nanoparticles.

3.5 Total phenolic content of selected metabolite extract

The total phenolic content of the screened and selected strain extract was quantified using a colorimetric method based on the Folin-Ciocalteu assay [55]. Briefly, 0.5 mL of diluted NL(C) extract was mixed with 0.2 mL of Folin-Ciocalteu reagent, followed by the addition of 0.2 mL of 20% sodium carbonate (Na_2CO_3). The reaction mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was subsequently measured at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800). Gallic acid was used as the standard reference compound, with a calibration curve prepared using concentrations ranging from 0.05 to 0.5 mg/mL.

3.6 Total flavonoid content of selected metabolite extract

The total flavonoid content of the screened and selected extract (NL(C)) was determined using the aluminium chloride colorimetric method. Briefly, 0.1 mL of NL(C) extract was dissolved in methanol, followed by the addition of 0.1 mL of 10% aluminum chloride ($AlCl_3$) and 0.1 mL of 1.0 M sodium acetate. The mixture was incubated in the dark at room temperature for 45 minutes. After incubation, absorbance was recorded at 415 nm using a UV-visible spectrophotometer. Quercetin was used as the reference standard, with a calibration curve prepared in the concentration range of 0.1–1.0 mg/mL using the same protocol.

3.7 Extraction of exopolymer substance (EPS)

The purified fungal strain was initially cultivated on Modified Melin-Norkans (MMN) medium supplemented with 100 µg/mL ampicillin, and subsequently transferred to MMN broth (PDB) for liquid culture. Fermentation was carried out under shaking conditions at 25 ± 5°C for 10 days. Following incubation, the mycelia were separated using a simple filtration method with a double layer of muslin cloth. The resulting broth was concentrated by heating at 60°C in a water bath for one hour and then allowed to cool to room temperature. Chilled absolute ethanol was added to the concentrated broth in a 1:3 (broth: ethanol) ratio under aseptic conditions to prevent contamination. The mixture was thoroughly vortexed and stored at 4°C overnight to promote complete precipitation of the exopolysaccharides (EPS). The precipitated EPS was then recovered by centrifugation at 10,000 rpm for 20 minutes at 4°C, and the resulting pellet was dried overnight at ambient temperature to remove residual ethanol. To purify the EPS, dialysis

was performed overnight against deionized water using 14 kDa molecular weight cut-off dialysis tubing (HiMedia, India), followed by reprecipitation using the same ethanol treatment protocol.

3.8 Biochemical characterization of selected EPS extract from the screened strain

To get an initial assessment of the carbohydrate and protein content in the EPS sample, protein and total carbohydrate estimations were conducted as outlined below.

3.8.1 Bradford assay

Bradford assay was used for protein determination, wherein the binding of Coomassie Brilliant Blue G-250 (Sigma Aldrich, Germany) present in Bradford reagent to the protein results in a change of the absorption maximum from 465 nm (brownish-red) to 595 nm (blue), with the increase in absorption at 595 nm being the parameter evaluated. This test is highly reproducible and quick, with the dye binding process nearly complete in around 2 minutes, exhibiting good colour stability for 1 hour. Bovine serum albumin, i.e., BSA (Sigma Aldrich, Germany), was used as a standard, in a concentration range of 0.005-0.1 mg/mL.

3.8.2 Phenol sulfuric acid method

Concentrated sulfuric acid dehydrates carbohydrates to produce furfural derivatives, namely furfural from pentoses and hydroxymethylfurfural from hexoses. These compounds interact with phenol to form a yellow-orange complex, which may be quantified spectrophotometrically at a wavelength of 490 nm. A glucose standard was used from 0.1 to 1.0 mg/mL. During the testing, 1mL of samples or glucose standard was taken, respectively, and was added to the test tubes. Followed by the addition of 0.5mL of phenol (5%) and the immediate addition of 2.5 mL of concentrated sulfuric acid. The samples were incubated at 30 ºC for 20 minutes. The absorbance of the sample was then measured at 490 nm.

3.9 Synthesis of SeNPs with selected fungal extract (NLC) and EPS

3.9.1 Synthesis of fungal extract capped SeNPs- The dried screened extract i.e., from NL(C) strain was reconstituted in Milli-Q water. To synthesize selenium nanoparticles (SeNPs), 5 mL of 5 mM sodium selenite (Na_2SeO_3) was mixed with 5 mL of the aqueous NL(C) solution, followed by the addition of 1 mL of 20 mM ascorbic acid as a reducing agent (Fig 3.2). The reaction mixture was incubated at room temperature in the dark for

one hour. A visible colour changes to an orange-red hue indicated the formation of selenium nanoparticles, hereafter referred to as NL(C)-SeNPs due to capping by the NL(C) extract.

The formation of NL(C)-SeNPs was further confirmed by UV-visible spectroscopy, with spectra recorded across a wavelength range of 200 to 800 nm at regular intervals.

3.9.2 Synthesis of exopolysaccharide-capped SeNPs - An alternative method for selenium nanoparticle (SeNPs) synthesis involved the use of a secondary metabolite, exopolysaccharide (EPS) extract, as a stabilizing and capping agent (Fig. 3.3). EPS was dissolved in Milli-Q water and stirred vigorously to obtain a homogeneous solution. A 5 mM sodium selenite (Na_2SeO_3) solution, serving as the selenium precursor, was added to the EPS solution under continuous stirring for 15 minutes. Following thorough mixing, 20 mM ascorbic acid was added dropwise as a reducing agent, and the reaction mixture was stirred for an additional 30 minutes at ambient temperature in the dark to prevent photo-oxidation of the nanoparticle surface.

The appearance of a characteristic crimson reddish-yellow colour indicated the successful synthesis of EPS-capped selenium nanoparticles (EPS-SeNPs). The resulting nanoparticles (NL(C)-SeNPs and EPS-SeNPs) were purified by centrifugation at 12,000 rpm for 40 minutes, followed by four successive washes with ultrapure water using centrifugal filters (Macrosep Advance Centrifugal Device, Pall Corp., USA) with a 10 kDa molecular weight cut-off. The filtrate obtained during the washing steps was assessed for residual ascorbic acid using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay; a colour shift from purple to yellow or colourless indicated effective removal of free radicals and unreacted reductants. After purification, the EPS-SeNPs were lyophilized using a tabletop freeze dryer (Martin Christ Alpha 1-4, Germany) at a condenser temperature of −55°C for further use.

3.10 Physicochemical characterization of NL(C)-SeNPs and EPS-SeNPs

3.10.1 UV-Visible Spectroscopy

The formation of selenium nanoparticles (SeNPs) was preliminarily confirmed by the characteristic colour change to an orange to brick-red solution, indicative of surface plasmon resonance. Aliquots of NL(C)-SeNPs and EPS-SeNPs were placed in quartz cuvettes with a 1 cm path length and scanned across the wavelength range of 200–800 nm using a UV-visible spectrophotometer (Shimadzu UV-1800). Milli-Q water was used to establish the baseline.

3.10.2 Dynamic Light Scattering (DLS) and Zeta Potential

Dynamic light scattering (DLS) was employed to determine the hydrodynamic diameter and particle size distribution of NL(C)-SeNPs and EPS-SeNPs at 27 ± 2°C. The zeta potential (ζ) was measured using a Zetasizer Nano ZS (Malvern Panalytical, United Kingdom) to assess the surface charge and stability of the nanoparticles in suspension.

3.10.3 Transmission Electron Microscopy (TEM) and Elemental Analysis

To determine nanoparticle morphology, shape, and size, transmission electron microscopy (TEM) was performed. The SeNP suspensions were sonicated for 15 minutes, drop-cast onto carbon-coated copper grids, and air-dried before imaging. Particle size distribution was analyzed using Carl Zeiss AxioVision software (version 4). Elemental composition was determined using energy-dispersive X-ray spectroscopy (EDX) on the QUANTAX 200 system (Bruker, USA), operated at an accelerating voltage of 20 kV.

3.10.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted using the attenuated total reflectance (ATR) technique to identify functional groups present in the NL(C) and EPS extracts, and to observe chemical modifications following nanoparticle capping. Spectra were recorded in transmission mode using an IRTracer-100 spectrometer (Shimadzu Ltd, Japan), in the range of 4000–400 cm^{-1}, with a resolution of 0.01 cm^{-1} and wavenumber accuracy of ±2 cm^{-1}. A platinum diamond ATR crystal was used as the internal reflectance element.

3.10.5 X-ray Diffraction (XRD)

Crystallinity of the synthesized SeNPs was evaluated using X-ray diffraction on a PANalytical X'Pert PRO diffractometer. The instrument was equipped with a copper anode X-ray source (λ = 1.5405 Å), operated at 45 kV and 40 mA, and fitted with a Ni filter to suppress Cu Kβ radiation. Diffraction patterns were recorded over a 2θ range of 9.89° to 79.99°, with a step size of 0.0262°. The average crystallite size (La) was estimated using the Scherrer equation:

$$L_a = \frac{K\lambda}{\beta \cos(\theta Bragg)}$$

where K is the shape factor (typically 0.9), λ is the X-ray wavelength, β is the full width at half maximum (FWHM) in radians, and θ is the Bragg angle.

3.10.6 Raman Spectroscopy

Raman spectroscopy was used to further confirm the crystalline nature of NL(C)-SeNPs and EPS-SeNPs. Lyophilized nanoparticle samples were placed on clean glass slides, and spectra were recorded using a LabRAM HR Evolution Raman spectrometer (Horiba Scientific Ltd, Japan) with a 785 nm excitation laser at 30 mW. Spectra were collected across the range of 150–2100 cm^{-1} and averaged over 10 scans. The spectral data were processed and visualized using Origin8 software.

3.11 *In-vitro* bioactivity assays for synthesized NL(C)-SeNPs and EPS-SeNPs

The bioactive properties of SeNPs, including antimicrobial activity (both antibacterial and antifungal) and antioxidant potential, were systematically evaluated. Additionally, their cytotoxic effects were assessed against the HepG2 human liver carcinoma cell line.

3.11.1 *In-vitro* antioxidant property

DPPH Radical Scavenging Assay - The DPPH assay was used to evaluate the antioxidant activity of NL(C)-SeNPs and EPS-SeNPs. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with delocalized electrons that prevent dimerization, resulting in its characteristic deep purple color and a strong absorption maximum at 517 nm [57]. The assay was performed using various concentrations of NL(C)-SeNPs and EPS-SeNPs, with the corresponding fungal metabolites NL(C) and EPS serving as controls. Absorbance was recorded at 517 nm using a UV-visible microplate reader. Ascorbic acid was used as a reference standard.

The radical scavenging activity (RSA) was calculated using the following equation:

$$RSA = [(A_{\text{DPPH}} - A_{\text{Analyte}})/A_{\text{DPH}}]\, x\, 100$$

ABTS Radical Scavenging Assay -The ABTS radical scavenging assay was performed to evaluate the antioxidant activity of the selenium nanoparticles (SeNPs) based on their ability to neutralize the ABTS radical cation [58]. The $ABTS^{\bullet+}$ radical (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was generated by reacting 7 mM ABTS (HiMedia Labs, India) in 0.1 M phosphate-buffered saline (PBS, pH 7.4) with 2.45 mM potassium persulfate in equal volumes. The reaction mixture was incubated in the dark at room temperature for 16 hours, resulting in the formation of a dark green solution.

The working $ABTS^{\bullet+}$ solution was prepared by diluting the stock with PBS (pH 7.4) to achieve an absorbance of 0.9 ± 0.05 at 734 nm. Various concentrations of NL(C)-SeNPs and EPS-SeNPs were tested for their scavenging activity, with their respective fungal metabolites

(NL(C) and EPS) serving as controls. Absorbance was measured at 734 nm using a UV-visible spectrophotometer. Trolox was used as the standard antioxidant for comparison.

3.11.2 MTT Assay for Antibacterial Activity

The antibacterial activity of NL(C)-SeNPs and EPS-SeNPs was evaluated using the MTT assay method (Shi et al., 2007). The assay was conducted against *Escherichia coli* (MTCC 448), *Staphylococcus aureus* (MTCC 902), and *Enterococcus faecalis* (MTCC 6845). Bacterial cultures were grown in Mueller-Hinton broth (HiMedia Labs, India) and incubated at 37°C until an optical density (OD) of 0.1 at 600 nm was achieved.

In a sterile 96-well microtiter plate, 100 µL of serially diluted nanoparticle suspensions (concentration range: 2–2000 µg/mL) were dispensed into designated wells. Subsequently, bacterial inoculum was added to each well, bringing the final volume to 150 µL. Three control groups were included:

- Culture control (broth + bacterial inoculum)
- Negative control (broth only)
- Positive control (ampicillin + bacterial inoculum)

The plate was incubated at 37 °C for 24 hours. After incubation, 20 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 2 mg/mL) was added to each well and incubated for an additional 30 minutes at 37 °C. The resulting purple formazan crystals were solubilized using DMSO, and absorbance was measured at 540 nm using a microplate reader.

The percentage of antibacterial activity was calculated using the following formula:

$$Bacterial\ inhibition(\%) = \frac{[Abs.Cul.Control - Abs\ sample]}{[Abs.Cul.Control]}\ x100$$

Where the culture (Cul.) control contained bacterial inoculum and broth.

The anti-bacterial activity was compared between SeNPs with respective metabolite (NL(C) and EPS) alone to examine if the difference is statistically significant (GraphPad Prism V5.01, Dotmatics, USA).

3.11.3 Anti-fungal activity assay using a neutral red staining assay

The antifungal efficacy of NL(C)-SeNPs and EPS-SeNPs was assessed against four filamentous fungal strains: *Aspergillus niger*, *Fusarium lateritium*, *Alternaria alternata*, and *Aspergillus flavus*. These strains were cultivated on potato dextrose agar (PDA) slants and incubated for two weeks at room temperature to ensure adequate sporulation. Following incubation, conidial suspensions were prepared by flooding the culture plates with phosphate-buffered saline (PBS) containing 2% Tween-20 and gently scraping the surface to release conidia. Conidia were counted using a hemocytometer with a 20 µL suspension, and the final concentration was adjusted to 3×10^6 conidia/mL in Sabouraud dextrose broth (SDB).

For the antifungal assay, 100 µL of serially diluted NL(C)-SeNPs or EPS-SeNPs (ranging from 2 to 2000 µg/mL) was added to the wells of a sterile 96-well microtiter plate, followed by 100 µL of the conidial suspension. The plate was incubated in the dark at 28 °C for 24 hours. After incubation, the supernatant was carefully removed, and 200 µL of neutral red dye solution (50 µg/mL) was added to each well. The plate was then incubated for an additional 1 hour at 28 °C to allow dye uptake by viable fungal cells.

