Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Formulation, characterization and antidepressant evaluation of phyto-assisted selenium nanoparticles synthesized using *Tinospora cordifolia* aqueous extract

Radhika CHAURASIA[1], Surya Prakash GUPTA[1]*

[1] Rajiv Gandhi Institute of Pharmacy, Faculty of Pharmaceutical Science & Technology, AKS University, Satna (MP)-India.

*Corresponding Author. E-mail: suryatony@yahoo.co.in (S.P.G); Tel. +91-992-610 07 03.

Received: 17 November 2023 / Revised: 19 March 2024 / Accepted: 20 March 2024

ABSTRACT: Tinospora cordifolia extracts are extensively used in various herbal preparations for the treatment of different ailments for its anti-periodic, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritic, anti-allergic, and anti-diabetic properties. In the present investigation the aqueous extract of leaf of Tinospora cordifolia was used as reducing agent for the synthesis of Selenium nanoparticles. The total phenolic content of the extract was determined by derivatization with Folin-ciocalteu reagent and measuring the absorbance in UV-visible spectrophotometer at 765 nm. The SeNPs prepared by reduction of sodium selenite by the extract were assessed in terms of FTIR, UV absorption, size, and form and antidepressant action using forced swim test in mice. The extract solution was dark green in color and contained 1.3 ± 0.003 GAE/mg of phenolics. Se NP was produced rapidly with ascorbic acid as well as *Tinospora cordifolia* extract. The formation of Se NP is indicated by an absorbance at 226.0 nm in the ultraviolet spectrum. The FTIR spectrum revealed the stretching and bending vibrations of O-H, C-H, C-C, N-O, C-N, and other groups due to the presence of phytoconstituent composition. It was discovered that the concentration of the extract, or reducing agent, had an impact on the size of the Se NP nanoparticles, which ranged in size from 46 to 137 nm. The SEM images showed smooth particles with a spherical structure. The Se NPs synthesized using *Tinospora cordifolia* leaf extract exhibited antidepressant action in a concentration dependent manner. The lowest immobility time was depicted by Se NP_{E4} (1.115 ± 0.0213 min)..

KEYWORDS: Selenium nanoparticles; *Tinospora cordifolia*; Biogenic reduction; green synthesis; sodium selenite.

1. INTRODUCTION

Selenium, one of the essential minor elements, has been demonstrated to augment or restore the activity of glutathione peroxidase and selenium-catalyst in the prevention of free radical damage to cells and tissues in vivo1. Supplemental selenium can guard against diseases such as viral infections, weakened immune systems, and loss of brain function. Selenium nanoparticles (SeNPs) can be synthesized at a lower cost than metal nanoparticles (NPs) of Au and Ag. Moreover, SeNPs can be combined with other biological agents to improve their biological properties. The particles' surfaces are more exposed due to their higher surface-to-volume ratio at the nanoscale, which more deeply enhances selenium's activity in the nanoscale. In biological applications, SeNPs exhibit promising potential as drug carries, cancer therapeutic agents and antioxidants. Numerous studies have supported their anticancer, antioxidant, antimicrobial, and anti-biofilm properties [1].

Several scientists have noted that further investigation into SeNPs is imperative due to their remarkable biomedical applications and occasionally even higher antibacterial activity than Ag NPs. SeNPs have been synthesized in various forms, such as nanowires, nanorods, and nanotubes, using a range of techniques, such as sonochemical, refluxing, microwave, hydrothermal, gamma irradiation, pulsed laser ablation, and physical evaporation methods [2]. The main precursors used in these processes are sodium selenite, sodium selenate, sodium selenosulphate, sodium oxide, and sodium selenous acid. Using these precursors is often preferred because selenium in its selenite and selenate forms is hazardous even at very low concentrations and accumulates in the biosystem [1].Selenium is an essential biochemical component of glutathione peroxidase, an enzyme that functions as an antioxidant by degrading peroxides and safeguarding critical SH-groups. Selenium has bactericidal qualities because it can catalyze the oxidation of

How to cite this article: Chaurasia R, Gupta SP. Formulation, characterization and antidepressant evaluation of phyto-assisted selenium nanoparticles synthesized using Tinospora cordifolia aqueous extract. J Res Pharm. 2025; 29(1): 352-359.

https://dx.doi.org/10.29228/jrp.2022.00

ISSN: 2630-6344

intracellular thiols, which destroys microscopic organisms. Many scientists have noted that further study is desperately needed on SeNPs due to their incredible biomedical applications and occasionally even higher antibacterial activity than Ag NPs. SeNPs have been reported after being synthesized via a range of methods. These methods can be divided into two primary groups: biological reduction and chemical reduction. Biological agents such as bacteria or plant extracts are used in biological reduction techniques to transform various organic and inorganic selenium compounds into beneficial and non-toxic selenium nanoparticles (SeNPs).

