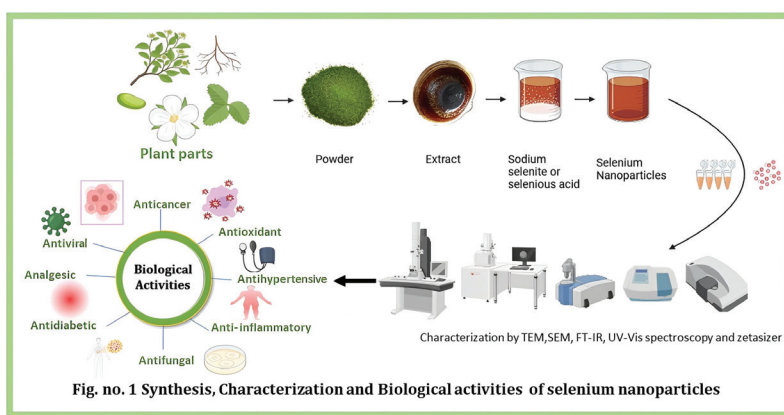


# Recent Progress in the Green Synthesis, Characterization, and Applications of Selenium Nanoparticles

Sajeda Samreen Sayyed Ibrahim<sup>1,\*</sup>, Yunus N. Ansari<sup>2</sup>, Abhijeet V. Puri<sup>3</sup>, Vikas V. Patil<sup>2</sup>, Sharad S. Gaikwad<sup>1,\*</sup> and R. Ansari Haroon<sup>1</sup>

## Graphical abstract



The graphical abstract summarizes the eco-friendly synthesis of Se-NPs by mainly using plant extracts followed by characterization thoroughly highlighting their biological activity. The green route followed to synthesize the nanoparticles is depicted in the synthesis chart, and characterizations have been done using UV-Vis spectroscopy, FTIR, Zetasizer, SEM, and TEM for further evidence of particle formation, stability, morphology, and size distribution as well. The recently synthesized nanoparticles' biological activities, derived from a range of plant sources, are summarized, highlighting their pharmacological potential and diverse therapeutic applications as reported in this review paper.

# Recent Progress in the Green Synthesis, Characterization, and Applications of Selenium Nanoparticles

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## Abstract

Selenium nanoparticles (Se-NPs) have attracted researchers' attention because of their unique attributes and potential for application in diverse areas, such as biological medicine, environmental remediation, and energy generation. This review summarizes recent progress in the green synthesis and characterization of Se-NPs. It elaborates on the fabrication of Se-NPs through chemical, biological, and physical techniques, including advantages and challenges. Moreover, techniques for evaluating the chemical and physical characteristics of NPs are described. The promising applications of Se-NPs are emphasized, including antioxidant, anticancer, and antimicrobial applications, and treatment of neurodegenerative diseases. Because of their exceptional properties and biocompatibility, Se-NPs are used in diverse industries. Recently, plant-extract synthesized Se-NPs have become increasingly used because of their benefits over chemically synthesized Se-NPs, including lower cost and greater environmental friendliness.

## Keywords

Characterization, green synthesis, selenium, selenium nanoparticles.

## Significance Statement

Recently synthesized Se-NPs from plant extracts are described, and their mechanisms, applications, advantages, toxicity, and other aspects are summarized. These Se-NPs are notable for their environmental sustainability, cost-effectiveness, and enhanced biocompatibility. Eco-friendly synthesis approaches eliminate hazardous chemicals, decrease production costs, and yield biocompatible nanoparticles with superior antioxidant and antimicrobial properties. Compared to chemically synthesized nanoparticles, the produced Se-NPs with the help of green synthesis are safer due to their reduced toxicity and are more suitable for a range of biomedical and environmental applications. This review advances green nanotechnology by highlighting innovative synthesis methods and various applications, and promoting sustainable research practices.

## Introduction

Richard Feynman's statement that "There's plenty of room at the bottom" has opened new doors for the scientific community, leading to research interest in nanotechnology, which pertains to the properties of matter at the atomic scale [1]. "Nanotechnology," a term originated by Norio Taniguchi, refers to the production of materials with one or more dimensions at the nanoscale. The goal of nanotechnology is to improve manufacturing processes while producing superior-quality products. Nanoparticles (NPs), nanocomposites, and nanowires are the structural and functional components of nanotechnology [2], the use of small materials and systems. Nanotechnology is expected to play a critical role in solving a variety of challenges,

including advancements in healthcare, environmental sustainability, and improving industrial efficiency and has the potential to influence countries' global economic standing [3]. Nanotechnology involves working with matter at the atomic and molecular scale, which is extremely small—usually between 1 and 100 nm [4]. Materials at the nanoscale have unique properties that make them suitable for commercial applications that benefit humanity, such as biological probes, diagnosis, catalysis, display devices, and optoelectronics [5].

In the field of nanotechnology, matter can be modified at the molecular and atomic scales, to produce materials with unique characteristics that can be applied to a range of challenges [6, 7]. Nanoscale materials have properties different from those of their larger counterparts, and can be

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Received: August 1 2024

Revised: August 27 2024

Accepted: September 2 2024

Published Online: November 5 2024

Available at: <https://bio-integration.org/>

commercialized [8, 9]. Synthesis of nano-materials, particularly metallic NPs, can be achieved through various methods, such as laser pyrolysis, supercritical fluid synthesis, spinning, the sol-gel method, mechanical milling, chemical vapor deposition, molecular condensation, chemical reduction, green synthesis, etching, sputtering, laser ablation, and electro-explosion [10]. The most economical and sustainable method among those discussed is the green synthesis of metallic NPs [11]. This method, compared with chemical methods, poses less risk of biological threats that might result in environmental toxicity. This method applies biological agents, such as plant parts and other microorganisms including bacteria and fungi, as reducing and stabilizing agents [12, 13].

Using living cells to produce NPs via biological pathways is a highly efficient and effective technique with greater mass yield than similar methods. Biochemicals and other components that can serve as stabilizing and reducing agents for the synthesis of green NPs are abundant in plants. This method is economical, safe, environmentally beneficial, and also more stable than other physical, chemical, and biological methods. The green synthesis of NPs can be categorized into three types: extracellular, intracellular, and phytochemical. The extraction of NPs from plant extracts is an economical process that achieves high yields, because of the abundance of phytochemical components that act as reducing and stabilizing agents in converting metal ions into metal NPs [14].