Following dye incubation, the neutral red solution was aspirated, and the wells were washed with a fixative solution containing 4% formaldehyde and 1% calcium chloride. After removing the wash solution, 200 µL of an extraction solution (1% acetic acid in 50% ethanol) was added to solubilize the retained dye from viable cells. The plate was incubated on an orbital shaker at room temperature for 20 minutes. The resulting filtrate was transferred to a fresh 96-well plate, and absorbance was measured at 550 nm using a microplate reader.

The assay was performed in triplicate, and three experimental groups were included:

- Negative control: broth only
- Culture control: broth + fungal inoculum
- Treatment group: broth + fungal inoculum + SeNPs at various concentrations

The percentage of antifungal activity was calculated using the following formula:

$$fungal\ inhibition(\%) = \frac{[Abs.\,Cul.\,Control - Abs\ sample]}{[Abs.\,Cul.\,Control]}\ x100$$

3.11.4 SEM visualization of structural alterations caused by SeNPs in microbial cells

Ultrastructural and morphological changes of the treated bacterial cells on exposure to TeNPs were observed via FE-SEM (Carl Zeiss Sigma 500, Germany). Bacterial strains grown in their logarithmic growth phase were inoculated into the 100 mL fresh medium (MHB). The cells were then exposed to a previously selected concentration based onto the minimum inhibitory concentration of NL(C)-SeNPs and EPS-SeNPs. The cells were centrifuged at 5000 rpm and at 4ºC for 10 min to obtain the pellet. The pellet was washed twice with 0.2 Mm of Phosphate buffer (pH 7). The pellet was then fixed with glutaraldehyde (2.5% strength in 0.2 mM phosphate buffer) for 1 h. The samples were dehydrated with increasing ethanol concentrations, i.e. 25, 50, 75 and 100%, for 10 minutes each. The 100% cell suspension was further processed with critical point drying i.e., CPD (Leica-EM CPD300, Germany) in which ethanol was substituted with a transitional fluid, such as liquid CO_2, in the CPD instrument as per the manufacturer's specifications. Further CO_2 exchange cycles were performed, and critical point transitioning was done, and samples were removed slowly for further processing. The samples were then gold-coated via a sputter coater (Quorum technologies-Q150R, UK) and observed under FE-SEM to understand the impact of mentioned nanoparticles on microbial morphology.

3.11.5 Peripheral blood mononuclear cells (PBMC) viability and Cytotoxicity Assay

Fresh peripheral whole blood was obtained from a consenting human volunteer and collected in citrated tubes, following institutional ethical approval (Ethical Clearance No. TIET/EC/2024-09). Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using the Ficoll-Histopaque method (HiSep™ LSM 1077, HiMedia), as per the manufacturer's instructions. The isolated cells were washed with sterile phosphate-buffered saline (PBS), and viability and cell count were determined using the trypan blue exclusion method with an automated cell counter (Farscope B, Curiosis, Republic of Korea).

The PBMCs were resuspended in RPMI-1640 medium supplemented with 5% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, and 25 µg/mL amphotericin B. Cells were seeded into 96-well microplates at a density of 2×10^5 cells/mL. The cultures were then treated with varying concentrations (0–800 µg/mL) of both nanoparticles i.e., NL(C)-SeNPs and EPS-SeNPs, simultaneously and incubated for 24 hours at 37 °C in a humidified atmosphere containing 5% CO_2.

Following incubation, cell viability was assessed using the MTT assay. The results were expressed as a percentage relative to the untreated control group, indicating the cytotoxic potential of the nanoparticles.

3.11.6 *In-vitro* Cytotoxicity Assessment of SeNPs on HepG2 Cells

The cytotoxic potential of NL(C)-SeNPs and EPS-SeNPs was evaluated against the human hepatoma cell line HepG2. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well in Dulbecco's Modified Eagle's Medium (DMEM, low glucose) and incubated at 37°C for 24 hours in a humidified atmosphere containing 5% CO_2. The culture medium was supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, and 25 µg/mL amphotericin B.

Following the initial incubation, cells were treated with varying concentrations (0–800 µg/mL) of NL(C)-SeNPs, EPS-SeNPs, and their respective metabolites (NL(C) and EPS), which served as controls. After 24 hours of treatment, 20 µL of MTT solution (5 µg/mL in DMEM, pH 7.0) was added to each well. The plates were covered with aluminium foil and incubated for an additional 4 hours at 37 °C under the same humidified conditions to allow the formation of formazan crystals.

After incubation, 150 µL of the medium was carefully removed from each well to avoid disturbing the cell monolayer. An equal volume (150 µL) of dimethyl sulfoxide (DMSO) was then added to each well to solubilize the formazan crystals, and the contents were thoroughly mixed. The optical density (OD) was measured at 540 nm using a spectrophotometer (Thermo Scientific, Waltham, MA, USA), with an appropriate blank as reference.

$$Cell\ viability\ (\%) = \frac{[Abs.of\ treated\ cells]}{[Abs.of\ control\ cells]}\ x100$$

3.12 Functionalization of sterile cotton gauze fabrics with EPS-SeNPs

In brief, EPS-SeNPs nanoparticles exhibited significant antibacterial efficiency, particularly against Staphylococcus aureus and Escherichia coli. EPS-SeNPs demonstrated bacterial inhibitory efficacies of 72% and 64% at the highest tested dosage of 2000 µg/mL, and these nanoparticles had an IC_{50} concentration of 500 µg/mL.

Consequently, due to the pronounced antibacterial properties demonstrated by EPS-SeNPs over NL(C)-SeNPs, they were chosen for further use in the biofunctionalization of sterile cotton gauze fabric. Building upon these findings, the present study aimed to harness the

bioactive potential of EPS-SeNPs by incorporating them into commercially available sterile cotton gauze. The objective was to evaluate the antibacterial performance of chitosan-coated gauze functionalized with EPS-SeNPs, as well as to investigate the mechanism of nanoparticle adherence and retention on the gauze matrix.

3.12.1 Cationization of sterile cotton gauze

Sterile cotton gauze (CG) was initially cleaned using ultrasonic treatment in deionized water for 30 minutes, followed by air drying at room temperature (RT). To cationize the gauze surface, a dip-and-dry method was employed using low molecular weight chitosan (CH) with a degree of deacetylation of 75%. A 2% (w/v) chitosan solution was prepared by dissolving CH in 1% (v/v) aqueous acetic acid, followed by overnight stirring at room temperature to ensure complete dissolution.

Rectangular pieces of cotton gauze (10 × 10 cm^2) were immersed in the chitosan solution for 10 minutes. To enhance coating adherence, the immersed gauze was subjected to ultrasonic treatment in a water bath at 50°C for 30-40 minutes. After sonication, excess solution was gently removed, and the treated gauze was dried at 60°C for 30 minutes. The resulting cationized cotton gauze was designated as CH_CG.

3.12.2 Impregnation of EPS-SeNPs onto cationized cotton gauze

Following the drying step, the cationized cotton gauzes (CH_CG) were functionalized with EPS-SeNPs using a dip-coating method. The gauze samples were immersed in the EPS-SeNP suspension and stirred at 200 RPM for 2 hours at room temperature (Fig. 3.4).

After nanoparticle loading, the treated gauzes designated as EPS-SeNPs@CH_CG, along with control samples (CH_CG and untreated CG, the latter representing sterile cotton gauze without any treatment), were thoroughly rinsed with autoclaved distilled water to remove unbound residues. Subsequently, all gauze specimens were dried under laminar airflow for 30 minutes and stored under aseptic conditions for future experimental analyses.

3.13 Physicochemical characterization (FTIR and XRD) of functionalized cotton gauze

64 Fourier Transform Infrared (FTIR) spectroscopy using the attenuated total reflectance (ATR) method was employed to investigate changes in functional group moieties on cotton gauze following chitosan pretreatment and subsequent EPS-SeNPs coating [60]. Spectral analysis 1 was conducted using an IRTracer-100 spectrometer (Shimadzu Ltd, Japan) operated in transmission mode. Infrared spectra were recorded over the range of 4000–400 cm^{-1} with a

wavenumber accuracy of ±2 cm^{-1} and a resolution of 0.01 cm^{-1}. For each sample untreated gauze (CG), chitosan-treated gauze (CH_CG), and nanoparticle-coated gauze (EPS-SeNPs@CH_CG) a total of 65 scans were accumulated to ensure high signal-to-noise ratio.

To assess the crystalline structure of the cotton gauze samples, X-ray diffraction (XRD) analysis was performed using a PANalytical X'Pert PRO diffractometer. A copper (Cu) anode served as the X-ray source, generating characteristic Cu Kα and Kβ radiation with wavelengths of 1.540 Å and 1.544 Å, respectively, at an operating voltage of 45 kV and tube current of 40 mA. A nickel (Ni) filter was employed to eliminate the Kβ component. Diffraction patterns were recorded using a continuous scan mode with a step size.

3.14 FE-SEM and EDS analysis of EPS-SeNP-coated gauze

Field Emission Scanning Electron Microscopy (FE-SEM) was carried out using a Carl Zeiss Sigma 500 instrument to evaluate the surface morphology of cotton gauze and to confirm the deposition of EPS-SeNPs onto chitosan-treated gauze (CH_CG). Gauze samples were sectioned into 1 × 1 cm^2 pieces and mounted onto aluminum SEM pin stubs using conductive carbon tape. The samples were then sputter-coated with a thin layer of gold using a Quorum 150R ES Plus coater to enhance conductivity.

FE-SEM imaging was employed not only to visualize the morphological characteristics of the cotton fibres but also to assess the uniformity and adherence of EPS-SeNPs on the gauze surface. Elemental mapping and composition analysis were further conducted using Energy Dispersive X-ray Spectroscopy (EDS) integrated with the QUANTAX 200 system (Bruker, USA). EDS analysis was performed at an accelerating voltage of 20 kV to detect selenium signals and confirm nanoparticle localization along the fibre surfaces. Image acquisition and data processing were conducted using SmartSEM software.

3.15 Selenium release kinetic via ICP-OES

To evaluate the release profile of selenium, gauze samples (2 × 2 cm^2) of EPS-SeNPs@CH_CG were immersed in 15 mL of phosphate-buffered saline (PBS, pH 7.4). The samples were incubated under continuous agitation at 100 rpm in a rotary shaker. At predetermined time intervals of 8 hours, aliquots of the release medium were collected and replaced with an equal volume of fresh PBS to maintain sink conditions.

The cumulative release of selenium was quantified using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Prior to analysis, selenium content in the collected samples

was digested according to the standardized protocol outlined by the American Public Health Association (APHA), specifically following Method 3120 B of the 24th Edition (2017).

3.16 Antibacterial study of functionalized cotton gauze

To investigate the antibacterial properties of the functionalized cotton gauze coated with EPS-SeNPs, two bacterial strains were chosen as preliminary targets for inhibitory studies, i.e., *Escherichia coli* and *Staphylococcus aureus*. Despite EPS-SeNPs previously shown significant antibacterial efficacy against these strains, it was essential to investigate if their activity was preserved following functionalization with nanoparticles. Furthermore, *Escherichia coli* and *Staphylococcus aureus* were chosen as representative Gram-negative and Gram-positive bacteria, respectively, owing to their worldwide clinical significance, differing cell envelope layouts, and designation as standard reference strains for antimicrobial susceptibility testing(Clinical and Laboratory Standards Institute, 2023; International Organization for Standardization, 2019), facilitating comprehensive activity evaluation.

3.16.1 Bacterial Growth Inhibition Study of EPS-SeNPs@CH_CG

Sterilized samples of EPS-SeNPs@CH_CG (2 × 2 cm^2) were prepared by exposing them to UV light. Each sample was then immersed in 20 mL of bacterial suspension containing either *Escherichia coli* or *Staphylococcus aureus*, adjusted to an initial optical density (OD_{600}) of 0.06. The suspensions were incubated under shaking conditions at 100 rpm to promote bacterial interaction with the treated gauze.

At predetermined time intervals, 1 mL aliquots were withdrawn from the culture medium, and the bacterial growth was monitored by measuring the OD at 600 nm using a UV-visible spectrophotometer (Shimadzu UV-2600). This analysis enabled the assessment of bacterial proliferation inhibition over time in response to the EPS-SeNPs-coated gauze.

3.16.2 Assessment of Bacterial Viability via CFU Counting

Gauze samples of EPS-SeNPs@CH_CG and CH_CG were sterilized by exposure to UV light. The sterilized samples were then incubated in 10 mL bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus* at 37°C for 1 hour to evaluate antimicrobial efficacy. After incubation, 10-fold serial dilutions of the bacterial suspensions were prepared using sterile saline buffer.

From each dilution, 0.1 mL aliquots were plated onto Nutrient Agar (NA) plates and incubated at 37°C to allow colony development. Following incubation, colony-forming units (CFUs)

were counted to determine the viability of bacteria in the presence of treated and control gauze samples.

$$\text{CFU/mL} = \frac{Colony\ forming\ unit\ (CFU)\ x\ Total\ dilution\ factor}{Volume\ of\ the\ culture\ plated\ (mL)}$$

3.16.3 Protein and polysaccharide leakage study of targeted bacteria

Protein Leakage Assay: The antibacterial effect of EPS-SeNPs@CH_CG was further evaluated by assessing protein leakage from bacterial cells, an indicator of membrane disruption. The assay was performed in comparison with control samples (CG and CH_CG) using the Folin–Lowry method. Bacterial suspensions of *E. coli* and *S. aureus* at a concentration of 10^7 CFU/mL were incubated with 2 × 2 cm² gauze samples (EPS-SeNPs@CH_CG and controls) at 37°C for 8 hours. Post incubation, the culture broths were centrifuged at 12,000 rpm for 30 minutes at 4°C. The resulting supernatants were stored at −20°C for subsequent analysis.

For protein quantification, 1 mL of supernatant was collected from each sample and subjected to the Folin–Lowry assay. Absorbance was measured at 660 nm using a spectrophotometer. A bacterial culture without any gauze sample served as the reference control. All experiments were performed in triplicate.

Polysaccharide Leakage Assay: Polysaccharide leakage was quantified using the dinitrosalicylic acid (DNS) method to assess the release of reducing sugars from bacterial cells exposed to the gauze treatments. Bacterial cultures at 10^7 CFU/mL were incubated with 2 × 2 cm² gauze samples (EPS-SeNPs@CH_CG and controls) at 37°C for 8 hours. A parallel control without gauze was maintained under identical conditions.