Selenium is a crucial micronutrient for the proper functioning of the body's metabolic and biological processes. Many severe illnesses, such as cancer, neurological, musculoskeletal, immunological, etc., are brought on by a selenium deficiency. Selenium is typically only shown at very narrow concentration ranges due to its toxic doses, physiological effects, and deficiency. At higher doses, se exhibits pro-oxidant activity; at optimal levels, se functions as an antioxidant. To solve this issue, a precisely calibrated dosage of Se is typically advised. Selenium has many health advantages due to its lower molecular weight and bioavailability in the form of selenoproteins. Many selenoproteins exhibit redox activity and control redox reactions in cells. These include glutathione peroxidases, thioredoxin reductases, and Se1P, Se1F, and Se1S. Selenium nanoparticles have recently been widely synthesized using plants as the reducing agents [2-17]. *Tinospora cordifolia* is a climber that has been extensively used in traditional medicine. The plant has been blessed with a plethora of pharmacological actions and is home of several antioxidant compounds, terpenoids, lignans, alkaloids and sterols [18]. Green synthesis of selenium nanoparticles has been previously reported using microbial and herbal extracts. The presence of terpenoids, sterols, flavonoids and phenolics have been found to be beneficial for the green synthesis of nanoparticles [19]. Hence in the present investigation was attempted to formulate selenium nanoparticles using the aqueous extract of *Tinospora cordifolia* leaves and assess the antidepressant action of the nanoparticles.

2. RESULTS AND DISCUSSION

2.1. Extraction of phytoconstituents

The aqueous extraction of the leaf powder was carried out using cold maceration method. Twice the amount of distilled water was used for extraction of the soluble constituents. The presence of saponins, alkaloids, phenolics, tannins, steroids, cardiac glycosides and carbohydrates has been previously reported in the aqueous extract [20]. Hence the aqueous extract was considered for the present study as tannins and phenolics may act as reducing component for the selenium salt.

The extract solution was dark green in color and was stored in solution form for further use (Figure 1).

2.2. Total Phenolic Content

The total phenolic content of the extract was determined by measuring the absorbance of a Folin-Ciocalteu derivatized solution extract using UV visible spectrophotometer at 765 nm employing gallic acid as the standard. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be $y = 0.0046x + 0.0024$.The total phenolic content in the extract was calculated to be 1.3 ± 0.003 GAE/mg. Previous studies on the leaf extract have also revealed the total phenolic content in the aqueous leaf extract equivalent to our results [21].

2.3. Biosynthesis of Selenium nanoparticles

SeNP was produced through the reduction of sodium selenite in the presence of the extract or ascorbic acid. SeNP formed rapidly upon combination with ascorbic acid and *Tinospora cordifolia* extract. There was a discernible color change from pale yellow to red as the selenium salt was reduced (Figure 2). In order to carry out the synthesis of SeNP, sodium selenite was reduced in parallel using ascorbic acid or *Tinospora cordifolia* extract.

PVA was used as the stabilizing agent during the synthesis of SeNPs. PVA molecules help prevent nanoparticle agglomeration and regulate particle size [22]. Studies from the past have also recommended using bovine serum albumin or polysorbate 80 to stabilize the SeNPs [13]. Precursors such as sodium selenite, sodium selenosulfate, and selenium acid are employed in the chemical synthesis of SeNPs. Different concentrations of *Tinospora cordifolia* leaf extract were used as the reducing agent, which caused the SeNP solutions to have different colors. This demonstrates how reducing agents work during the selenium

nanoparticle synthesis process. The ascorbic acid solution yielded a bright red solution, while the extracts produced yellowish-orange solutions.