Selenium (Se) is an essential trace element in various physiological processes, such as metabolism and immune function [15]. Selenium NPs (Se-NPs) have unique physicochemical properties and biocompatibility, and consequently are useful in biomedicine, catalysis, and biotechnology and pharmaceutical sectors [16]. Recently, Se-NPs produced from plant extracts have become frequently used because of their advantages over chemically synthesized Se-NPs, including lower toxicity and higher sustainability [17]. Using plant extracts as reducing agents for Se-NPs is more cost-effective and environmentally friendly than conventional synthesis methods [18]. Active phytoconstituents in plant extracts act as capping agents and accelerate the conversion of selenite to fundamental selenium, thus yielding Se-NPs with diverse applications [19].

Selenium's valuable properties render it useful in various scientific fields including medicine, biology, physics, and chemistry. Se-NPs are of special interest because they interact with a variety of proteins and have strong biological activity. Functional groups such as C–O, C–N, NH, and COO– found in proteins are responsible for this interaction. Additionally, Se-NPs demonstrate high adsorption capacity [20]. Many studies have successfully produced Se-NPs from extracts of plants, such as *Terminalia arjuna* [21], *Vitis vinifera* (raisin), *Capsicum annum* [22], and fenugreek seeds [23]. This discovery has offered a new path for the environmentally friendly synthesis of Se-NPs by using plant extracts; this path may be valuable in many industries. Consequently, the use of plant extracts as reducing and stabilizing agents in Se-NP production is currently a major topic of scientific research [24].

The use of plant extracts in synthesizing Se-NPs advantageously enables precise control over the size and shape of the particles. Additionally, this process is straightforward

and can be replicated consistently, and therefore is suitable for large-scale industrial production [25]. The biogenic synthesis of Se-NPs by using plant extracts is a promising alternative to traditional methods that provides a sustainable and environmentally friendly option, while still preserving traditional knowledge, and has the potential to revolutionize multiple fields [26]. Because of their cost-effectiveness, sustainability, and eco-friendliness, plant extracts are increasingly used for the green synthesis of Se-NPs. This technique enables specific control over particle dimensions and therefore is highly suitable for diverse applications in biotechnology and medicine, such as biosensors, cancer therapy, antimicrobial agents, and targeted drug delivery [27–29].

## Synthesis methods of Se-NPs

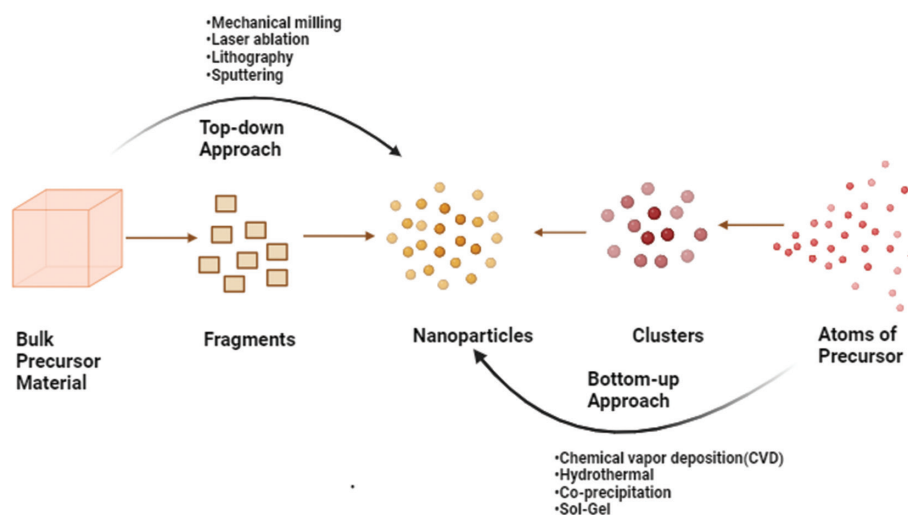
### Top-down approach

Selenium nanoparticles (Se-NPs) can be synthesized through biological methods utilizing the reducing and stabilizing properties of entities like plant extracts, microorganisms, and enzymes. Synthesis can be achieved through either top-down or bottom-up approaches, as shown in **Figure 1**. The first step in the top-down approach involves converting larger structures into nano-sized materials [30]. The top-down approach for the synthesis of nanoparticles (NPs) involves breaking down larger bulk materials into nanoscale particles. This method contrasts with the bottom-up approach, which builds NPs from atomic or molecular precursors. The top-down method has several drawbacks like the particles might not be uniform in size and shape, because of mechanical stress, vigorous shaking, and deformation during production. Although NPs can be produced on a larger scale, the top-down approach is not optimal in all cases [31]. When creating NPs, choosing the proper method is essential. The bottom-up approach tends to produce NPs with more distinct physical and chemical properties than the top-down approach. Therefore, the specific needs and objectives for NP synthesis must be carefully considered before selection of the most suitable method, to ensure that the desired results are achieved [32].

### Bottom-up approach

To synthesize NPs from molecules, certain materials are combined with agents that promote stability. Subsequently, the materials are subjected to specific conditions, such as heating, mixing, or chemical reactions [33]. NPs can be produced through a process called self-assembly, which enables control of the dimensions of the particles and addition of any necessary coatings or stabilizers. The production of metallic NPs starts with metal salts, which are broken into tiny atomic-sized particles. These particles then adhere and form NPs. This method is useful for producing NPs of the same size and shape, for applications such as drug delivery or catalysis. Self-assembly aids in control of the process and the production of uniform particles [34].

The bottom-up approach offers enhanced control over NP composition and surface characteristics. This technique also



**Figure 1** Illustration of the top-down and bottom-up approaches for the synthesis of nanoparticles.

enables addition of specific coatings or functional groups to the NP surface, thus improving the particles' stability and ability to interact with other materials. The bottom-up method is flexible and adaptable, thereby allowing for the production of NPs with various functionalities through the optimization of synthesis parameters, such as pH, temperature, and concentration, or use of different precursor materials [35]. In contrast, the top-down approach reduces larger structures, such as bulk materials or thin films, to nanoscale dimensions to form NPs. In creating NPs from molecules, specific stabilizing agents are combined with precursor materials and subjected to certain conditions, such as heating, mixing, or chemical reactions [36]. NP size and shape can be precisely controlled through a process called self-assembly, which includes adding necessary coating and stabilizing agents. Metallic NPs can be synthesized through a bottom-up synthesis method, in which metal salts are reduced to produce atomic-sized materials. These materials then undergo self-assembly through nucleation and growth, thus producing NPs with the desired dimensions. This method has many benefits, including the ability to create highly uniform NPs, thus aiding in applications that require particle uniformity, such as drug delivery systems or catalysis [37].

NPs can be made through either a bottom-up or top-down approach. The external appearance and composition of the particles can be more precisely controlled through the bottom-up method, which allows for specific coatings or functional groups to be added. Consequently, the particles are less prone to degradation and are better able to interact with other materials. The bottom-up approach can produce NPs with various functionalities through adjustment of synthesis parameters or use of different precursor materials. In contrast, the top-down approach reduces larger structures to nanoscale dimensions to synthesize NPs [38].