Following incubation, the culture broths were centrifuged at 12,000 rpm for 30 minutes at 4°C, and the supernatants were stored at −20°C. For analysis, 1 mL of each supernatant was mixed with 3 mL of DNS reagent and heated at 90°C for 15 minutes. The absorbance was then recorded at 540 nm to evaluate reducing sugar leakage. All tests were conducted in triplicate to ensure reproducibility.

3.16.4 Bacterial Adherence Assay via FE-SEM Analysis

To evaluate the anti-adhesive properties of EPS-SeNPs@CH_CG against *Escherichia coli* and *Staphylococcus aureus*, comparative studies were conducted using untreated cotton gauze (CG) as the control. Gauze samples measuring 1 × 1 cm² were placed in the wells of a sterile

24-well plate. Each well was then inoculated with 1 mL of bacterial suspension containing 1 × 10^8 CFU/mL and incubated at 37°C for 8 hours to facilitate bacterial adhesion.

Following incubation, the samples were carefully removed using sterilized tweezers and sequentially washed five times in 5 mL of sterile phosphate-buffered saline (PBS) to remove non-adherent bacteria. The gauze specimens were then fixed in 3% glutaraldehyde at 4°C for 4 hours. Post-fixation, the samples were dehydrated through a graded ethanol series and air-dried. The dried fabrics were sputter-coated with gold using a Quorum Technologies Q150R coater (UK).

Surface morphology and bacterial attachment were examined using field emission scanning electron microscopy (FE-SEM) at 10,000× magnification. For quantitative assessment, six SEM fields (each measuring 42 × 28 μm²) per sample were analyzed. The number of adherent bacteria was counted, and the adhesion-to-anti-adhesion ratio was calculated using the following equation.

$$\text{Bacterial anti-adhesion rate} = \frac{cells_{control}cm^{-2} - cells_{sample}cm^{-2}}{cells_{control}cm^{-2}}$$

4.0 Results and Discussion

4.1 Isolation of endophytic fungi

Medicinal plants are known for their therapeutic properties and play a vital role in the Indian Ayurvedic system. Among these, endophytic fungi microorganisms that reside asymptomatically within plant tissues are of particular interest due to their ability to biosynthesize bioactive compounds that mimic or complement those produced by their host plants. The isolation and identification of novel endophytic fungal species hold significant potential for drug discovery and development.

In the present study, endophytic fungi were then isolated from the plant samples as outlined in Fig. 4.1 (A–D). A total of six distinct fungal strains were successfully isolated and designated as NL(A) through NL(F). These strains were subsequently evaluated for their ability to facilitate the biosynthesis of selenium nanoparticles (SeNPs), as illustrated in Fig. 4.1E. Screening was performed based on the bioactivity of secondary metabolites produced by each isolate, employing the micro-broth dilution method. Plant samples of *Nerium oleander* were collected from the Thapar Institute (TIET) campus in Patiala and immediately transferred into sterile containers. Upon arrival at the laboratory, samples underwent surface sterilization followed by fungal isolation procedures under aseptic conditions.

The isolated fungal strains were subjected to morphological analysis using lactophenol cotton blue staining and observed under an optical microscope. Cultures were grown on Potato Dextrose Agar (PDA) plates, and staining was performed on the 4th or 5th day of incubation to assess hyphal characteristics (septate or aseptate) and sporulation patterns. Distinct morphological variations were noted among the six strains.

Strain NL(A) exhibited darkly pigmented conidia, either solitary or arranged in short chains on geniculate conidiophores. These conidia displayed both transverse and longitudinal septation, forming a muriform structure, and ranged in shape from club-like to ovoid with a swollen apex, features consistent with the genus *Alternaria* spp. (Fig. 4.2).

Strain NL(B) demonstrated a septate hyphal arrangement with conidia forming characteristic brush- and broom-like structures. The conidiophores appeared in a radiating pattern, and the

metulae bore phialides from which the conidia radiated outward a distinguishing feature of *Penicillium* spp.

Strain NL(C) exhibited delayed sporulation, with microscopic examination conducted on the 7th day of incubation. Slightly hyaline hyphae and dark-stained pigmented conidia were observed. The conidia had a strict globular structure, which is a characteristic feature of *Nigrospora* spp.

Strain NL(D) was identified as a fast-growing fungus compared to the other isolates. Microscopic analysis revealed aseptate, unbranched hyphae with terminal sac-like structures containing smooth-walled spores, indicative of sporangia. A similar morphology was observed in strain NL(F). These features are characteristic of fungi belonging to the phylum *Zygomycota*, specifically *Rhizopus* spp., suggesting that both NL(D) and NL(F) likely belong to this genus.

In strain NL(E), hyaline, septate, and branched hyphae were observed. At the hyphal termini, short chains of phialides gave rise to small, globular conidia arranged in a radiating, chain-like fashion. Based on these morphological traits, NL(E) was inferred to belong to the genus *Aspergillus* spp.

4.2 Preliminary screening of endophytic strains

The extracts from the above fungal isolates were screened for their antimicrobial activity using the micro-broth dilution method. Preliminary screening revealed that strain NL(C) exhibited notable antimicrobial activity against both Gram-positive bacteria *Enterococcus faecalis* and *Staphylococcus aureus* as well as Gram-negative bacteria *Escherichia coli* and *Salmonella enterica*. Additionally, strain NL(E) demonstrated inhibitory effects against *Acinetobacter calcoaceticus* and *Escherichia coli*, suggesting a broad-spectrum antimicrobial potential.

In screening of anti-fungal activity of the said extracts, strain NL(C) demonstrated significant inhibitory activity against *Fusarium lateritium* and *Aspergillus niger*. While NL(E), which had shown antibacterial potential, did not exhibit any antifungal activity, strain NL(A) displayed selective antifungal efficacy against *Aspergillus niger*. The primary objective of screening these fungal metabolites for antimicrobial properties was to evaluate their potential role in stabilizing or capping selenium nanoparticles (SeNPs). The aim was to determine whether biologically active capping agents could enhance the bioactivity of the nanoparticles.

4.3 Molecular characterization of the fungal strain

Molecular identification of the endophytic fungal strain was performed at the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The strain NL(C) was identified as *Nigrospora spp.* (Fig. 4.3). The Accession number (GenBank) of the said strain is OP248797. There have been reports in which *Ascomycota* sp. were isolated frequently from *N.oleander* (W.-Y. Huang et al., 2007). Several biologically active metabolites are associated with *N. oleander,* although it is a plant used mainly for ornamental purposes (Fu et al., 2005; Gioia et al., 2020). Gioia et al. reported a survey about endophytic fungi associated with ornamental plants, in which *Nigrospora* spp. have been revealed to be isolated from the roots and other parts of plant parts of *N.oleander* (Gioia et al., 2020).

4.4 Biochemical analysis of fungal extract and EPS of NL(C) strain

The NL(C) extract of *Nigrospora* spp. showed a total phenolic content of 121.9 ±1.88 µg/mL (gallic acid equivalent), and the regression equation obtained from the gallic acid calibration standard (R^2=0.9903) was used for the calculation of TPC. Furthermore, in flavonoid content, NL(C) showed 59.34 ± 2.47 µg/mL of total flavonoid content (quercetin equivalent), and the regression equation obtained from the quercetin calibration standard (R^2=0.9908) has been used for the calculation of TFC. The phenolics, flavonoids, and terpenoids are the primary metabolites that are responsible for the antioxidant, antibacterial, and anticancer activity. Phenolics have been documented to mitigate oxidative damage in biological systems via oxygen scavenging, free radical suppression, metal inactivation, and peroxide breakdown (Sathiyaseelan et al., 2021; Verma et al., 2022). It is assumed that higher the phenolic and flavonoid content, the higher will be the antioxidant activity, which in turn can show a potent cytotoxic activity as well with reference to antibacterial or anti-cancer activity (Verma et al., 2022).

NL(C) fungal strain was further utilized to extract another secondary metabolite i.e., exopolysaccharide (EPS). The extracted EPS had a carbohydrate and protein content of 395 ± 13.20 mg/g and 121 ± 3.21 mg/g, respectively. Exopolysaccharides are mostly heteropolysaccharides composed of monomers including galactose, rhamnose, and uronic acids. The uronic acid component of the exopolysaccharide may contribute to the negative charge on EPS, enhancing its activity for metal chelation and radical scavenging action, hence promoting antibacterial activity (Y. Cheng et al., 2017; Mahapatra & Banerjee, 2013; Sajna et al., 2021).

Endophytic fungi represent a vast and largely unexplored source of bioactive secondary metabolites, with estimates suggesting the existence of millions of unique species. Many of these fungi are capable of synthesizing compounds analogous to those of their host plants for example, Taxol, offering added value for nanoparticle synthesis through biologically derived pathways (Adeleke et al., 2024). Therefore, such biologically driven synthesis systems can be regarded as a form of "green chemistry," integrating principles of microbial biotechnology and nanotechnology for the eco-friendly synthesis of nanoparticles. Transitioning from conventional nanoparticle synthesis methods to more sustainable, biologically driven approaches holds promise for enhancing therapeutic applications (Naik, 2020).

Therefore, in light of the promising results from the preliminary screening, strain NL(C) emerged as the most potent candidate, displaying broad-spectrum antimicrobial activity. Consequently, both NL(C) and its exopolysaccharide (EPS) were selected as bioactive capping agents for the green synthesis of selenium nanoparticles. This selection was made to assess whether these biologically derived components could enhance the stability and therapeutic efficacy of the resulting SeNPs.

4.5 Concomitant synthesis of NL(C)-SeNPs and EPS-SeNPs

4.5.1 NL(C)-SeNPs – The study investigated the potential of the NL(C) fungal extract in stabilizing selenium nanoparticles (SeNPs) during their biosynthesis, using sodium selenite as the selenium source and ascorbic acid as the reducing agent. Successful synthesis of NL(C)-capped SeNPs (NL(C)-SeNPs) was confirmed by UV–Visible spectroscopy, which exhibited a characteristic absorption peak (λ_{max}) at 265 nm (Fig. 4.4A), indicative of the Surface Plasmon Resonance (SPR) typically associated with SeNPs (Shahabadi et al., 2021, Tabibi et al., 2023). NL(C)-SeNPs were further examined for their stability under different pH conditions (Fig. 4.4B), it was observed that the nanoparticles showed a decrease in size from pH 4 to 6 and a slight increase in size from pH 7 to 9, which may denote aggregation, further contributing towards decreased colloidal stability.

Biochemical characterization of the extract from strain NL(C), as discussed earlier, which was identified as *Nigrospora* spp., revealed substantial levels of bioactive compounds. Phenolic compounds, characterized by aromatic rings bearing hydroxyl (-OH) groups, are known to function as efficient stabilizing and capping agents in nanoparticle synthesis. These groups can chelate metal ions and interact with the nanoparticle surface, thus enhancing stability and

preventing agglomeration. Flavonoids, similarly, contribute to the capping process through hydrogen bonding and electrostatic interactions, further supporting nanoparticle dispersion.

Fungal extracts, particularly those derived from endophytic fungi are recognized as rich reservoirs of secondary metabolites with broad bioactivity profiles, including antioxidant, antimicrobial, and anticancer properties (Phongpaichit et al., 2007). In recent advancements, these metabolites have shown considerable promise in green nanotechnology, particularly for the synthesis of biocompatible nanoparticles (Adeleke et al., 2024, Bogas et al., 2022). Among these, phenolic and flavonoid compounds have emerged as key players due to their dual roles in both reducing metal precursors and stabilizing the formed nanoparticles (Dasauni, Singh, and Nailwal, 2023). Their involvement not only contributes to the eco-friendly nature of the synthesis process but also imparts added therapeutic potential to the resulting nanomaterials.

To expand the search for bio-potent capping agents, NL(C) isolate was also screened for its EPS secretion, and the EPS was extracted via chilled ethanol precipitation. NL(C)'s EPS extract had a favourable outcome in antibacterial screening using the microbroth dilution test. Inhibitory activity was observed against three Gram-positive strains (*B. subtilis*, S. *aureus,* and *E. faecalis*) and two Gram-negative strains (*E. coli* and *S. enterica*).

EPS polysaccharides are broadly classified based on their ionic charge as anionic (e.g., hyaluronic acid, heparin, pectin), cationic (e.g., chitosan), or non-ionic (e.g., starch, cellulose). Among these, polyanionic EPS are particularly effective in nanoparticle stabilization due to their functional groups' ability to bind metal ions and prevent aggregation (Dey et al., 2023). The extensive hydrogen bonding networks of EPS are believed to contribute significantly to surface passivation, reducing nanoparticle agglomeration and enhancing colloidal stability.

4.5.2 EPS-SeNPs - The purified EPS was employed to cap and stabilize selenium nanoparticles (SeNPs), with the reaction carried out at room temperature under dark conditions to prevent photo-degradation. During the process, selenite ions (SeO_3^{2-}) from the precursor salt were reduced to elemental selenium through a redox reaction mediated by ascorbic acid. The polysaccharide groups in the EPS provided effective surface coverage, resulting in the formation of stable EPS-SeNPs (Li et al., 2021).

The EPS-SeNPs were collected via centrifugation, thoroughly washed with sterile distilled water, and subjected to characterization using spectroscopic techniques and X-ray diffraction (XRD) analysis. Initial confirmation of nanoparticle formation was obtained from UV–visible

spectroscopy, which displayed a distinct absorption peak (λ_{max}) at 265 nm, consistent with the surface plasmon resonance (SPR) of SeNPs similar to NL(C)-SeNPs.

Additionally, a minor absorbance peak at 280 nm was observed in the EPS, indicating the presence of proteinaceous components characteristic of exopolymeric substances, which are primarily composed of carbohydrates and proteins. It was observed that a pH range higher than 7 resulted in an increased size of the nanoparticles with the possibility of agglomeration, and a pH range between 4 to 6 showed a nanoparticle of decreased size (Fig. 4.5B). Although SeNPs exhibited appreciable colloidal stability, especially in the case of EPS-SeNPs. For extended bioactivity analyses and storage, the nanoparticles, i.e., NL(C)-SeNPs and EPS-SeNPs were subsequently lyophilized and preserved.

4.6 Physicochemical observations of SeNPs

4.6.1 NL(C)-SeNPs - Transmission electron microscopy (TEM) analysis revealed that the NL(C)-SeNPs were predominantly spherical, with an average size ranging from approximately 55 to 60 nm (Fig. 4.6A and B), and exhibited minimal size variation, as indicated by a standard deviation of ±7.0 nm. Energy-dispersive X-ray (EDX) spectroscopy further confirmed the elemental composition of the nanoparticles, showing a prominent Se(0) signal at 1.35 keV (Fig. 4.6C), which is characteristic of elemental selenium.