Figure 1. Menstruum containing soluble components from ***Tinospora cordifolia*** leaf

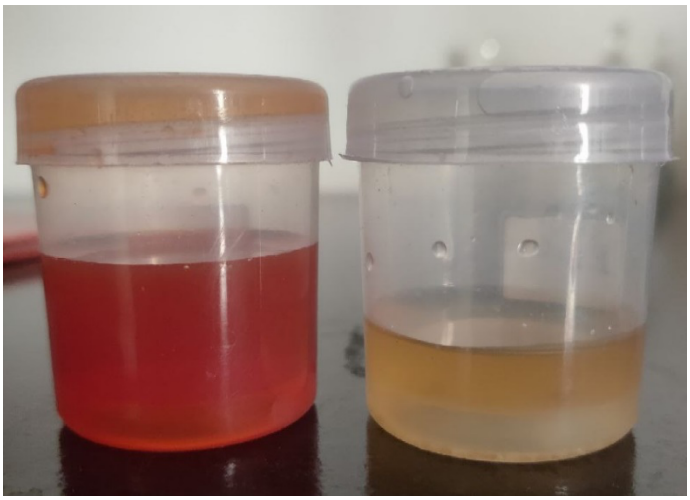

Figure 2. Se NP synthesized using ***Tinospora cordifolia***

2.4. Characterization of Se NP

2.4.1. UV absorption

The formation of SeNP is indicated by the UV spectrum showing absorbance at 226.0 nm (Figure 3). Previously few authors have reported that the suspension of the SeNPs of 20 nm diameter had a yellowish-orange appearance and showed the absorption maximum below 250 nm. When the particle sizes increase, the characteristic red-shift of the absorbance peak maxima is observed [23]. The high absorption peaks result due to Surface Plasmon Resonance (SPR) of Se NPs.

The UV spectrum of various SeNP synthesized using the extract of *Tinospora cordifolia* also revealed absorption maxima at 224-227 nm suggesting the completed synthesis of SeNP (Figure 4).

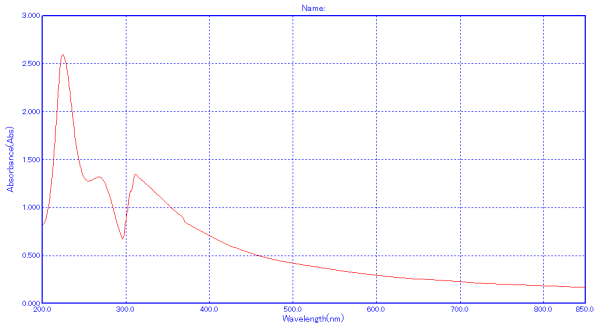

Figure 3. UV spectrum of Se NP synthesized using ascorbic acid as reducing agent

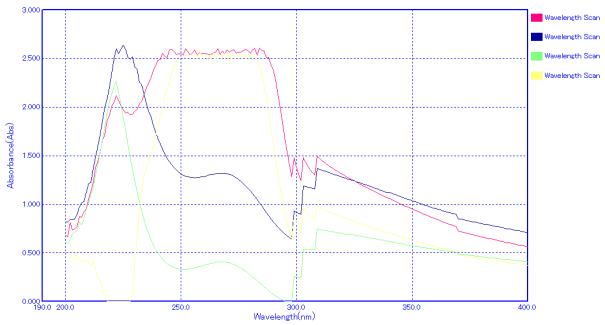

Figure 4. UV spectrum of SeNP synthesized using *Tinospora cordifolia* leaf extract as reducing agent

2.4.2. Infrared spectral study

The O-H stretch of alcohols or phenol groups is linked to a broad vibration peak observed at 3423 cm^{-1} in the synthesized Se NP Fourier Transform Infrared Spectrometer (FTIR) spectrum. Vibration peaks at 3125, 3019, 2983, and 2827 cm^{-1} represent the corresponding C–H stretch of the alkynes. The peak appears at 2341 cm−1, indicating that the compound contains nitro compounds, which are an asymmetric stretch of N and O. The strong band at 1522 cm^{-1} represents the aromatic ring (C–C and C–H stretching). The small vibrational peaks 1358 to 1156 cm^{-1} correspond to the bending C–H, C–N, O–H, C–X, and C–N–C stretching attributed to alkanes, amines, and carboxylic groups.(Figure 5).