## Biogenic synthesis of Se-NPs

Se-NPs can be produced by biological systems including fungi, bacteria, enzymes, and plant parts. Biogenic synthesis methods are increasingly used because of their eco-friendly

and sustainable qualities. Unlike chemical approaches, which produce hazardous waste, biogenic synthesis converts soluble selenium ions, such as selenate or selenite, into NPs. This process uses biological agents such as plant extracts or microbial cells. Temperature, pH, concentration, and biological agent type all affect the process [39]. Biogenic synthesis is a cost-effective, environmentally friendly, highly stable, and biocompatible method for producing Se-NPs [40].

## Plant-mediated Se-NPs synthesis

Se-NPs can be synthesized biologically using plant extracts, which facilitate the reduction of soluble selenium ions into nanoparticles. This green synthesis technique, known for its ease of use, low cost, and environmental friendliness, has captured substantial research attention [41]. Important plant parts, including leaves, seeds, flowers, stems, and roots, either dried or fresh, can be used in the synthesis process. Bioactive compounds that are phytochemicals, such as flavonoids, anthocyanins, or carotenoids, are extracted by boiling or sonication in water or organic solvents. To promote the reduction of selenium ions into NPs, selenium salts (precursors), such as selenite or selenate, are mixed with the plant extracts under optimal conditions, such as controlled pH and temperature [42]. Flavonoids and terpenoids are used in the synthesis to stabilize the metallic Se-NPs created by reducing selenium ions. The plant type, selenium ion concentration, and reaction time are several variables affecting the dimensions of Se-NPs [43]. The evaluation of metallic Se-NPs can be performed with methods including ultraviolet-visible (UV-vis) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) [44]. The synthesis of Se-NPs with plants offers several advantages, such as excellent biocompatibility; non-toxicity; and potential applications in health-care, agriculture, and environmental remediation [45].

The method for synthesis of Se-NPs with selenious acid and plant extracts is as follows. The plant extract is prepared by mixing the plant material with water and allowing it to

stand for a period of time. The extract is then filtered to remove any solid particles. The plant extract is mixed with selenious acid, which acts as a selenium-containing precursor. The mixture is stirred at room temperature for a specific time interval, typically 12–72 hours. The plant extract contains biomolecules that act as reducing agents and stabilizers for the Se-NPs. The reduction of selenious acid to Se-NPs occurs under the influence of these biomolecules. The Se-NPs are separated from the reaction mixture by centrifugation at high speed, and are subsequently washed thoroughly with water and solvent to remove any residual plant extract or other impurities [51, 52].

### Bacteria-mediated Se-NP synthesis

Bacteria can be used to synthesize Se-NPs by converting soluble selenium ions into NPs with bacterial cells or cell-free extracts. This technique has attracted interest because of its simplicity, high yield, and potential for massive production. To increase the reduction of selenium ions into NPs, the synthesis process involves incubating bacterial cells or cell-free extracts with selenium salts, such as selenite or selenate, under controlled pH and temperature conditions. The resulting Se-NPs can be analyzed with various analytical methods, including UV-vis spectroscopy, TEM, and XRD. *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are among the bacterial species demonstrated to be able to produce Se-NPs. The synthesis process involves bacterial cells secreting reducing agents, such as proteins, enzymes, or metabolites, which aid in the transformation of selenium ions into NPs. The dimensions of Se-NPs are influenced by variables such as the type of bacteria, concentration of selenium ions, and reaction time. The biological synthesis of Se-NPs with bacteria offers numerous advantages, including high stability, potential therapeutic and environmental applications, eco-friendliness, and possibilities for future use [79].

### Fungi- mediated Se-NP synthesis

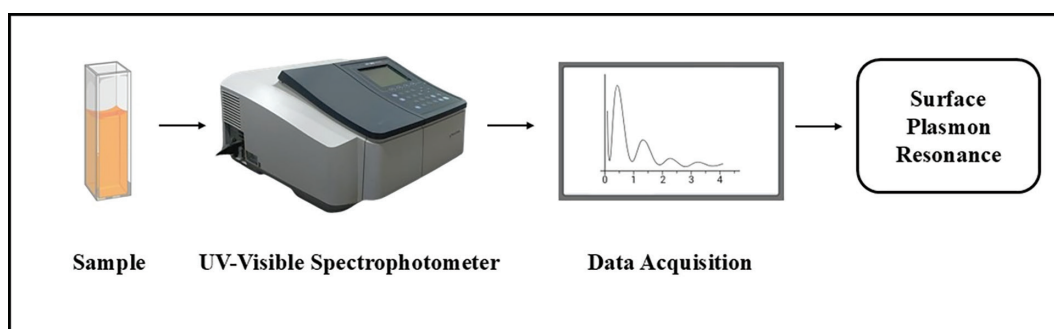
During the fabrication of Se-NPs with fungi, fungal cells or fungal extracts are used to transform soluble selenium ions into NPs. This technique is considered eco-friendly and

sustainable, because of its affordability and high yield. For synthesis of Se-NPs, fungal extracts or cells are incubated with selenium salts under suitable pH, temperature, and incubation time conditions. The enzymes, polysaccharides, and proteins in the fungal extracts or cells act as reducing agents in the conversion of selenium ions to NPs. The generated Se-NPs can be analyzed with analytical methods such as UV-vis spectroscopy, XRD, and TEM. Fungi such as *Candida glabrata*, *Aspergillus niger*, and *Penicillium* species have been used to produce Se-NPs. The dimensions of Se-NPs are influenced by many factors, such as the type of fungus, selenium ion concentration, and reaction conditions. The advantages of producing Se-NPs biologically with fungi include high stability; low toxicity; and potential biomedicine, biotechnology, and environmental remediation applications. Se-NPs generated from fungi have been demonstrated to possess anticancer, antifungal, antibacterial, and antioxidant properties, and can be used as drug delivery systems [79, 80].

## Characterization of Se-NPs

### Ultraviolet-visible spectroscopy

UV-vis spectroscopy is a tool used to estimate the optical properties of NPs. **Figure 2** shows the UV-Vis absorption spectra of the synthesized nanoparticles, illustrating the surface plasmon resonance. This method provides valuable information regarding particle size, shape, and composition. NPs have quantized energy levels because of their small size [81]. The size of Se-NPs affects their electronic structure, and consequently how they absorb and scatter light. These effects are particularly noticeable in the UV-vis region. This method helps researchers examine how NPs interact with light, thus providing essential information for understanding NP size and quantum properties [82]. UV-vis spectroscopy studies have shown that Se-NPs have unique electrical structures that cause them to absorb light in a distinct manner. Surface plasmon resonance information is essential for understanding how light interacts with NPs. Researchers have confirmed the presence of Se-NPs according to peaks in the UV region (200–400 nm) [83], as depicted in **Table 1**.