In addition to selenium, signals corresponding to carbon and oxygen were detected, which are attributed to the natural ligand coating (NL(C)) functioning as a capping agent on the nanoparticle surface. These observations are consistent with the previous findings of Xu et al. (2019), who reported that fungal filtrates contain a diverse array of bioactive molecules that mediate both the reduction of metal salts and the stabilization of nanoparticles (Xu et al., 2019). Such fungal-derived metabolites, including flavonoids, phenolic compounds, and tannins, not only serve as reducing agents but also play a critical role in determining nanoparticle morphology and stability.

The uniform size distribution of the synthesized SeNPs suggests that the fungal extract offers a stable and consistent reaction milieu, likely due to the synergistic interactions among various bioactive molecules. As noted by Philip, fungal extracts are enriched with reducing sugars, quinones, and amino acids, which not only influence the crystal structure of the nanoparticles but also play a crucial role in preventing uncontrolled agglomeration. These biomolecular constituents contribute to a controlled nucleation and growth process, resulting in well-dispersed nanoparticles with uniform morphology (Philip, 2011).

4.6.2 EPS-SeNPs - SEM analysis of the EPS capping agent alone (Fig. 4.7A) showed a dense, irregularly shaped morphology. EDX analysis of EPS (Fig. 4.7B) further confirmed its carbohydrate-rich nature, with carbon and oxygen constituting 59% and 30% of the elemental composition, respectively. These results highlight the efficacy of EPS as a capping agent, as it facilitates the synthesis of smaller, more uniformly dispersed SeNPs by inhibiting excessive particle growth and aggregation. In addition to the primary elemental signals, trace amounts of phosphorus, sulfur, potassium, and nitrogen were also detected.

A similar elemental profile was reported by Zaghloul et al. (Zaghloul & Ibrahim, 2022), where EPS produced by *Lactiplantibacillus plantarum* exhibited an irregular, rough surface dominated by carbon and oxygen as the major constituents. In another study, Tilwani et al. observed that EPS produced by an *Enterococcus faecium* strain isolated from fish gut displayed a globular morphology with a smooth and porous structure (Tilwani et al., 2021). These variations in EPS microstructure can be attributed to differences in the monosaccharide composition and the glycosidic linkage patterns, which influence the physical properties and surface characteristics of the EPS (Singh et al., 2019).

The morphological characteristics of EPS-SeNPs were examined using TEM. The analysis revealed predominantly spherical nanoparticles with an average diameter of 43.7 ± 13.7 nm (Fig. 4.8A and B). The EDX spectroscopy confirmed the presence of elemental selenium (Se^0) through a characteristic signal at 1.35 keV (Fig. 4.8C). In addition to selenium, the EDX spectra also displayed peaks corresponding to carbon, oxygen, and nitrogen. These signals are likely attributed to the extracellular polymeric substances (EPS) serving as the capping matrix, which predominantly consists of polysaccharides and proteins.

Quantitative analysis revealed that nitrogen constituted approximately 3% by mass, whereas carbon and oxygen accounted for 40% and 50%, respectively. This elemental distribution aligns with earlier biochemical assays, reinforcing the conclusion that the polysaccharide content in the EPS is substantially higher than that of proteins.

The synthesized EPS-SeNPs were found to be well dispersed, exhibiting a polydispersity index (PDI) of 0.312 and a zeta potential of -28.2 mV. The PDI, also known as the heterogeneity index, is a dimensionless value derived from the cumulant analysis of dynamic light scattering data. It reflects the distribution width of particle sizes in suspension. While highly monodisperse systems typically exhibit PDI values below 0.05, values above 0.7 indicate a broad and heterogeneous size distribution. For polymer- or lipid-based

nanomaterials, a PDI ≤ 0.2 is generally considered ideal, although a threshold of ≤ 0.3 is often acceptable for drug delivery applications involving nanoliposomes or similar carriers (Danaei et al., 2018). With a PDI of 0.312, the EPS-SeNPs demonstrate moderate polydispersity and were deemed suitable for further in vitro evaluation.

Zeta potential is a key physicochemical parameter that reflects the electrostatic interactions at the interface between nanoparticles and the surrounding liquid medium. It serves as a reliable indicator of colloidal stability by measuring the degree of electrostatic repulsion or attraction between particles. Typically, nanoparticles with zeta potential values exceeding ±30 mV are considered highly stable, as the strong repulsive forces prevent aggregation. In this study, the EPS-SeNPs exhibited a zeta potential of –28.2 mV, which, although slightly below the conventional stability threshold, still indicates reasonably strong electrostatic repulsion and suggests near-optimal colloidal stability.

Additional characterization was performed on the control samples i.e., uncapped selenium nanoparticles (SeNPs), using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). SEM imaging of the uncapped SeNPs (Fig. 4.9A) revealed the formation of substantially larger particles, with diameters ranging from approximately 240 to 500 nm. The corresponding EDX spectrum (Fig. 4.9B) exhibited prominent peaks for selenium (47%), carbon (37%), and oxygen (14%), confirming the elemental composition of the nanoparticles.

4.6.3 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy was performed on NL(C)-SeNPs samples to identify the functional groups present in the fungal extract that may have contributed to the reduction and capping of SeNPs. A prominent shift from 3270 cm^{-1} to 3208 cm^{-1} was observed, indicating the interaction of SeNPs with hydroxyl (–OH) groups in the extract, suggestive of hydrogen bonding between selenium and these functional groups. Additionally, a shift from 2909 cm^{-1} to a weaker band at 2804 cm^{-1} was noted, corresponding to C–H stretching vibrations (Fig. 4.10, Table 4.3). Similar shifts have been reported in a study showing the synthesis of SeNPs using *Terminalia arjuna* extract (Prasad & Selvaraj, 2014) , and another study showing green synthesis of SeNPs using *Allium sativum* (garlic) extract (Anu et al., 2017).

Further spectral changes included shifts in peaks from 1655, 1575, and 1316 cm^{-1} in the native fungal extract to 1701, 1581, and 1391 cm^{-1} in NL(C)-SeNPs, attributed to C=C stretching, C–

N stretching, and O–H bending, respectively. A minor shift from 1180 to 1185 cm^{-1} was observed, likely due to C–O stretching, which is consistent with previous findings (Kannan et al., 2014). Additional low-frequency shifts from 822 cm^{-1} in the fungal extract to 741 cm^{-1} in the NL(C)-SeNPs can be attributed to the interaction among the biomolecules in the extract and the SeNPs surface. According to a previous report, such strong interactions between selenium and hydroxyl groups may enhance the stability of NL(C)-SeNPs (Yu et al., 2016).

EPS-SeNPs - The stabilization and capping of SeNPs by EPS, facilitated through its diverse functional moieties, were also confirmed via FTIR spectroscopy (Fig. 4.11, Table 4.4). A broad absorption band observed in the range of 3400–3427 cm^{-1} corresponds to O–H and N–H stretching vibrations, which can be attributed to hydroxyl groups in polysaccharides and amine groups in proteins present within the EPS matrix. A medium-intensity band near 2922 cm^{-1}, corresponding to C–H stretching vibrations, was found to be diminished in the EPS-SeNP spectrum, suggesting potential surface functionalization of SeNPs by these groups.

A prominent band around 1628 cm^{-1}, assigned to C=O stretching vibrations, is indicative of carbonyl groups that may exist in both free and bound states, depending on intra- or intermolecular interactions (Touliabah, El-Sheekh, and Makhlof, 2022). Bands in the region of 1318–1328 cm^{-1} are likely associated with C–N stretching vibrations from protein moieties, while the –CH bending vibrations, typically attributed to polysaccharides, also exhibited decreased intensity upon formation of EPS-SeNPs (Jain et al., 2015).

In native EPS, the band at 3427 cm^{-1} is predominantly linked to O–H groups from sugar residues, and the band at 2926 cm^{-1} represents C–H stretching within the sugar ring structure (Ramachandran et al., 2023). A notable vibrational band around 1070 cm^{-1}, observed in both EPS and EPS-SeNPs, is characteristic of α-glycosidic linkages in the EPS framework (Hamidi et al., 2023). The persistence and modification of these spectral features in the EPS-SeNPs confirm the effective stabilization and surface capping of SeNPs by functional groups inherent to EPS.

4.6.4 XRD and Raman spectroscopy analysis

The morphological characteristics of NL(C)-SeNPs were assessed using X-ray diffraction (XRD) and Raman spectroscopy (Fig. 4.12A and B). The XRD analysis revealed the absence of distinct Bragg reflection peaks, indicating that the red-coloured NL(C)-SeNPs possess an

amorphous structure. This finding was further substantiated by Raman spectroscopic analysis, which displayed sharp peaks at 254 cm^{-1} and 234 cm^{-1} (Fig. 4.12B), corresponding to the amorphous and trigonal phases of selenium, respectively. These spectral features suggest that while the nanoparticles are predominantly amorphous, localized structural rearrangements indicative of trigonal selenium may occur.

The Raman spectral profile of NL(C)-SeNPs aligns with observations reported by Kora et al. demonstrating that selenium nanoparticles synthesized by a *Bacillus* isolate (mean size ~92.6 nm) exhibited a trigonal configuration in Raman analysis, despite appearing amorphous in XRD (Kora, 2018). This apparent phase transition is attributed to the effect of the Raman laser, which is known to induce structural conversion from amorphous selenium to its more thermodynamically stable trigonal form. A similar phenomenon was reported by Liu et al. where repeated Raman analysis of colloidal selenium nanospheres in the α-Se phase led to a gradual transformation into the trigonal phase, driven by the high-energy laser exposure used during spectral acquisition (Liu et al., 2025).

EPS-SeNPs - The X-ray diffraction (XRD) (Fig 4.13A) and Raman spectral studies (Fig 4.13B) demonstrated that EPS-SeNPs and SeNPs possess an amorphous structure. Selenium has three allotropic forms, i.e., trigonal form (t-Se), amorphous form (α-Se), and the least stable monoclinic form (m-Se). Trigonal form consists of helical morphology, and simultaneously, amorphous exists in a disordered chain array morphology. In the following study, Raman spectral analysis of EPS-SeNPs (Fig. 4.13B) revealed features similar to those of NL(C)-SeNPs

Among the various forms of selenium, amorphous α-selenium (α-Se) is particularly valued for its advantageous properties in applications such as solid-state imaging and X-ray detection (Kasap & Rowlands, 2000). In addition to its functional utility, α-Se exhibits lower cytotoxicity and demonstrates enhanced anticancer and potent antibacterial properties (Kasap & Rowlands, 2000). Similar results were reported by Gao et al. where selenium nanoparticles (SeNPs) coated with *Polyporus umbellatus* extract showed comparable biological activities (Gao et al., 2020).

The structural phase of SeNPs can vary significantly depending on the capping or stabilizing agents used during synthesis. For instance, amorphous SeNPs coated with β-lactoglobulin or ferulic acid were reported (Cui et al., 2018, Zhang et al., 2018). In contrast, SeNPs synthesized with lentinan or polysaccharide complexes exhibited a trigonal crystalline phase (Jia et al.,

2015). These discrepancies underscore the influence of surface-stabilizing biomolecules on the resulting structural conformation of SeNPs. In the Raman analysis for EPS-SeNPs, features observed were consistent with NL(C)-SeNPs. This divergence observed in the case of both nanoparticles can be due to laser-induced phase transition by the Raman laser.

The capping material, EPS, exhibited a predominantly amorphous structure, as revealed by XRD analysis, with minor crystalline features indicated by weak diffraction peaks at 21.0°, 26.6°, and 32.0° (Fig. 4.13A). The primarily amorphous nature of EPS can be attributed to the irregular and disordered arrangement of its polysaccharide chains, which hinders the formation of well-defined crystalline structures. Similar amorphous characteristics have been reported for EPS produced by Lactic acid bacteria (Amini et al., 2022), whereas EPS derived from *Aeribacillus pallidus* displayed a more crystalline nature (Genc et al., 2024).

Amorphous materials generally possess higher solubility, faster dissolution rates, and improved bioavailability compared to their crystalline counterparts. These physicochemical properties often translate into enhanced biological activity, making the amorphous form particularly advantageous for biomedical applications. The observed amorphous structure of EPS in this study, therefore contributes not only to the stabilization of SeNPs but may also enhance their therapeutic potential.

Raman spectral analysis provided additional insight into the compositional nature of EPS. The deconvoluted Raman spectra revealed key vibrational features indicative of the structural components of the EPS (Fig. 4.14, Table 4.5). In the spectral range of 200–500 cm^{-1}, weak vibrational signals corresponding to skeletal vibrations of sugar backbones were observed, which are typically associated with endo- and exocyclic ring deformations in carbohydrates (Tahir et al., 2020). Further Raman bands detected in the range of 700–760 cm^{-1} can be attributed to C_1–H_1 stretching vibrations, characteristic of glucan- or fructan-based sugar residues (Brezeștean et al., 2021).

Additionally, prominent bands in the range of 1100–1380 cm^{-1} were identified and assigned to C–O and C–C stretching vibrations, as well as C–O–H bending modes features commonly associated with polysaccharide-rich biopolymers (Tahir et al., 2020). Notably, no distinct peaks were observed in the 1600–1700 cm^{-1} region, which typically corresponds to amide I vibrations in proteins. This absence suggests a relatively low protein content in the EPS under investigation, reinforcing earlier biochemical observations.

4.7 Bioactive properties of NL(C)-SeNPs

NL(C)-SeNPs were further evaluated for their bioactive potency through various optimised *in-vitro* assays.

4.7.1 Anti-oxidant properties

NL(C)-SeNPs demonstrated significant antioxidant activity against free radicals in a dose-dependent manner, with scavenging efficiency increasing proportionally with nanoparticle concentration. This activity was evaluated using two 96-well-established assays: DPPH and ABTS. In the DPPH assay, approximately 80% radical quenching was observed at 100 µg/mL of NL(C)-SeNPs, compared to the quercetin control (Fig. 4.15A). In the ABTS assay, NL(C)-SeNPs exhibited even stronger activity, achieving ~72% quenching at the same concentration, while quercetin reached ~90% (Fig. 4.15B). The superior performance in the ABTS assay may be attributed to the aqueous environment, which enhances nanoparticle dispersion and interaction with radicals.

The half-maximal effective concentration (EC_{50}) for the NL(C) capping agent alone was 80 µg/mL, whereas the EC_{50} for NL(C)-SeNPs was notably lower, i.e., 60 µg/mL in the ABTS assay and 70 µg/mL in the DPPH assay, indicating enhanced antioxidant potency upon nanoparticle formation. While the NL(C) extract itself exhibited inherent antioxidant properties, likely due to its rich phenolic and flavonoid content as confirmed by TPC and TFC assays, the incorporation of selenium further amplified the effect. This synergistic enhancement is attributed to both the antioxidant potential of selenium and the stabilizing, bioactive functional groups from the capping agent.

In contrast, uncapped SeNPs showed markedly lower antioxidant activity, likely due to their intrinsic instability. Their high surface energy promotes aggregation, which reduces surface area and diminishes reactive potential. This underscores the critical role of NL(C) as an

effective capping agent that not only stabilizes SeNPs but also enhances their functional bioactivity, making them more suitable for practical biomedical applications.

Numerous studies support our findings, highlighting that the antioxidant activity of SeNPs is closely influenced by their particle size and the nature of the capping or stabilizing agents employed. Qiu et al. synthesized pectin-decorated SeNPs with an average size of 41 nm and reported an EC_{50} value of 500 µg/mL (Qiu et al., 2018). In contrast, Kokila et al. developed smaller SeNPs (~16 nm) stabilized using *Diospyros montana* extract, which exhibited a significantly lower EC_{50} of 22.5 µg/mL, demonstrating how green synthesis and effective stabilization enhance biological activity (Kokila et al., 2017). Similarly, in a study synthesized SeNPs were synthesized using a polysaccharide-protein (PSP) complex derived from *Corbicula fluminea*, yielding particles of 43 nm in size (Wang et al., 2018). When stabilized at a Se/PSP ratio of 1:2, these nanoparticles showed optimal antioxidant performance with an EC_{50} of 80 µg/mL.

These findings collectively reinforce the conclusion that the antioxidant activity of NL(C)-SeNPs in our study is significantly influenced by both nanoparticle size and the surface functionalization derived from the bioactive metabolites present in the *N. guilienensis* extract. The synergistic effects of selenium and phytochemical-rich capping agents contribute to the enhanced free radical scavenging efficiency observed.

4.7.2 Anti-microbial property of NL(C)-SeNPs

Further studies have also increasingly highlighted the antimicrobial potential of SeNPs, with many reports documenting pronounced activity, particularly against Gram-negative bacteria. In our study, the NL(C)-SeNPs exhibited greater inhibitory effects against one Gram-negative, i.e., *Escherichia coli* (Fig. 4.16A) and two Gram-positive bacteria, i.e., *Enterococcus faecalis* and *Staphylococcus aureus* (Fig. 4.16 B and C). The MTT-dye-based antibacterial test evaluated NL(C)-SeNPs at concentrations ranging from 2 to 2000 µg/mL with minimum inhibitory concentration, i.e., MIC, as follows 62.5 µg/mL (*E. coli*), 125µg/mL (*E. faecalis*), 250µg/mL (*S. aureus*). Two-way ANOVA associated with the Bonferroni test carried out between SeNPs, NL(C), and NL(C)-SeNPs indicated that there was a significant difference ($p<0.001$) in the percent inhibitory effect of NL(C)-SeNPs when compared with the other two fractions. A comparison between SeNPs and NL(C) showed that there was a significant difference ($p<0.001$) only on exposure to 500 mg/mL and beyond. Observations by other

researchers also support the present findings. Al-Hakimi et al. investigated SeNPs stabilized with polyvinyl alcohol and carboxymethyl cellulose and demonstrated their antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* (Gram-positive), as well as *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative) (Al-Hakimi et al., 2022). A similar trend was observed in another study that synthesized SeNPs using plant extracts such as onion, acerola, and boldo, and reported stronger antibacterial activity against Gram-positive species (Dos Santos Souza et al., 2022). Their study also emphasized the role of zeta potential in modulating antibacterial efficacy.

In one significant study explained that bacterial surface charge is influenced by cell wall composition, which contributes to this variation, i.e., nanoparticles and bacterial cell interaction (Arakha et al., 2015). Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides (LPS), which can lead to different electrostatic interactions compared to Gram-positive bacteria, whose thicker peptidoglycan layers exhibit different surface charges. Supporting this, a study reported that SeNPs synthesized using *Azadirachta indica* (neem) extract and possessing a high negative zeta potential (-46 mV) displayed effective antibacterial activity against both Gram-positive and Gram-negative strains (Mulla et al., 2020).

These findings collectively indicate that surface charge is a critical determinant of nanoparticle-bacteria interactions. However, it is to be noted that other parameters, such as nanoparticle size, surface coating, and the nature of the stabilizing agents, also play a significant role in influencing antibacterial activity.

The antifungal activity of NL(C)-SeNPs was evaluated against filamentous fungi, including *Aspergillus niger*, *Fusarium laterium*, *Alternaria alternata*, and *Aspergillus flavus,* using the neutral red staining assay. Among the tested strains, NL(C)-SeNPs exhibited potent antifungal effects, particularly against *A. niger,* i.e., ~46% growth inhibition at 250 µg/mL (Fig. 4.17A) and *F. laterium,* i.e., ~40% growth inhibition at 250 µg/mL (Fig. 4.17B). The minimum fungicidal concentration (MFC) was determined to be approximately 125 µg/mL for *A. niger* and 62.5 µg/mL for *F. laterium*.

Simultaneously, in the control group, both the NL(C) capping agent and the uncapped SeNPs displayed noticeable antifungal activity. However, when linked together NL(C)-SeNPs demonstrated superior efficacy, suggesting a synergistic effect between the selenium

nanoparticles and the bioactive constituents of the *N. guilienensis* derived capping agent. Although elemental selenium is known for its intrinsic fungicidal properties, the enhanced antifungal response observed in the capped nanoparticles indicates that surface functionalization plays a crucial role in amplifying biological activity. Furthermore, a statistically significant difference ($p < 0.05$) was observed in inhibition of fungal growth across different treatment concentrations, confirming the enhanced inhibitory effect of NL(C)-SeNPs in comparison to NL(C) and uncapped SeNPs administered individually.

There are relatively few studies specifically addressing the antifungal activity of selenium nanoparticles (SeNPs), although emerging evidence highlights their broad-spectrum antimicrobial potential. Fouda et al. demonstrated the antifungal efficacy of biosynthesized SeNPs against clinical isolates of *Candida* species (Fouda et al., 2022). Similarly, Filipović et al. reported the antimicrobial activity of SeNPs capped with chitosan and bovine serum albumin (BSA), suggesting that the mechanism of action may involve the generation of reactive oxygen species (ROS) (Filipović et al., 2021). ROS production can disrupt essential cellular processes by inhibiting DNA replication, interfering with amino acid synthesis, and damaging the cell membrane.

Gunti et al. further confirmed the antifungal activity of phyto-fabricated SeNPs synthesized using *Emblica officinalis*, showed strong inhibitory effects against a wide range of foodborne fungal pathogens (Gunti et al., 2019). Notably, selenium itself is recognized for its potent fungicidal properties, often exceeding its bactericidal effects. This has led to its incorporation in commercial antifungal products, such as anti-dandruff shampoos, where it helps suppress fungal growth on the scalp (Oliveira et al., 2025).

The findings of our study are consistent with these reports, particularly in demonstrating that the incorporation of bioactive capping agents can significantly enhance the antimicrobial efficacy of SeNPs. The increased surface area and the presence of functional groups on the nanoparticle surface likely facilitate stronger electrostatic interactions with microbial cells, thereby amplifying their inhibitory effects on fungal and bacterial growth.

4.7.3 SEM morphological observation on NL(C)-SeNPs impact

NL(C)-SeNPs impact on microbial cells was observed on the MIC dose concentration of 62.5 µg/mL for *E.coli* and 125 µg/mL for *A.niger*. On SeNPs exposure, varying morphological

changes were observed in the microbial cells. For instance, in *E.coli*, cell surfaces were distorted with noticeable wrinkling and the presence of pores, indicating significant disruption to the cell membrane. Certain membrane blebs indicating damage were also observed. Simultaneously, in fungal cells, it was observed that on exposure to SeNPs, *A.niger* hyphae appeared shrunken, collapsed, and wrinkled, and seemed to indicate loss of osmotic pressure, and in certain areas, grooves were also observed, indicating hyphal damage due to the antifungal effect of SeNPs.

Nanoparticles (NPs) exhibit various mechanisms for antibacterial action. The abrupt onset of drug resistance is a principal factor driving the synthesis of nanoparticles to leverage their nanomedicine potential. The entry of nanoparticles and their impact on microbes are influenced by various factors, including the structure of the cell wall or membrane. One method of bacterial inhibition involves the capacity of nanoparticles to generate reactive oxygen species at elevated concentrations, thereby facilitating their penetration into the bacterial cell through disruption of the cell wall or membrane (Oliveira et al., 2025). Numerous reports indicate that positively charged nanoparticles induce ionic interactions with bacterial cells, facilitating their penetration and destabilizing various cellular processes, ultimately leading to lysis (Parvin et al., 2025; Dai et al., 2022). However, a study by Pescuma et al. found that biogenic SeNPs synthesized by *Delftia spp*. exhibited an antimicrobial effect specifically against the wood-rotting fungus *Oligoporus pelliculosus*. Upon exposure to a dose of SeNPs, it was observed microscopically that the growth of the fungal hyphae was significantly impeded, and the hyphae's morphology appeared to be affected or disrupted due to selenium exposure (Pescuma et al., 2023).

4.7.4 *In-vitro* anti-cancer activity of NL(C)-SeNPs

The cytotoxic effects of NL(C)-SeNPs and uncapped SeNPs were evaluated against HepG2, i.e., liver carcinoma cell lines. Both nanoparticle formulations exhibited a similar trend of dose-dependent inhibition of cell viability, with NL(C)-SeNPs showing enhanced cytotoxic activity compared to uncapped SeNPs (Fig. 4.19A). In contrast, the capping agent alone, NL(C), did not exhibit significant cytotoxicity after 24 hours of incubation (Fig. 4.19B), suggesting that the observed cytotoxic effects were primarily due to the selenium nanoparticles rather than the capping material. A concentration-dependent reduction in cell viability was observed as the dose increased from 100 to 800 µg/mL for both NL(C)-SeNPs and uncapped SeNPs. While the difference in cytotoxicity between capped and uncapped SeNPs at each concentration was

69 marginal and not statistically significant ($p > 0.05$), a highly significant difference was observed across the concentration range tested ($p < 0.001$), confirming a dose-dependent effect.

The enhanced cytotoxicity of NL(C)-SeNPs may be attributed to the potential oxidation of zero-valent selenium (Se^0) into its ionic forms, selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), during 1 cellular interactions. These ionic forms are known to contribute to increased reactive oxygen species (ROS) generation and oxidative stress, leading to apoptosis and cell death. The similarity in cytotoxic trends between capped and uncapped SeNPs suggests that the selenium core plays the dominant role, while the NL(C) capping may further modulate nanoparticle stability and bioavailability, enhancing their overall cytotoxic efficacy. The highest inhibition of 67% was observed for NL(C)-SeNPs at a concentration of 800 µg/mL, while a slightly higher inhibition of 74% was noted for uncapped SeNPs (Fig. 4.19A). Selenium is widely recognized for its anticancer activity against various malignancies, including breast, lung, kidney, and osteosarcoma (Kuršvietienė et al., 2020; Wang et al., 2025). However, the direct use of naturally occurring selenium compounds such as selenomethionine and selenocysteine in cancer therapy is limited due to their toxicity and poor bioavailability (Kuršvietienė et al., 2020). In contrast, selenium nanoparticles (SeNPs) offer superior bio-dispersion, enhanced surface area, and porosity, making them more suitable for biomedical applications (Vijayakumar et al., 2022).

The cytotoxic potential of SeNPs has been demonstrated in numerous studies. Haddadian et al. reported a significant dose-dependent anticancer effect of niosome-loaded SeNPs against MCF-7 breast cancer cells (Haddadian et al., 2022). Similarly, Cittrarasu et al. observed dose-dependent cytotoxicity of SeNPs in HBL100 and MDA-MB-231 cell lines (Cittrarasu et al., 2022). Beyond direct cytotoxicity, selenium also exhibits chemopreventive properties by mitigating the toxic side effects of certain antibiotics. SeNPs capped with phytochemicals from *Terminalia arjuna* were shown to protect human lymphocytes from arsenic (As^{3+})-induced DNA damage and cell death (Prasad et al., 2014). In another study, oral administration of *Lactobacillus brevis* conjugated with SeNPs in a mouse model of metastatic breast cancer enhanced interferon production and delayed immune hypersensitivity responses, contributing to improved therapeutic outcomes (Yazdi et al., 2013). These findings collectively support the potential of SeNPs as a chemotherapeutic and chemopreventive agent. When functionalized with bioactive moieties or co-administered with drugs, SeNPs can facilitate controlled selenium release, thereby improving efficacy while minimizing systemic toxicity.

4.7.5 Viability of PBMC studies by NL(C)-SeNPs

Peripheral blood mononuclear cells (PBMC) represent the body's first line of systemic defense, as they consist of T cells, B cells, NK cells, and monocytes. Across the tested concentration range (100–800 µg/mL), NL(C)-SeNPs exhibited a significant reduction in PBMC viability beyond 200 µg/mL of dose concentration (Fig 4.20). In contrast, the capping agent alone (NL(C)) demonstrated no observable cytotoxicity. The assay was performed in triplicate, and statistical evaluation using two-way ANOVA revealed no significant difference in cell viability between treatments with NL(C)-SeNPs and NL(C) ($p < 0.001$). Furthermore, in comparison, uncapped SeNPs, NL(C)-SeNPs showed no significant cytotoxicity at lower concentrations (100 and 200 µg/mL); however, at higher concentrations (400 and 800 µg/mL), a significant reduction in PBMC viability was observed, suggesting a concentration-dependent cytotoxic response. The data suggest that NL(C)-SeNPs had a concentration-based dosage effect on cell viability. As it goes without saying that too much of anything is bad, a proper dosage of toxicity in any formulation is necessary to assess, to prevent any sort of systemic toxicity if the formulation (in this case, NL(C)-SeNPs) is intended to be used for injection or topical application. Although the anti-viral activity of the *Nerium oleander* extract is well known, it is also thoroughly studied for its immuno-modulatory activity by Jensen et al. in their study. PBMC studies were done, and it was observed that the *N.oleander* extract directly activated the natural killer cell response, which, in a chain of reaction, triggered the production of cytokines (Jensen et al., 2023).

However, in the following observation, it was seen that the fungal extract of *N.oleander* has not shown any significant inhibition towards the cell viability. In certain cases, endophytes may carry silent gene clusters that can result in certain immunologically inactive metabolites production (Schulz et al., 2002). Another possibility is that the concentration dose of the extract may be low or the metabolites may lack pattern recognition ligands, which are somewhat vital to induce a cytotoxic response against PBMCs (Tariq et al., 2017).