**Figure 2** Characterization of nanoparticles (NPs) by UV-Vis spectroscopy.

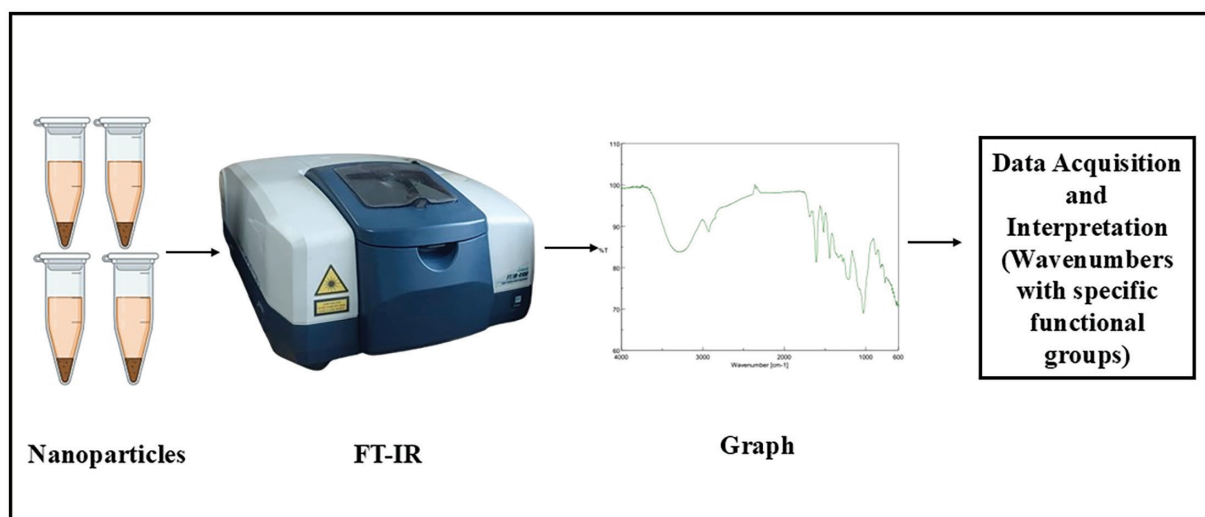
**Table 1** Plant-Mediated Fabrication of Se-NPs.

Sr. No	Plant Name	Plant Parts Used	UV (nm)	Average Size (nm)	Biological Activity	References
1	<i>Allium sativum</i>	Clove	260	100	Antimicrobial	[46]
2	<i>Azadirachta indica</i>	Leaves	286	168	Anthelmintic, antibacterial	[47]
3	<i>Brassica oleracea</i>	Florets	370	25	Antimicrobial	[48]
4	<i>Carica papaya</i>	Fruit	364	101	Antimicrobial	[49]
5	<i>Cassia angustifolia</i>	Seed	286	00–00	Antibacterial, antifungal	[50]
6	<i>Cassia auriculata</i>	Leaves	252	50	Anti-proliferative	[53]
7	<i>Citrus lemon</i>	Fruit juice	400	90	Antioxidant	[54]
8	<i>Citrus paradise</i>	Peel	550	10	Antibacterial	[20]
9	<i>Citrus reticulata</i>	Peel	265	70	Antimicrobial	[55]
10	<i>Citrus sinensis</i>	Peel	250–300	20	Antibacterial	[56]
11	<i>Clausena dentata</i>	Leaves	420	80	Larvicidal	[57]
12	<i>Cleistocalyx operculata</i>	Leaves	302	200	Antibacterial	[58]
13	<i>Clitoria ternatea</i>	Flower	635	106	Antibacterial	[59]
14	<i>Diospyros montana</i>	Bark	289	150	Antibacterial	[60]
15	<i>Encicostema axillare</i>	Leaves	325	98	Antibacterial	[61]
16	<i>Hibiscus sabdariffa</i>	Leaves	320	50	Antioxidant	[62]
17	<i>Moringa oleifera</i>	Leaves	530	20	Antioxidant	[63]
18	<i>Moringa peregrina</i>	Leaves	279	150	Antibacterial, anticancer	[64]
19	<i>Nigella sativa</i>	Seed oil	530	75	Larvicidal	[65]
20	<i>Ocimum gratissimum</i>	Leaves	300	50	Antimicrobial	[66]
21	<i>Opuntia basilaris</i>	Peel	280	90	Antibacterial	[67]
22	<i>Portulaca oleracea</i>	Leaves	266	30	Antimicrobial	[68]
23	<i>Psidium guajava</i>	Leaves	381	20	Antibacterial	[69]
24	<i>Punica granatum</i>	Peel extract	330	145	Antioxidant	[70]
25	<i>Ribes nigrum</i>	Fruit	265	50	Antioxidant	[71]
26	<i>Solanum lycopersicum</i>	Seed	350	100	Antimicrobial	[72]
27	<i>Terminalia arjuna</i>	Bark	289	150	Anticancer	[73]
28	<i>Theobroma cacao</i>	Seed	276	50	Antioxidant	[74]
29	<i>Tinospora cordifolia</i>	Stem	285	200	Antioxidant, anticancer	[75]
30	<i>Trigonella foenum-graecum</i>	Seed	200–400	50–150	Anticancer	[76]
31	<i>Vitis vinifera</i>	Fruits	280	100	Antioxidant	[77]
32	<i>Withania somnifera</i>	Root	622	22	Antioxidant	[78]

## Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) is a powerful technique for analyzing a material's functional groups

and chemical bonds, thereby providing information on Se-NP composition and surface properties. **Figure 3** presents the procedure to get FTIR spectra of synthesized nanoparticles. FTIR analysis reveals details regarding the surface chemistry and organic capping agents [84]. This method

**Figure 3** Characterization of nanoparticles (NPs) by fourier transform infrared spectroscopy (FTIR).

can help confirm the synthesis process and understand NPs' chemical environment. Plants rich in polyphenolic constituents are generally selected for synthesis. The active involvement of O–H, N–H, C=O, and C–O functional groups in the formation of Se-NPs can be validated by FTIR spectroscopy [85]. After the green synthesis of Se-NPs, the characteristic peaks observed in the FTIR spectrum are at 1375  $\text{cm}^{-1}$  (indicating phenolic OH), 1030  $\text{cm}^{-1}$  (corresponding to aromatic in-plane C–H bending), 1462  $\text{cm}^{-1}$  (representing asymmetric C–H bending in  $\text{CH}_3$  and  $\text{CH}_2$ ), and 1250  $\text{cm}^{-1}$  (indicating secondary O–H) [86]. Between 3200 and 3500  $\text{cm}^{-1}$ , bonded O–H stretching can be confirmed. Analysis of the FTIR spectrum of Se-NPs synthesized from plant extracts, provides insights into the chemical composition, surface functionalization, and surface modifications of NPs.