4.8 Bioactive properties of EPS-SeNPs

Subsequent to the assessment of NL(C)-SeNPs, additional quantitative bioactivity experiments were conducted to ascertain the potential antioxidant capacity, antibacterial efficacy, and anticancer properties of EPS-SeNPs.

4.8.1 Antioxidant activity of EPS-SeNPs

EPS-SeNPs exhibited significant antioxidant activity as assessed by the DPPH assay, with an EC_{50} value of 70 µg/mL, compared to the quercetin standard (Fig. 4.21B). In comparison, uncapped SeNPs showed a maximum antioxidant potential of only 10% at 100 µg/mL, while EPS alone demonstrated moderate activity, achieving 40% scavenging at the same concentration. These findings suggest that the combination of selenium and EPS substantially enhances radical scavenging efficiency.

In the ABTS assay, EPS-SeNPs displayed strong antioxidant activity in a concentration-dependent manner, with an EC_{50} of 80 µg/mL, as compared to the quercetin standard (Fig. 4.21A). The progressive increase in radical inhibition with rising EPS-SeNPs concentration highlights the effectiveness of EPS as a capping agent in enhancing the bioactivity of SeNPs.

Overall, EPS-SeNPs showed better antioxidant performance compared to SeNPs and EPS alone, confirming the synergistic role of the EPS coating in improving nanoparticle stability and bioactivity. When benchmarked against the quercetin standard, the antioxidant activity of EPS-SeNPs was found to be significantly different ($p < 0.05$), further underscoring their potential as effective antioxidant agents. The antioxidant activity of EPS-SeNPs observed in this study is consistent with findings reported by Prasathkumar et al. where photo-fabricated SeNPs derived from the non-edible parts of *Senna auriculata,* designated as SAF-SeNPs and SAL-SeNPs, exhibited dose-dependent antioxidant activity as assessed by both DPPH and ABTS assays.

In their study, the EC_{50} values for the DPPH assay were 62.14 µg/mL for SAF-SeNPs and 43.63 µg/mL for SAL-SeNPs, while in the ABTS assay, the EC_{50} values were 75.19 µg/mL and 58.52 µg/mL, respectively (Prasathkumar et al., 2022). Similarly, Ramya et al. reported the antioxidant activity of biosynthesized selenium nanoparticles produced using actinobacteria. Their study emphasized that seleno-compounds possess potent antioxidant potential and can enhance cellular defense mechanisms by protecting cells and tissues from free radical-induced damage (Ramya, Shanmugasundaram, & Balagurunathan, 2015).

4.8.2 Antimicrobial activity of EPS-SeNPs

There is a growing demand in pharmaceutical research for molecules that simultaneously exhibit both antioxidant and antimicrobial properties. This interest stems from the hypothesis that compounds with strong antioxidant potential may also exert antibacterial effects. One proposed mechanism is that certain bacteria rely on nascent oxygen for survival; thus, antioxidants that scavenge free radicals and reactive oxygen species (ROS) can indirectly inhibit bacterial viability by disrupting redox balance.

Simultaneously, EPS-SeNPs demonstrated antibacterial activity against both Gram-positive and Gram-negative bacterial strains. Notably, significant inhibition was observed against *Bacillus subtilis* (Fig. 4.22A), *Staphylococcus aureus* (Fig. 4.22B), and *Enterococcus faecalis* (Fig. 4.22D) among Gram-positive bacteria, as well as against *Escherichia coli* (Fig. 4.22E) and *Salmonella enterica* (Fig. 4.22C) among Gram-negative strains. The MIC among the Gram-negative strains was 62.5 µg/mL (*E. coli*) and 125 µg/mL (*S. enterica*), and for Gram-positive strains, it was 31.2 µg/mL (*S. aureus*), 125 µg/mL (*E. faecalis*), and 62.5 µg/mL (*B. subtilis*). A statistically significant difference ($p < 0.05$) in antibacterial efficacy was observed between EPS-SeNPs and the individual components (uncapped SeNPs and EPS) across all tested strains.

While conventional antibiotics are generally effective against bacterial infections, the rise of multi-drug-resistant (MDR) strains poses a significant challenge. Metal-based nanoparticles, such as SeNPs, offer promising alternatives due to their multifaceted mechanisms of action. These include cell wall disruption, enzyme inactivation, and interaction with intracellular targets, which make them less susceptible to resistance development (Zhang et al., 2021). SeNPs have been proposed as effective agents against a range of nosocomial infections, owing to their ability to release selenium ions in a controlled and targeted manner, thereby damaging bacterial structures (Zhang et al., 2021).

The antimicrobial activity of nanoparticles is heavily influenced by their physicochemical characteristics, particularly size, shape, and concentration. Smaller nanoparticles have been shown to possess superior penetration capabilities, allowing them to breach bacterial cell walls and membranes more efficiently (Sirelkhatim et al., 2015). In this study, the dose-dependent

antibacterial efficacy of EPS-SeNPs is likely attributable to the higher selenium content. At elevated concentrations, selenium exerts a dual mode of action: promoting cell wall lysis and compromising membrane integrity. This leads to intracellular imbalance, disruption of homeostasis, and ultimately, microbial cell death (Yuan et al., 2023).

However, clinical trials investigating the supplementation of antioxidants such as tocopherol, selenium, and β-carotene to reduce cancer risk have yielded inconclusive or even adverse outcomes, including increased mortality rates (Bardia et al., 2008). These limitations may be overcome by incorporating antioxidant compounds into nanoparticle systems or designing nanoparticles with intrinsic bioactive capabilities. Such systems can offer improved metabolic stability, controlled drug release, and targeted delivery, thereby enhancing therapeutic efficacy.

1 EPS-SeNPs also exhibited notable antifungal activity against filamentous fungal strains, including *Alternaria alternata*, *Fusarium laterium*, and *Aspergillus niger*. Among the tested strains, EPS-SeNPs exhibited potent antifungal effects, particularly against *A. alternata* at 250 µg/mL, i.e., ~23% growth inhibition (Fig. 4.23A) and *F. laterium* (Fig. 4.23B), i.e., ~30% growth inhibition (Fig. 4.23B) and ~10% growth inhibition for *A. niger* (Fig. 4.22C). The 1 minimum fungicidal concentration (MFC) values were determined to be 62.5 µg/mL for *A. alternata* (Fig. 4.22A), 125 µg/mL for *F. laterium*, and 250 µg/mL for *A. niger*. A statistically significant difference ($p < 0.05$) in antifungal activity was observed between EPS-SeNPs and 1 the individual components (uncapped SeNPs and EPS) across all tested strains. A similar level of statistical difference was noted among the various concentrations examined.

The enhanced antifungal efficacy of EPS-SeNPs underscores the importance of capping or stabilizing agents as therapeutic enhancers. When biocompatible nanoparticles are functionalized with bioactive capping agents like EPS, a synergistic effect is often observed. This is attributed to covalent or non-covalent interactions between the capping ligands and the nanoparticle surface, which introduce steric hindrance and improve colloidal stability (Javed et al., 2022). At the nanoscale, the proportion of selenium atoms exposed at the particle surface increases significantly, and this surface effect is further amplified by the stabilizing properties of the capping material.

Yip et al. demonstrated the efficacy of nano-selenium modified with biogenic polysaccharide–protein (PSP) complexes from *Pleurotus tuber-regium*, which effectively inhibited the growth of *Staphylococcus aureus* and *Trichophyton rubrum* when applied to fabric surfaces (Yip et al., 2014). Similarly, Pescuma et al. reported that SeNPs suppressed the growth of the wood-decaying fungus *Oligoporus pelliculosus* (Pescuma et al., 2023). In another comparative study, SeNPs capped with different agents showed significant antifungal activity against several plant-pathogenic fungi, including *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, and *Diaporthe longicolla*, outperforming silver nanoparticles (AgNPs) in certain cases (Vrandečić et al., 2020). While AgNPs are widely recognized for their biological utility, concerns over the environmental toxicity associated with the release of ionic silver limit their widespread application. In contrast, SeNPs are increasingly favoured in therapeutic contexts due to their enhanced bioavailability, reduced toxicity, and environmentally safer profile.

4.8.3 SEM morphological observation of microbial cells on EPS-SeNPs exposure

EPS-SeNPs demonstrated a clear dose-dependent antimicrobial effect. To observe the impact of EPS-SeNPs onto the microbial cells, SEM analysis was done of the treated microbial cells. EPS-SeNPs were given at a dose correlating to its MIC i.e, 31.2 µg/mL for *S.aureus* and 125 µg/mL for *F.laterium*. On observation, *S. aureus* treated cells showed pleomorphic morphology with imperative disruption in the membrane, which can be observed viz., pit and grooves seen on the bacterial cell surface. Nanoparticles seemed to result in membrane damage, or they can further result in interaction with intracellular membrane components which can result in bactericidal effect (Sinha et al., 2011). Furthermore, *F. laterium* hyphae appeared shrunken, suggesting a loss of osmotic imbalance (Fig. 4.24). These disruptions in morphology suggest ROS-mediated damage, increased membrane permeability, or mechanical stress from nanoparticle deposition, all of which can result in microbial destabilization.

Our findings are consistent with earlier research group observations, such as those made in a study employing SeNPs stabilized by bovine serum albumin (BSA) appeared to exhibit essential membrane disruption in bacterial strains (such as *Listeria moncytogens* and *Enterobacter cloacae*) similar to ours, i.e., via pits and grooves (Yuan et al., 2023). Additionally, a study by Desouky et al. found that chitosan-capped selenium nanoparticles exhibited a strong antifungal effect (Desouky et al., 2025). Further, in one study, by Gahlawat

et al. it was observed that the exopolysaccharide-capped silver nanoparticles also seemed to show a prominent antibacterial effect against *Vibrio cholerae* (Gahlawat et al., 2016). SEM observation of the fungal hyphae impact revealed that the hyphae appeared to have lost their osmotic balance, which resulted in a shrunken and membrane-damaged state of the hyphae. This observation is consistent with our hyphal disruption observation.

4.8.4 *In-vitro* anticancer activity of EPS-SeNPs on HepG2 cell line

A cell viability assay was performed to evaluate the cytotoxic effects of EPS-SeNPs on the HepG2 liver carcinoma cell line. The assay was conducted using the MTT method across a concentration range of 100 to 800 µg/mL. A dose-dependent decrease in cell viability was observed, with EPS-SeNPs demonstrating a maximum inhibition rate of 68.7% at 800 µg/mL (Fig. 4.25). In comparison, uncapped SeNPs exhibited a slightly higher inhibition rate of 74%. EPS alone showed negligible cytotoxicity across all tested concentrations, indicating minimal effect on cell proliferation. Statistical analysis revealed significant differences ($p < 0.001$) in cytotoxicity between EPS-SeNPs and uncapped SeNPs at most concentrations. However, no significant variation was observed in the cytotoxic effect of EPS alone across the tested range, reinforcing the conclusion that selenium, rather than the EPS matrix, was responsible for the observed cytotoxicity. Moreover, a significant difference was observed between both SeNP formulations (capped and uncapped) and EPS when analysed across multiple exposure concentrations.

Although the precise anticancer mechanism of SeNPs remains to be fully elucidated, several studies suggest that their cytotoxicity may stem from intracellular oxidative stress. Cancer cells are known to maintain an acidic microenvironment and exhibit disrupted redox homeostasis. This intracellular milieu may facilitate the peroxidative modification of selenium nanoparticles, resulting in increased free radical generation. On the one hand, this induces mitochondrial membrane breakdown, leading to the release of pro-apoptotic proteins (Zhuang et al., 2020; Sonkusre & Cameotra, 2017). On the other hand, it triggers endoplasmic reticulum (ER) stress, further contributing to apoptosis. The combined effects of mitochondrial disruption and ER stress ultimately activate caspase enzymes, which are central to the execution of programmed cell death.

4.8.5 Viability of PBMC studies of EPS-SeNPs

To evaluate the cytotoxicity of EPS-SeNPs on immune cells. Peripheral blood mononuclear cell (PBMC) viability assay analogous to the one employed for NL(C)-SeNPs was conducted to assess the immune compatibility of EPS-SeNPs (Fig. 4.26). PBMC assays as suggested previously, serve as predictive models to investigate potential cytokine responses and immune activation following exposure to test agents, in this case, EPS-SeNPs. Across the tested concentration range (100–800 µg/mL), EPS-SeNPs did not exhibit any significant reduction in PBMC viability. Similarly, the capping agent alone (EPS) demonstrated no observable cytotoxicity. The assay was performed in triplicate, and statistical evaluation using two-way ANOVA revealed no significant difference in cell viability between treatments with EPS-SeNPs and EPS ($p < 0.001$). In comparison, uncapped SeNPs showed no significant cytotoxicity at lower concentrations (100 and 200 µg/mL); however, at higher concentrations (400 and 800 µg/mL), a significant reduction in PBMC viability was observed, suggesting a concentration-dependent cytotoxic response. These findings align with previous results from this study, where EPS-SeNPs demonstrated selective cytotoxicity against HepG2 liver carcinoma cells while sparing normal immune cells.

The favourable cytocompatibility of EPS-SeNPs may be attributed in part to the redox balance in healthy cells. SeNPs, despite their pro-oxidant properties, typically do not induce oxidative stress in normal cells due to the well-regulated redox homeostasis, which prevents excessive reactive oxygen species (ROS) generation (Bisht et al., 2022).

Given these findings, it is imperative to thoroughly assess the immunological and cytotoxic profiles of nanoparticle-based systems, especially when considering their integration into therapeutic or biomedical applications. Based on the compelling evidence of biological efficacy and biocompatibility, EPS-SeNPs were deemed suitable for further evaluation as a bioactive coating for gauze matrices in wound healing applications.

4.9 Biofunctionalization of cotton gauze with EPS-SeNPs

As observed, EPS-SeNPs synthesized from the fungal strain *N. guilinensis* exhibited an average particle size of 43.7 nm, and owing to the significant antibacterial activity exhibited by EPS-SeNPs, they were selected for further application in the biofunctionalization of sterile cotton gauze fabric. The coating was performed using a simple dip-coating method. However, initial washing with autoclaved distilled water resulted in a significant leaching of EPS-SeNPs from

the gauze surface (as illustrated previously in Fig 3.3), indicating that the nanoparticles were only loosely adsorbed. This instability can be attributed to electrostatic repulsion between similarly charged surfaces. The cellulose fibers of cotton gauze carry negatively charged hydroxyl (-OH) groups, and the zeta potential of the EPS-SeNPs was previously measured to be –28.2 mV, confirming their negative surface charge. The interaction between two negatively charged surfaces, cotton and EPS-SeNPs, likely resulted in poor adhesion, consistent with the principle that "like repels like."

To enhance the adhesion of EPS-SeNPs to cotton gauze. During the deposition process, sonication was employed to promote the formation of microbubbles. The subsequent collapse of these microbubbles generated localized high pressure and temperature, thereby improving the penetration and uniform distribution of chitosan within the cotton fiber matrix. Chitosan is a cationic polysaccharide derived from the deacetylation of chitin and is composed of two repeating subunits: N-acetylglucosamine and glucosamine. Due to its wide-ranging biomedical applications, chitosan has garnered considerable interest as a biopolymer. Both chitosan and its depolymerized derivatives, including oligomers, exhibit diverse bioactivities, particularly bactericidal and bacteriostatic effects (No et al., 2002). The hydroxyl and amino functional groups present in chitosan are largely responsible for its bioactive properties, contributing to its mucoadhesive behaviour and antimicrobial efficacy.

At acidic pH, the amino groups in chitosan become protonated, resulting in a net positive charge on the molecule. This cationic nature facilitates electrostatic interactions with negatively charged materials, such as exopolysaccharides (EPS) that are commonly used to stabilize or cap selenium nanoparticles (SeNPs). These interactions enhance the compatibility between chitosan and EPS-SeNPs, enabling better surface integration and functional coating.

4.10 Characterization study of functionalized cotton gauze EPS-SeNP@CH_CG

Most microbial EPS, including those secreted by fungi, tend to carry a net negative charge. This is primarily due to the presence of uronic acid residues such as galacturonic and glucuronic acids, which contribute to the anionic nature of the polymer (Nguyen et al., 2024). This charge differential plays a crucial role in facilitating the interaction between cationic chitosan and anionic EPS, thus improving the structural stability and functional performance of EPS-SeNP-coated gauze.

The functional modification of cotton gauze through chitosan coating followed by encapsulation with EPS-SeNPs (EPS-SeNP@CH_CG**)** is expected to significantly alter the surface characteristics of the material, such as surface area and roughness, thereby improving its antibacterial efficacy.

Additionally, the hygroscopic nature of chitosan may enhance the moisture absorption capacity of the gauze, potentially influencing its effectiveness in absorbing wound exudates. However, it is essential to maintain the structural integrity of the gauze to preserve its suitability as a medical dressing, while simultaneously optimizing its antimicrobial performance. To assess these functional changes, X-ray diffraction (XRD) and Fourier-transform infrared (FTIR) spectroscopy analyses were conducted.

The X-ray diffractograms (Fig. 4.27A) revealed changes in the crystallinity of gauze samples. The control gauze exhibited characteristic peaks at 15.3°, 22.6°, and 34.5°, corresponding to the (110), (200), and (004) lattice planes of cellulose I (Iβ), indicating the presence of a crystalline cellulose structure. These crystalline patterns were consistently observed in the untreated cotton gauze (CG), chitosan-coated gauze (CH_CG), and EPS-SeNP-functionalized gauze (EPS-SeNPs@CH_CG), suggesting that chitosan and SeNPs were amorphously deposited onto the cotton surface without disrupting the core crystalline structure. Complementary insights were obtained from FTIR spectroscopy (Fig. 4.27B, Table 4.6). In the EPS-SeNPs@CH_CG sample, an increase in the broad band near ~3300 cm^{-1} was observed, corresponding to hydroxyl (-OH) stretching vibrations. This increase is attributed to the disruption of intra- and intermolecular hydrogen bonds within the cellulose matrix, leading to the exposure of additional free –OH groups (Lang et al., 2021) . These hydroxyl groups can interact with the protonated amine groups ($-NH_2$) of chitosan, facilitating strong intermolecular hydrogen bonding.

A slight increase in crystallinity was noted in the CH_CG sample, likely due to the ordered alignment of chitosan molecules along the cellulose chains. This molecular arrangement promotes a more organized structure, which may enhance the overall crystallinity of the material. These observations are consistent with previous studies. For instance, Ali et al. reported that chitosan-coated cellulose microfiber cotton supported metal nanoparticles through amorphous surface layering, which also resulted in a subtle increase in crystallinity (Ali et al., 2017). Similarly, Raza et al. observed increased crystallinity upon chitosan loading,

which was slightly reduced after subsequent incorporation of α-tocopherol onto the modified surface (Raza et al., 2020).

Crystallinity in cotton fibers is a critical factor for their performance in therapeutic applications. Cotton's structural composition includes both amorphous and crystalline regions. While higher crystallinity contributes to improved durability, reduced swelling, and enhanced thermal stability, it may also decrease flexibility and fluid absorption attributes that are crucial for wound dressing materials. Furthermore, excessive crystallinity could lead to fabric brittleness, thereby affecting user comfort and handling.

Further spectral shifts in the 1000–1100 cm^{-1} region are attributed to interactions between chitosan and the C–O stretching vibrations of cellulose. A slight reduction in CH stretching intensity indicated successful deposition of chitosan onto the cotton surface. Peaks observed in the 700–800 cm^{-1} region in EPS-SeNPs@CH_CG are consistent with selenium-oxygen (Se–O) stretching vibrations (Lang et al., 2021), confirming the presence of selenium interactions. Notably, the hydrogen bonding between –OH groups in cellulose and both –OH and $–NH_2$ groups in chitosan despite the absence of a chemical cross-linker likely contributes to the observed broadening or shifting of the 3300–3500 cm^{-1} band, as previously reported (Gao et al., 2023).

4.11 Morphological characterization of the functionalized gauze EPS-SeNPs@CH_CG

Initially, a chitosan (CH) layer was deposited onto the cotton gauze (CG) to impart cationic surface properties. While this modification did not lead to any distinct visual colour change, it rendered the gauze surface slightly rougher in texture, indicating a physical alteration of the surface morphology. Subsequently, EPS-capped selenium nanoparticles (EPS-SeNPs) were deposited onto the dried, chitosan-treated gauze (CH_CG) using a simple dip-coating method.

This step resulted in a noticeable and uniform colour change from white to orange-red, which visually confirmed the successful deposition of nanoparticles on the gauze surface. The homogenous coloration across the treated gauze further suggested an even chitosan coating, which likely facilitated uniform EPS-SeNP adherence.

To verify these observations, both untreated gauze (CG) and nanoparticle-functionalized gauze (EPS-SeNPs@CH_CG) were characterized using field emission scanning electron microscopy

(FESEM) (Fig. 4.28A) and elemental mapping (Fig. 4.28B). A strong selenium signal was observed on the fiber surfaces of the treated gauze, confirming the successful and uniform deposition of SeNPs onto the modified substrate.

Numerous strategies have been reported for the functionalization of gauze materials aimed at enhancing antibacterial performance, wound healing, and drug delivery properties. For instance, Rehan et al. developed a biomedical cotton gauze functionalized through cationization using 3-chloro-2-hydroxypropyl trimethyl ammonium chloride. In a parallel approach, they also employed partial carboxymethylation using monochloroacetic acid to enable the controlled release of oxytetracycline hydrochloride (Rehan et al., 2017). Similarly, Liu et al. fabricated antimicrobial gauze by employing a mussel-inspired surface modification method using a polydopamine/polyethyleneimine matrix to facilitate silver nanoparticle adhesion (Liu et al., 2020).

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Another relevant approach was proposed by Said et al. who functionalized cotton gauze through dual treatment: cationic modification with chitosan and anionic carboxymethylation (Said et al., 2021). This method enabled effective immobilization of silver nanoparticles and resulted in gauze materials exhibiting strong antimicrobial activity along with enhanced UV protection properties. Collectively, these functionalization strategies demonstrate the versatility of cotton gauze as a substrate for biomedical applications. The current study's EPS-SeNPs@CH_CG system, with confirmed nanoparticle adherence and bioactivity, aligns well with and adds to this growing body of functional gauze materials.

4.12 Antibacterial activity of EPS-SeNPs@CH_CG

E. coli and *S. aureus* possess global clinical relevance, and given that EPS-SeNPs have demonstrated considerable antibacterial efficacy against both strains. Henceforth, they were chosen to examine the antibacterial capabilities of functionalized cotton gauze coated with EPS-SeNPs.

4.12.1 Growth curve assay and Colony count assay

As previously described and shown, EPS-stabilized selenium nanoparticles (EPS-SeNPs) demonstrated excellent bioactive properties, including strong antibacterial, antifungal, and cytotoxic activity against HepG2 liver cancer cell lines (Singh et al., 2024). These promising bioactivities formed the foundation for exploring EPS-SeNPs functionalization of cotton gauze for anti-bacterial application. For cotton gauze to be suitable for wound dressings, it must possess sustained antibacterial efficacy to inhibit microbial growth over extended periods.

One of the key methods used to evaluate this property is the bacterial growth curve assay, which provides time-resolved, quantitative data on bacterial proliferation in response to treatment. In the present study, an 8-hour growth curve analysis was performed against *Escherichia coli* (Fig. 4.29A) and *Staphylococcus aureus* (Fig. 4.29B). The control groups, untreated cotton gauze (CG) and chitosan-coated gauze (CH_CG), both showed a steady increase in bacterial growth, indicating minimal inhibitory effects. In contrast, the functionalized gauze (EPS-SeNPs@CH_CG) exhibited significant antibacterial activity, effectively suppressing the growth of both bacterial strains throughout the incubation period. A two-tailed t-test revealed that *E. coli* proliferation was significantly inhibited upon EPS-SeNP functionalization ($p < 0.001$), confirming the enhanced antibacterial efficacy of the modified gauze. Similarly, CH_CG exhibited moderate inhibition compared to untreated CG, suggesting that chitosan alone provides a partial barrier to bacterial growth. Comparable inhibition was observed for *S. aureus*, where EPS-SeNPs@CH_CG treatment significantly suppressed bacterial growth ($p < 0.01$) compared to the controls. These results were further validated using the colony-forming unit (CFU) count method. After exposure to EPS-SeNPs@CH_CG, both *E. coli* and *S. aureus* displayed a marked reduction in viable colonies compared to the CH_CG group, supporting the growth curve findings.

These results were further validated using the colony-forming unit (CFU) count method (Fig. 4.30 A, B, and C). After exposure to EPS-SeNPs@CH_CG, both *E. coli* (Fig. 4.30B) and *S. aureus* (Fig. 4.30C) displayed a marked reduction in viable colonies compared to the CH_CG group, supporting the growth curve findings. Upon CFU count after 24 h, it was observed that

the log reduction in *E. coli* was 2.71 (99.80% microbial reduction) and in *S. aureus* was 5.76 (99.99% microbial reduction).

Our results align with other reports on functionalized cotton gauzes. For instance, Sharma et al. demonstrated that sodium alginate/glycerol/tannic acid-coated gauze reduced viable *E. coli* and *S. aureus* colonies by more than 95% (Sharma et al., 2022). Similarly, Romero-Fierro et al. developed thermo- and pH-responsive gauze fabrics loaded with norfloxacin, showing strong antibacterial efficacy governed by diffusion-based drug release mechanisms (Romero-Fierro et al., 2023).

Additional studies have highlighted the utility of nanoparticle-coated gauzes. One notable example reported the long-lasting antibacterial activity of superhydrophobic gauze coated with chitosan nanoparticles, where the effects were attributed to contact-killing mechanisms and sustained selenium ion release. Another study by Cao et al. demonstrated the use of gold nanoparticle-coated gauze activated by light and heat, leveraging photothermal effects for efficient bacterial eradication (Cao et al., 2011).

Our results align with other reports on functionalized cotton gauzes. For instance, Sharma et al. demonstrated that sodium alginate/glycerol/tannic acid-coated gauze reduced viable *E. coli* and *S. aureus* colonies by more than 95% (Sharma et al., 2022). Similarly, Romero-Fierro et al. developed thermo- and pH-responsive gauze fabrics loaded with norfloxacin, showing strong antibacterial efficacy governed by diffusion-based drug release mechanisms (Romero-Fierro et al., 2023).

Additional studies have highlighted the utility of nanoparticle-coated materials such as gauzes or steel castings (Jadon et al., 2024). One notable example reported the long-lasting antibacterial activity of superhydrophobic gauze coated with nanoparticles, where the effects were attributed to contact-killing mechanisms and sustained selenium ion release (Liu et al., 2019). Another study by Cao et al. demonstrated the use of gold nanoparticle-coated gauze activated by light and heat, leveraging photothermal effects for efficient bacterial eradication (Cao et al., 2011). The rationale for integrating nanoparticles into cotton gauze extends beyond antimicrobial action it also supports potential applications in drug delivery and therapeutic interventions. While pharmaceutical applications emphasize bio-distribution, systemic

toxicity, and long-term biocompatibility, gauze-based applications must consider nanoparticle leaching, local cytotoxicity (e.g., skin irritation), and environmental safety.

In this context, the EPS-SeNPs@CH_CG formulation offers dual advantages: effective bacterial inhibition and reduced nanoparticle leaching due to strong surface immobilization. Moreover, the suppression of bacterial proliferation and potential inhibition of biofilm formation by EPS-SeNPs@CH_CG could enable a sustained antibacterial effect, making this material a promising candidate for advanced wound dressing technologies.

4.12.2 EPS-SeNPs@CH_CG impacted protein and polysaccharide leakage in cells

The microbial inhibition observed in the growth curve assay can be primarily attributed to the disruption of the structural integrity of bacterial cell walls and membranes, which normally act as protective barriers against environmental stressors. Selenium (Se) ions are known to compromise these defenses by damaging the bacterial cell wall, increasing membrane permeability, and disturbing intracellular homeostasis ultimately leading to microbial cell death (Wiita et al., 2024).

To evaluate the metabolic disruption induced by EPS-SeNPs@CH_CG on *E. coli* and *S. aureus*, cytoplasmic leakage of intracellular macromolecules, such as polysaccharides and proteins, were assessed. EPS-SeNPs@CH_CG exposure resulted in polysaccharide leakage levels of 40 ± 5.1 µg/mL for *E. coli* and 27 ± 9.9 µg/mL for *S. aureus* (Fig. 4.31C and 4.31D). Protein leakage levels were recorded at 94 ± 6.9 µg/mL for *E. coli* and 88 ± 12.7 µg/mL for *S. aureus* (Fig. 4.31A and 4.31B), indicating significant membrane damage in both strains.

Statistical analysis using a t-test confirmed that protein leakage, used as a marker for bacterial cell death, was highly significant in *E. coli* upon exposure to EPS-SeNPs@CH_CG ($p < 0.001$), with a similarly significant effect observed in *S. aureus* ($p < 0.05$). Intracellular sugar leakage was also statistically significant for both bacterial strains ($p < 0.05$), further validating the membrane-compromising effect of EPS-SeNPs@CH_CG.