### Transmission electron microscopy

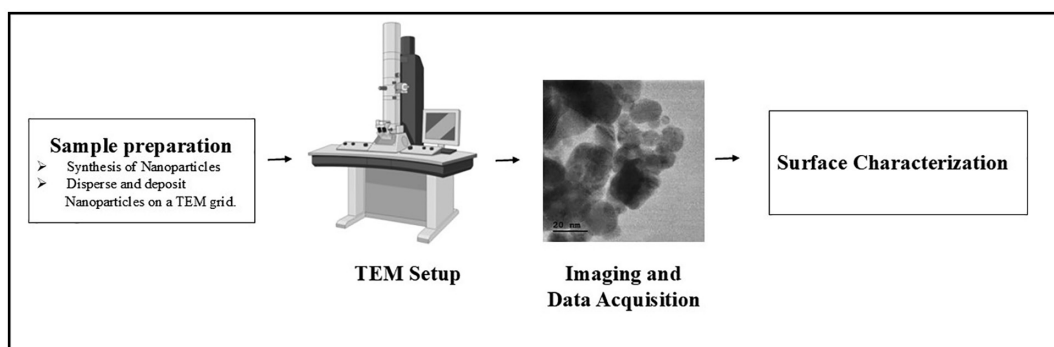
The fundamental morphology and dimensions of Se-NPs can be observed in high-resolution images produced by TEM. **Figure 4** showcases TEM image of the synthesized nanoparticles, providing detailed insights into their morphology, size, and distribution at the nanoscale. This information is essential for interpreting NPs' physical properties. NP size can be accurately measured with TEM. This aspect is important, because NPs' size significantly influences their properties, including their optical, electronic, and catalytic behavior [87, 88]. TEM can reveal details regarding the internal structure of Se-NPs and also provides insights into the spatial distribution of Se-NPs within samples. This information is valuable for understanding how well NPs are dispersed or aggregated, both of which influence NPs' activities and applications [88]. TEM can aid in identifying and visualizing any organic capping agents or ligands present on Se-NP surfaces. These coatings can potentially be incorporated during the synthesis process, and can affect NPs' stability and interactions with other substances [89]. TEM is used to characterize NPs with dimensions below 10 nm. Energy-dispersive X-ray spectroscopy can be coupled with TEM to analyze the chemical composition of NPs, thereby confirming the presence of selenium and any other elements in the NPs. This method also aids in identifying any additional elements introduced during synthesis [90].

### Scanning electron microscopy

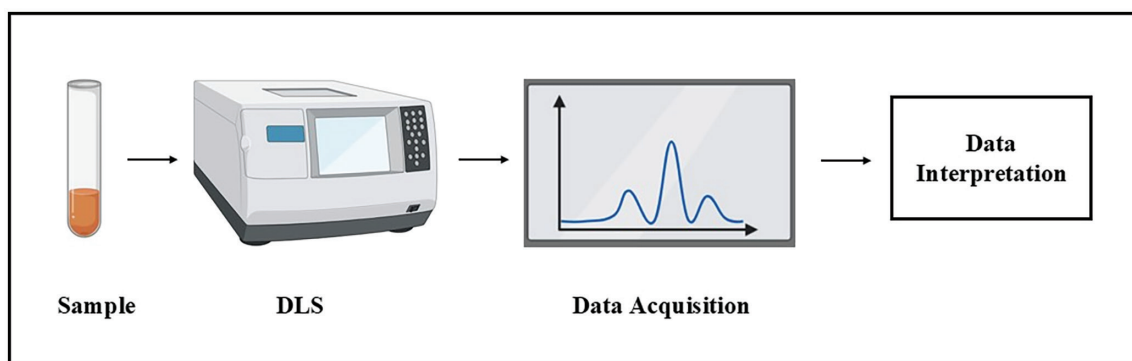
SEM is a valuable technique for visualizing NP morphology and providing details on particle size, shape, and surface characteristics. This technique enables high-resolution examination of the surface topography and structure of NPs. SEM scans NP surfaces with a focused electron beam. Different signals are produced as the electrons interact with the sample, including secondary and backscattered electrons. These signals are identified and used to generate an image of the NP surface. Consequently, researchers can examine NPs' structure, quantify their size and shape, and identify any surface defects. Alagesan et al. have revealed a distinct propensity of Se-NPs to aggregate—an observation substantiated by field-emission SEM images. These Se-NPs exhibit a spherical morphology, with diameters of 45–90 nm. During the synthesis of nanoparticles (NPs), particle aggregation becomes the dominant process, which masks the reduction of precursor atoms and the initial nucleation of these atoms [91].

### Dynamic light scattering

Dynamic light scattering (DLS) is a prominent approach for characterizing NPs in solutions, and providing information regarding their size distribution and mobility. **Figure 5** depicts the sample machine setup used for DLS analysis. The mechanism of characterizing Se-NPs with DLS is based on the principles of Brownian motion and the interaction of laser light with NPs in a solution [92]. This approach is widely used to characterize Se-NP size and distribution. This technique can determine the average size of NPs. The zeta potential of NPs can also be measured with DLS. The zeta potential measures NP surface charge and stability in a liquid medium, and is determined by analysis of the electrophoretic mobility of particles in an applied electric field [93]. Sani-e-Zahra *et al.* have used DLS analysis to calculate the average size of Se-NPs, thus demonstrating polydispersity in Se-NPs derived from tomato juice and seed extract sources. Two distinct model peaks were observed, at 989.5 nm and 151.7 nm, accompanied by a polydispersity index value of 0.432. The average size of the Se-NPs in the tomato juice extract was approximately 1020 nm [71].



**Figure 4** Characterization of nanoparticles (NPs) by transmission electron microscopy (TEM).



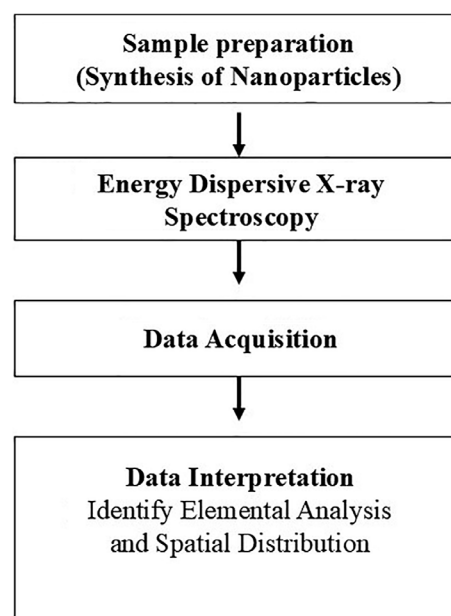
**Figure 5** Characterization of NPs by dynamic light scattering (DLS).