Interestingly, EPS-SeNPs@CH_CG demonstrated differential antibacterial effects, with *E. coli* appearing more susceptible to selenium exposure than *S. aureus*. This can be attributed to differences in cell wall architecture. The outer membrane of Gram-negative bacteria, such as *E. coli* contains a flexible lipopolysaccharide (LPS) layer, which is more prone to disruption

compared to the thicker peptidoglycan layer in Gram-positive bacteria, like *S. aureus*. Sahoo et al. reported that oxidative stress generated by SeNPs' photocatalytic activity caused more pronounced cytoplasmic leakage in *E. coli* than in *S. aureus*, leading to faster cell death (Sahoo et al., 2023).

These findings suggest that selenium present on the surface of EPS-SeNPs@CH_CG imposes oxidative stress on bacterial membranes, resulting in the leakage of intracellular contents and eventual microbial death. This supports the utility of EPS-SeNPs-functionalized gauze as a potent antibacterial material for biomedical applications.

The antibacterial mechanisms of SeNPs have been well-documented across multiple studies. Hernández-Díaz et al. reported that biosynthesized SeNPs adhere to bacterial membranes, increasing their permeability and resulting in cell lysis (Hernández-Díaz et al., 2021). Filipović et al. demonstrated that SeNPs with varying surface chemistries induce the generation of reactive oxygen species (ROS), which leads to oxidative stress and structural damage in bacterial cells (Filipović et al., 2021). Additionally, Sans-Serramitjana et al. highlighted that SeNPs effectively inhibit biofilm formation one of the primary defense strategies employed by bacteria (Sans-Serramitjana et al., 2023). Vahdati et al. further illustrated that lysozyme-capped SeNP nanohybrids interact with bacterial proteins and enzymes, impairing critical cellular functions and causing cell death (Vahdati & Tohidi Moghadam, 2020) .

4.12.3 Anti-adhesion and contact-killing of EPS-SeNPs@CH_CG

The growth curve and cytoplasmic leakage assays demonstrated that the functionalized gauze, EPS-SeNPs@CH_CG, possesses significant antibacterial activity. However, for medical applications, especially in wound care, another critical factor is the material's capacity to prevent bacterial adhesion to its surface. Bacterial attachment not only facilitates colonization but also serves as a precursor to biofilm formation, which can compromise the antibacterial efficacy of the dressing and exacerbate wound infections.

Sterile cotton gauze is widely used as a wound dressing material in clinical practice due to its favourable mechanical properties, biocompatibility, and low cost. Additionally, its porous structure and hydrophilic surface functional groups contribute to efficient moisture management in wounds. However, these same features also provide a conducive microenvironment for microbial colonization and proliferation, often leading to inflammation

and heightened immune responses. Thus, an ideal wound dressing should also possess anti-adhesive properties to inhibit biofilm formation and microbial attachment.

In the present study, the EPS-SeNPs@CH_CG composite gauze was evaluated for its anti-adhesion efficacy and contact-killing ability. Prior research has shown that altering surface charge from negative to slightly positive can electrostatically repel bacterial cells, thereby enhancing antimicrobial activity. This principle aligns with our earlier findings on EPS-SeNPs bioactivity (Singh et al., 2024). In this work, chitosan served as a positively charged intermediate, promoting the stable adhesion of EPS-SeNPs onto the gauze matrix while contributing to the antibacterial performance through its inherent bioactive properties.

Experimental observations from the growth curve assay (Fig. 4.29A and 4.29B), colony-forming unit (CFU) analysis (Fig. 4.30C and 4.30D), and anti-adhesion assessment (Fig. 4.32A and 4.32B) confirm the synergistic antibacterial effects of chitosan and EPS-SeNPs. While chitosan-coated gauze (CH_CG) alone demonstrated modest bacterial inhibition, its efficacy was markedly enhanced upon further functionalization with EPS-SeNPs, forming EPS-SeNPs@CH_CG. This enhancement is attributed to two primary mechanisms: (1) the shift in surface charge induced by the negatively charged EPS-SeNPs, which influences bacterial membrane interactions, and (2) the formation of a hydration layer by chitosan, which physically hinders microbial colonization.

To visualize bacterial adhesion, both CH_CG and EPS-SeNPs@CH_CG were incubated with *E. coli* and *S. aureus* suspensions at 37 °C for 8 hours, followed by saline rinsing and fixation for SEM imaging. As shown in Fig. 4.32A, CH_CG exhibited dense bacterial colonization, whereas EPS-SeNPs@CH_CG showed substantially fewer adherent bacterial cells. Quantitative anti-adhesion analysis (Fig. 4.32B) revealed that EPS-SeNPs@CH_CG reduced *E. coli* adhesion by 77.8% and *S. aureus* adhesion by 71.3%. In contrast, the unmodified gauze (CG) demonstrated only 38.6% and 35.6% reduction in adhesion for *E. coli* and *S. aureus*, respectively.

Statistical analysis using two-way ANOVA confirmed a significant difference ($p < 0.05$) between the strains with reference to the bacterial adhesion between CG and EPS-SeNPs@CH_CG treatments. Furthermore, a student t-test between CG and EPS-

SeNPs@CH_CG treatments also showed a significant difference ($p < 0.05$) for each of the strains tested.

These findings support the hypothesis that the EPS-SeNPs@CH_CG surface operates via a dual mechanism of bacterial repulsion and contact-mediated killing, graphically represented in Fig. 4.32C, endowing the material with excellent antibacterial and anti-fouling characteristics suitable for biomedical applications.

These observations are consistent with the findings of Liu et al., who employed a mussel-inspired polydopamine and polyethyleneimine coating strategy to enhance cotton gauze surfaces (Liu et al., 2020). Their modified gauze exhibited low hemocompatibility, minimal cytotoxicity, and strong antibacterial properties, demonstrating bacterial cell repulsion as an effective defence mechanism against microbial invasion. A clear contact-killing effect was observed (Fig. 4.32A), as evidenced by the presence of pits, grooves, and morphological shrinkage in *S. aureus* and *E. coli* upon direct interaction with the functionalized cotton gauze (EPS-SeNPs@CH_CG).

This morphological damage indicates that the gauze surface exerts a direct bactericidal effect upon contact. Furthermore, the sustained antibacterial activity of EPS-SeNPs@CH_CG was corroborated through the growth kinetics assay, reinforcing the material's long-term efficacy. This prolonged antimicrobial effect is likely attributed to the controlled and continuous release of selenium ions from the gauze matrix, as shown in Fig 4.33.

Managing the release of selenium nanoparticles (SeNPs) is critical to reducing environmental risks and minimizing adverse human health effects. Khurana et al., discussed that excessive selenium exposure can elevate reactive oxygen species (ROS) generation, leading to oxidative stress and mitochondrial dysfunction (Khurana et al., 2019). Similarly, Werkneh et al. raised concerns regarding SeNP bioaccumulation, noting its potential for trophic transfer and biomagnification in aquatic ecosystems, along with risks related to accumulation in edible plant tissues, which can compromise food safety (Werkneh et al., 2023).

To address these concerns, our study emphasizes the importance of safe, regulated selenium release from the gauze. Using inductively coupled plasma optical emission spectroscopy (ICP-OES), we confirmed a sustained and controlled release profile of SeNPs from EPS-SeNPs@CH_CG (Fig. 4.33). This release mechanism helps maintain antibacterial activity

while limiting environmental toxicity. Green synthesis routes offer a viable approach to addressing environmental and toxicity concerns. In our previous work (Singh et al., 2024), EPS-SeNPs were synthesized via an eco-friendly method using exopolysaccharides derived from *N. guilinensis* as a natural capping and stabilizing agent. This fungal-mediated strategy minimizes the use of hazardous chemicals, making it suitable for biomedical applications.

Several studies have demonstrated the value of nanoparticle-functionalized cotton gauze in enhancing antimicrobial efficacy and promoting wound healing. For instance, Lang et al. functionalized gauze with gallic acid, which significantly improved both antibacterial performance and wound healing properties (Lang et al., 2021). Likewise, mesoporous silica nanoparticles loaded with antibiotics have shown notable antimicrobial activity when applied to cotton fabrics (Lang et al., 2021). Kannan et al. used *Andrographis paniculata* extract to biosynthesize copper and silver nanoparticles, which were then applied to cotton gauze, resulting in improved bioactivity (Kannan et al., 2014).

While these studies demonstrate the potential of various nanoparticle systems, our formulation is distinct in employing a fungal-derived green synthesis route for SeNPs. The successful deposition of EPS-SeNPs on chitosan-modified cotton gauze not only confers broad-spectrum antibacterial efficacy but also contributes to anti-fouling properties by preventing microbial adhesion and biofilm formation. Taken together, these findings highlight the potential of EPS-SeNPs-functionalized gauze as a promising material for clinical wound care applications.

5.0 Conclusion

67 Selenium is an essential micronutrient; however, it possesses a narrow therapeutic window, which limits its therapeutic usage. In both formulations, i.e., bulk or ionic selenium, it tends to approach the critical range more readily. Therefore, the transformation of selenium in its nanoform is a distinctive opportunity to explore its therapeutic application. As is evident by the abundant ongoing research, nano-formulation also aids in addressing the narrow therapeutic index, as it enables increased biocompatibility, surface modification, and controlled release. The primary aim of this study was to create stable, physiologically compatible selenium nanoparticle systems by green synthesis methods and to investigate their antioxidant, antibacterial, and cytotoxic characteristics, ultimately intending to tap into its biomedical applications.

In the initial phase of the study, endophytic fungal strains were extracted from the widely recognized ornamental plant, *Nerium oleander*, also known as the Ganer plant. Subsequent to isolation and morphological identification, six strains (NL(A) to NL(F)) were evaluated for their bioactivities to serve as effective capping agents for selenium nanoparticles. During the screening phase, NL(C) exhibited potential bioactivity in the antimicrobial assessment. To investigate further potential biocompatible capping agents, the exopolysaccharide (EPS) extract from the same strain was subjected to antimicrobial screening, yielding favourable antimicrobial results.

Upon molecular characterization NL(C) strain was revealed to be *Nigrospora guilinensis*. Due to effective screening results, the NL(C) secondary metabolite resources were then tapped in for selenium nanoparticle (SeNP) synthesis. The reduction of sodium selenite using ascorbic acid and fungal metabolites produced nanoparticles averaging 55 ± 7 nm in size, as verified by transmission electron microscopy. Hydrodynamic measurements indicated a stable colloidal suspension with adequate zeta potential to prevent aggregation. FTIR examination of functional groups indicated the presence of varying functional groups on the nanoparticle surface, which can be attributed to flavonoids and phenolic moieties, corroborated by tests of total phenolic (121.9 ± 1.88 µg/mL (gallic acid equivalent)) and total flavonoid content at (59.34 ± 2.47 µg/mL (quercetin equivalent)). These physicochemical characteristics are closely associated with biological performance. In radical scavenging experiments, the metabolite-stabilized SeNPs showed significant activity in both DPPH and ABTS systems, with a dose-dependent impact, signifying their ability to neutralize free radicals and reduce oxidative stress.

The antimicrobial findings were notably significant; SeNPs had potent inhibitory effects against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*, in addition to demonstrating antifungal activity against *Aspergillus niger* and *Fusarium laterium*. Moreover, in cell culture experiments, metabolite-stabilized SeNPs induced a dose-dependent reduction in the viability of HepG2 carcinoma cells, ranging from 100 to 800 µg/mL, demonstrating selective anticancer activity. Collectively, these findings indicated that fungal metabolites served as capping agents while simultaneously conferring bio-functional characteristics, resulting in nanoparticles with broad-spectrum bioactivity.

The second step relied on utilizing extracellular exopolysaccharides derived from the same fungal source as stabilizing agents. EPS are high-molecular-weight biopolymers made of carbohydrates and proteins, with several functional groups that may adhere to nanoparticle surfaces and provide steric hindrance to prevent aggregation. In SeNP synthesis, EPS produced nanoparticles with a reduced average size of 43.7 ± 13.7 nm compared to NL(C)-capped, signifying enhanced colloidal stability. EPS-SeNPs nanoparticles demonstrated superior antibacterial efficacy, successfully suppressing a broad spectrum of harmful microorganisms, including *B.subtilis*, *S.aureus*, *E. coli*, *S.enterica*, and fungi strains such as *A. alternata*, *A.niger*, and *F. lateritium*. Moreover, they demonstrated an enhanced antioxidant capability, attributable to the synergistic interactions between selenium and the functional groups in EPS. Moving forward, EPS-SeNPs also exhibited enhanced biocompatibility and selective cytotoxicity against cancer cells, while minimizing damage to primary cells i.e., PBMC cells, hence augmenting their suitability for biomedical applications.

In the third stage of this study, synthesized bipotent SeNPs, i.e., EPS-SeNPs were translated for a biomedical application-based study. In which functionalization of EPS-SeNPs onto chitosan-coated cotton gauze (CH_CG) resulted in an antimicrobial wound dressing termed as EPS-SeNPs@CH_CG, which was characterized by stable nanoparticle immobilization (validated by FTIR, XRD, SEM) with reference to controls, viz., uncoated gauze (CG) and chitosan-coated gauze (CH_CG). Further, bacterial inhibitory efficacy of functionalized gauzes was studied against *E. coli* and *S. aureus* through various *in vitro* assays. The integration of selenium core activity (ROS production) with biopolymer surface chemistry (EPS, chitosan) amplifies biological interactions, resulting in enhanced bioactive capabilities. EPS-SeNPs@CH_CG exposure resulted in polysaccharide leakage levels of 40 ± 5.1 µg/mL for *E.*

coli and 27 ± 9.9 µg/mL for *S. aureus*. Protein leakage levels were recorded at 94 ± 6.9 µg/mL for *E. coli* and 88 ± 12.7 µg/mL for *S. aureus*, indicating significant membrane damage in both strains which was supported by the colony-forming unit and growth curve assay, in which EPS-SeNPs@CH_CG, when exposed to the targeted strains, resulted in enhanced growth inhibition as compared to the controls. The enhanced anti-bacterial effect of the gauze fabric is attributed to two primary mechanisms: (1) the shift in surface charge induced by the negatively charged EPS-SeNPs, which influences bacterial membrane interactions based on like-repels-like, and (2) the sustained release of selenium, which hinders microbial colonization viz., contact killing efficacy.

Thus, the following doctoral work illustrates a prefatory comprehensive pipeline from eco-friendly synthesis to real-world application, using nanotechnology, natural products, and biomedical engineering concepts.

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