## X-ray diffraction

XRD, based on Bragg's law, illustrates X-ray diffraction by crystal planes. When X-rays collide with a crystalline sample, they interact with the lattice and are dispersed at different angles. The scattering angles can be used to calculate the interatomic distances within the crystal lattice. XRD can be used to determine the crystalline structure of Se-NPs. Exposing the NPs to X-rays provides diffraction patterns revealing information about the arrangement of atoms in the NPs. Consequently, the crystal structure can be identified, although the synthesis method and conditions can introduce variations. For example, selenium can exist in different crystalline forms, including hexagonal and amorphous forms, which can be distinguished by XRD. XRD data can be used to estimate the average Se-NP particle size through analysis of peak broadening in the XRD pattern, which is associated with the size of the crystalline domains in the NPs. Moreover, XRD can reveal the presence of impurities in the synthesized Se-NPs and can confirm Se-NPs' chemical composition and stoichiometry [94]. Hashem *et al.* have reported the XRD analysis of green synthesized Se-NPs and described the crystal and amorphous composition for precursor and synthesized Se-NPs, respectively [95].

## Energy dispersive X-ray spectroscopy

Energy dispersive X-ray spectroscopy (EDX) provides useful information regarding NPs' elemental compositions and chemical characteristics, through analysis of the energy distribution of X-rays emitted by a sample. **Figure 6** illustrates the process of EDX in nanoparticle characterization. EDX provides quantitative data on the elemental composition, including the ratio of selenium to other elements present, which can aid in assessing NP purity. Examining the spatial distribution of elements within an NP and its elemental composition is helpful. This approach is based on the X-ray fluorescence principle. High-energy X-rays ionize and excite atoms in the sample, and characteristic X-ray spectra are emitted. Advanced EDX systems, such as X-ray photoelectron spectroscopy (XPS or ESCA), provide information regarding the chemical states of elements, thereby



**Figure 6** Characterization of NPs by energy dispersive X-ray spectroscopy.

aiding in identifying chemical bonds and understanding NP surface chemistry. EDX spectroscopy can also be coupled with scanning electron microscopy to further enhance NP characterization [96]. Shahbaz *et al.* identified the solid absorption peaks of selenium ions at 1.35 keV, 11.20 keV, and 12.40 keV during the synthesis of selenium nanoparticles (Se-NPs) from plant extracts, using EDX spectra. According to EDX analysis, selenium coexists as peaks with other elements in elemental form [97].

## Zetasizer analysis

Particle size remains a key determinant of NP biodistribution, uptake, and clearance from the body. A Zetasizer instrument can be used to measure Se-NP particle size in terms of hydrodynamic diameter. The size distribution can also be characterized by the polydispersity index, which is expressed as mutually exclusive and opposite values: the lower the index, the higher the monodispersity. Zeta potential refers to NP surface charge and is used to determine the stability of an NP dispersion. Particles with a high zeta potential, whether



positively or negatively charged, generate repulsive forces that prevent aggregation, thereby ensuring long-term stability in the solution. Zeta potential also affects the behaviors of NPs toward biological entities such as cell membranes and proteins. When Se-NPs are characterized with a Zetasizer, synthesis methods can be tailored to synthesize Se-NPs of known size, with surface charges suitable for a given application. This information is valuable for establishing proper Se-NP formulations for drug delivery, imaging, and therapeutic applications in which size and stability play important roles in effectiveness and safety [98].

## Factors affecting the synthesis of Se-NPs

### Sources of reducing and stabilizing agents

#### Plant extracts

The type and the part of the plant used (leaves, stems, or roots) affects the synthesis because of the varying concentrations of phytochemicals such as flavonoids, phenolic compounds, and terpenoids [99].

#### Microorganisms

Various strains of bacteria, fungi, and algae have enzymatic pathways that affect the reduction and stabilization of selenium ions [100].

### Concentrations of precursor and reducing agents

Higher concentrations of selenium salts (e.g., sodium selenite or selenious acid) lead to the formation of larger NPs or higher yield, but can also increase the risk of aggregation [75, 101].

### pH of the reaction medium

The pH of the synthesis medium affects the charge on the NPs and the ionization state of the reducing agents, and consequently influences the reduction rate, particle size, and stability. Neutral to slightly alkaline pH is favorable for Se-NP synthesis [102].

### Temperature

Higher temperatures accelerate the reduction process, and affect the size distribution and crystallinity of Se-NPs [103]. Excessively high temperatures may lead to uncontrolled growth and aggregation.

### Reaction time

The duration of the synthesis process influences the growth and stabilization of NPs [103]. Shorter reaction times may result in incomplete reduction, whereas longer times can lead to larger particles or aggregation. Finding an optimal reaction time is important for achieving desired NP characteristics.

### Agitation and mixing

Proper mixing ensures uniform distribution of reducing agents and selenium precursors in the reaction medium, thus promoting homogeneous nucleation and growth of NPs [104]. Agitation speed can influence particle size and distribution.

### Ionic strength and presence of additives

The ionic strength of the medium, influenced by the presence of salts or other additives, can affect the electrostatic interactions between particles, thereby influencing their stability and aggregation behavior. The choice of solvent can influence the solubility of the precursor and reducing agents, as well as the reduction kinetics. Aqueous solvents are frequently used in green synthesis, because of their eco-friendliness [24, 105].

## Cytotoxicity of Se-NPs

In evaluating the suitability of green synthesized Se-NPs for biomedical purposes, investigating their cytotoxicity is crucial. The green synthesis method uses natural and environmentally friendly sources, such as plant extracts, to produce Se-NPs, which are presumed to be less harmful than chemically synthesized Se-NPs [106]. Several investigations have been conducted to determine the cytotoxicity of Se-NPs produced through green synthesis with various cell lines, including normal and cancer cells. The cytotoxicity of Se-NPs produced through green synthesis varies according to the concentration, size, surface charge, and duration of exposure. Although some studies have concluded that Se-NPs produced through green synthesis have minimal cytotoxicity at low doses, others have reported heightened cytotoxicity at higher concentrations. Notably, the cytotoxicity of Se-NPs may also be influenced by the cell type and biological environment in which they are used. Assays such as MTT, LDH, and Annexin V/propidium iodide can be used to evaluate the cytotoxicity of Se-NPs produced through green synthesis. These tests measure cell viability, membrane integrity, and apoptosis/necrosis after exposure to Se-NPs. Although green-synthesized Se-NPs are less hazardous than chemically synthesized Se-NPs, their cytotoxicity must be thoroughly investigated to ensure their safety and efficacy in various biological applications [107].

## Future advances

Se-NPs have substantial potential in diverse applications, as illustrated in **Figure 7**. Se-NPs have a high surface-to-volume ratio, thus enhancing their activity and making them more effective than larger particles. Se-NPs have substantial potential in a range of biological applications, including medication delivery, cancer therapy, and antioxidants. Studies have demonstrated their anti-cancer, antioxidant, antimicrobial, and anti-biofilm properties. The application of nano-Se medications has shown promising results in treating Huntington's disease. Se-NPs have notable semiconducting, photoelectric, and X-ray-sensing properties; are used in photocells, photocopying, photometers, and xerography; and are also important in renewable energy devices. Se-NPs are valuable in environmental applications because of their mercury-capturing properties.

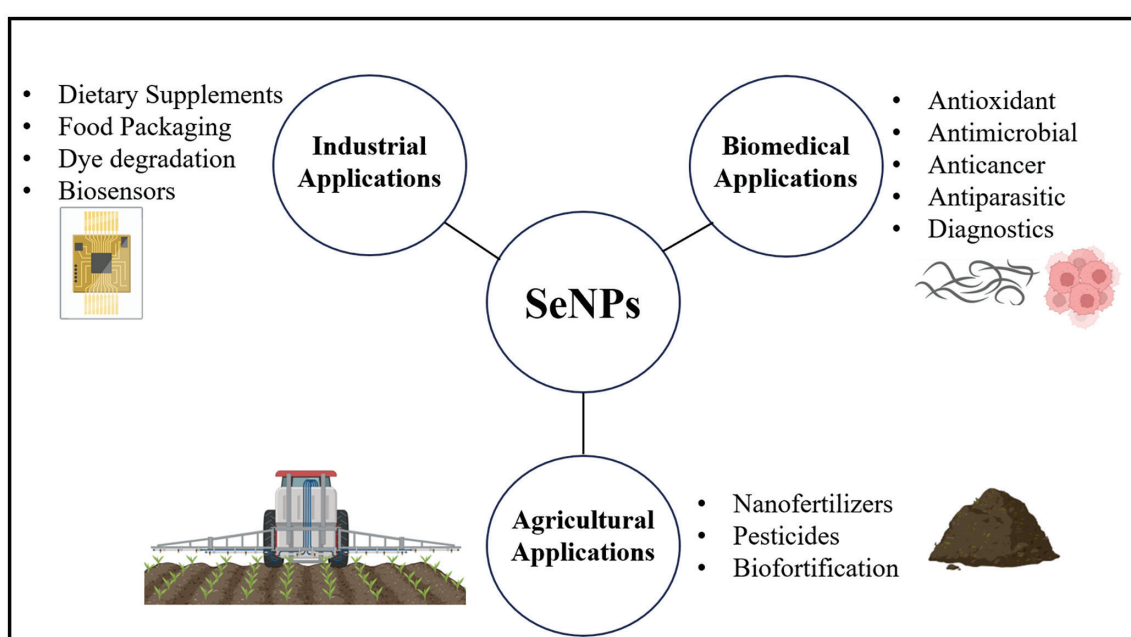
## Se-NPs in anticancer applications

Cancer is a major research focus, because it is the most destructive disease in the 21<sup>st</sup> century. Current challenges include problems of drug-induced toxicity and resistance. Various treatment methods are being tested to combat cancer. With the help of nanotechnology, personalized medicine has become more effective, by enabling better targeting while decreasing toxicity. Inorganic NPs, such as Se-NPs, have been successfully used to induce cytotoxicity in cancer cells. Se-NPs have the potential to decrease drug resistance and limit chemotherapeutic drug toxicity. Se-NPs derived from the probiotic bacterial strain *Lactobacillus casei* ATCC393 have been biogenically synthesized and demonstrated to suppress colon cancer cell proliferation, both in vitro and in vivo. At a treatment dose of 15 g/mL, Caspases 3/7 and

9 are activated by Se-NPs, thereby limiting the development of Caco-2 colon cancer cells. Moreover, Se-NPs have been found to activate intrinsic apoptotic pathway-associated apoptotic processes in CT26 and HT29 colon cancer cells [108]. The immunomodulatory effects of Se-NPs as an immunoadjuvant have been examined by Yazdi, et al., to develop a preventive tumor-associated antigen-based vaccine effective against breast tumors in mice [109]. For the prevention of cervical cancer, Se-NPs have been synthesized through green chemistry methods, and altered with a hydrophilic biocompatible polymer such as chitosan to incorporate anticancer drugs such as paclitaxel. Se-NPs act primarily by inducing apoptosis through caspase activation and mitochondrial dysfunction, thus generating reactive oxygen species (ROS), and leading to oxidative stress and DNA damage, inhibited cell proliferation via cell cycle arrest, and down-regulation of proliferative signaling pathways [110].

## Se-NPs in antimicrobial applications

Antimicrobials, including antibiotics, antivirals, antifungals, and antiparasitics, are currently essential in medical practice. Despite warnings regarding the adverse effects of antibiotic resistance, which was discovered in penicillin-resistant bacteria, antibiotics continue to be overused. Since the discovery of *Staphylococcus* in 1940, with extensive use of antimicrobials in food, medicine, and agriculture, multi-drug-resistant microorganisms have proliferated and have become more difficult to eradicate with potent antibiotics. The growth of antimicrobial resistance has necessitated development of alternative antimicrobials. Gold, silver, copper, titanium dioxide, and zinc oxide NPs are among those currently being researched. Many studies have shown excellent efficacy of Se-NPs as broad-spectrum antibacterial agents against bacteria, viruses, fungi, and parasites.



**Figure 7** Applications of green synthesized Se-NPs.

According to Sans-Serramitjana et al., the application of Se-NPs against oral pathogenic microorganisms such as *C. albicans*, *E. faecalis*, *P. gingivalis*, and *S. mutans* appears to be promising for in vitro reduction of planktonic and sessile microbial populations. Se-NPs exhibit antimicrobial activity by generating ROS that damage microbial cell walls and DNA, disrupting cell membranes to cause leakage, interacting with sulfur-containing proteins and consequently inhibiting microbial functions, and preventing biofilm formation. These mechanisms collectively enhance the effectiveness against various bacteria [111].

## NPs in antifungal applications

Se-NPs have antifungal properties and have found use in various biological applications. These NPs primarily interfere with essential fungal enzymes and proteins, thus disrupting metabolic processes. Lazcano-Ramirez et al. have conducted assays with Se-NPs at serial dilutions from 0 to 1.7 mg/mL, and have reported their antifungal activity against the commercially important plant pathogenic fungi *Fusarium oxysporum* and *Colletotrichum gloeosporioides*. Both Se-NPs showed antifungal activity against the plant pathogens at 0.25 mg/mL doses. Nile et al. have functionalized biogenic Se-NPs synthesized with the help of *Paenibacillus terreus* with nystatin (Se-NP@PVP nystatin nanoconjugates) and used them to inhibit *Candida albicans* growth, morphogenesis, and biofilm formation. Although Se-NPs produced during biological processes are inert, nanoconjugates have demonstrated antifungal activity against *C. albicans* by preventing growth, morphogenesis, and biofilm formation [112].

## Se-NPs in antidiabetic applications

Diabetes is a common metabolic disorder that affects many people and can greatly reduce their quality of life. According to the World Health Organization, diabetes is expected to affect 366 million people by 2030, and is associated with 1.5 million annual fatalities worldwide. Several factors contribute to the development of diabetes, including poor eating habits, stress, inactivity, obesity, inflammation, heredity, and age. However, various methods are available to manage diabetes and its associated complications, including dietary changes; engaging in physical activity; and closely monitoring blood pressure, glucose levels, and cholesterol. The protein hormone insulin is typically administered through subcutaneous injections to regulate blood glucose levels in people with diabetes. However, frequent insulin injections can cause discomfort, localized infection, fatty deposition, hypertrophy, and trypanosomiasis. Se-NPs have been used in studies to address diabetes, because of their strong ability to regulate blood glucose levels. Se-NPs elicit antidiabetic effects by enhancing insulin sensitivity, decreasing oxidative stress and inflammation, regulating glucose metabolism, and protecting pancreatic beta cells, thereby improving glucose control and mitigating diabetes complications. Gutierrez et al. have administered Se-NPs derived from luteolin (Lu) and diosmin (DIO) to mice with streptozotocin-induced

diabetes, to treat hyperlipidemia, hyperglycemia, and hepato-renal dysfunction. This treatment resulted in enhanced serum biochemical parameters, better glycemic control, and decreased lipotoxicity while maintaining  $\beta$ -cell function. The findings suggest that synthesized NPs can effectively manage diabetic diseases and have high potential for ameliorating the disorders associated with diabetes mellitus [113].

## Toxicity assessment

Se-NPs have greater effects on organisms than inorganic selenium forms. In addition, each individual's need for antioxidant defense determines how selenium affects health status. Selenium becomes toxic when present in excess. Selenium toxicity in general and Se-NP toxicity have been assumed to be related: both selenium and Se-NPs have pro-oxidative properties that increase ROS concentrations. The bioaccumulation phenomenon may amplify this effect in various tissues, among which the liver is most susceptible [114]. However, in the toxicological examination of Se-NPs, only the function of the antioxidant system; body weight; and bioaccumulation in the liver, kidneys, and heart have received substantial attention. The ways in which Se-NPs interact with the gastrointestinal tract, immunological system, muscles, and other indirect targets of selenium are poorly understood [115]. Se-NPs are less harmful than selenium in most tests. Sublethal doses of 20 nm Se-NPs at 0.05, 0.5, or 4 mg Se/kg body weight (BW)/d were not found to lead to differences in brain neurotransmitters or hematological markers between control and sodium selenite-treated groups (0.5 mg Se/kg BW/d) during a 28-day trial [116]. Se-NPs did not show more efficient bioaccumulation in blood and tissues after dietary administration of 10 mg Se/kg BW. Plasma, liver, and kidney GPx activity did not differ between Se-methionine and Se-NP treatment. Moreover, Se-NPs led to less immediate liver injury and less toxicity than Se-Met. A decrease in the dietary selenium stockpile and an increase in the lethal dosage in Se-NPs fed mice demonstrated the efficacy of Se-NPs in avoiding selenium toxicity. The hypothesized mechanism involves the cell's unique selenium uptake and phase 2 response. Despite the varying toxicological effects of Se-NPs, biologically or ecologically fabricated and altered NPs have been reported to enhance animal health, with diminished toxicity [117]. Specific doses of Se are believed to be harmful. Therefore, the toxicity of Se nanomaterials is believed to depend on both the size/shape and dosage of Se-NPs. Many studies have shown that biogenic Se-NPs are less harmful than sodium selenite in animals. Bano et al. have researched the toxicological effects of Se-NPs in animals and concluded that low concentrations of Se-NPs can be considered safe [118].

## Conclusion

Se-NPs can be fabricated through physical, chemical, and biological methods. The green synthesis approach is gaining

attention for its economical and eco-friendly advantages. Using natural sources such as extracts of plants and microorganisms as reducing and stabilizing agents in Se-NP synthesis is a promising technique. The green synthesis of Se-NPs has substantial potential in various fields, such as medicine, agriculture, and environmental remediation. Moreover, Se-NPs have potential in cancer therapy, wound healing, and drug delivery systems in medicine. In agriculture, Se-NPs have been found to improve plant growth and resilience to environmental challenges and diseases. In environmental remediation, Se-NPs have been demonstrated to remove contaminants from wastewater and soil. The future of Se-NPs will entail developing new green synthesis procedures and optimizing existing methods to increase Se-NP yield and stability. Combining Se-NPs with other nanomaterials and traditional medicines is expected to create more effective and targeted treatments for various ailments. The green synthesis of Se-NPs has excellent potential for diverse applications, and further study in this field will be critical for producing safe and effective nanomaterials that substantially benefit society. The phytoconstituents that cap selenium nanoparticles (Se-NPs) enhance their therapeutic effectiveness

in a dose-dependent manner, opening up new possibilities for use in the food, pharmaceutical, and biomedical industries. The biosynthesis of plant-based NPs is a relatively simple process that is easily scalable for large-scale production. This Review provided a comprehensive overview of the current status and future prospects of this emerging field. Future research on Se-NPs should focus on optimizing green synthesis methods for sustainability and scalability, advancing characterization techniques, and exploring the potential of Se-NPs in targeted drug delivery systems. In-depth mechanistic studies are needed to understand their biological activity, and comprehensive toxicity and environmental impact assessments will be essential to ensure safety. Efforts should also be directed toward clinical translation, including pre-clinical studies and regulatory framework development. By summarizing recent advances in synthesis methods, characterization techniques, and potential applications, this Review provides insights into the unique properties and promising therapeutic potential of Se-NPs. Exciting new avenues may enable the design and development of robust biogenic Se-NPs that can be produced, stored, and marketed globally without risk.

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