Figure 5. FTIR spectrum of SeNP

2.4.3. Particle Size and distribution

As different amounts of the extract solution were used for synthesizing the SeNP, it was found that a varying color was obtained in each condition. This indicates the effect of concentration of the reducing agent in the synthesis of SeNPs. Although each concentration was able to cause reduction and lead to synthesis of SeNP, but the size of the SeNP formed varied with each. The particle size was measured using a Malvern particle size analyzer. The particle size of the SeNP nanoparticles was found to increase with an increase in concentration of the reducing agent (extract) as indicated by a more reddish color of the solution. The particle size ranged from 46 to 137 nm in various samples with varying polydispersity index (PDI) (Table 1).

Table 1. Particle size of synthesized SeNPs

Formulation	Particle size (d.nm)	PDI
Se NP_A	137	0.611
Se NP_{E1}	46	0.583
Se NP_{E2}	64	0.794
Se NP_{E3}	86	0.519
Se NP_{E4}	98	0.773

Se NP_A – selenium nanoparticles using ascorbic acid, Se NP_{E1-E4} – selenium nanoparticles using *Tinospora cordifolia* extract

It was found that when ascorbic acid was used in synthesizing the Se NP, the particle size was highest. Smaller particles were obtained when *Tinospora cordifolia* extract was used for synthesis of the Se NPs. The PDI was not affected by the concentration of extract (Figure 6). A typical particle size by intensity graph obtained using the particle size analyzer for Se NP_{E1} is presented in Figure 7.

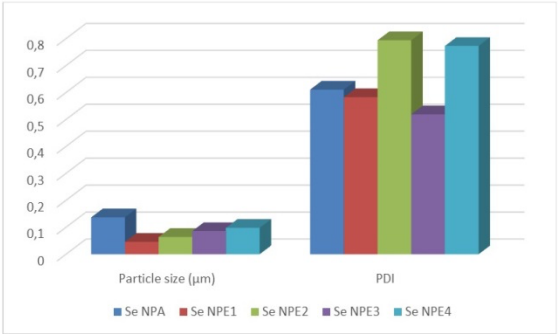

Figure 6. Particle size and PDI of the synthesized SeNPs

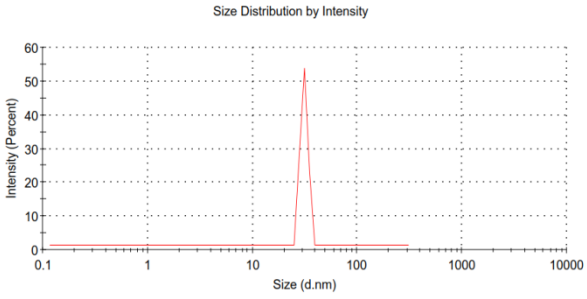

Figure 7. Particle size distribution by intensity for $SeNP_{E1}$

2.4.4. Surface Morphology of SeNPs

The surface study of the SeNPs was carried out using SEM imaging. The SEM images showed the smooth particles with spherical structure (Figure 8). The spherical morphology is vital for cellular uptake of the particles and is helpful in maintaining minimum energy thereby resulting in stable particles.

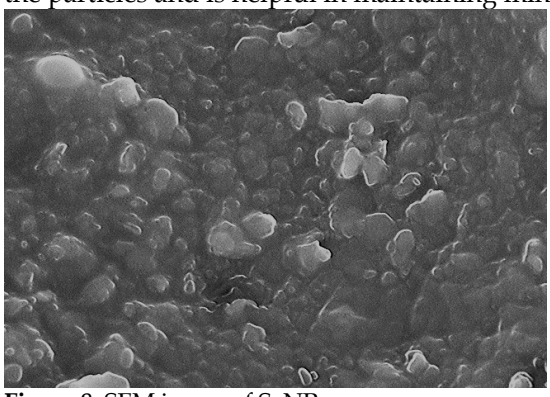

Figure 8. SEM image of $SeNP_{E1}$

2.5. Antidepressant action of SeNP

Forced swim test is a widely used animal model for evaluating the antidepressant action of drugs and substances. The synthesized Se NPs were evaluated for their ability to tackle depressive conditions. The SeNPs synthesized using *Tinospora cordifolia* leaf extract exhibited antidepressant action in a concentration dependent manner (Table 2). On the other hand $SeNP_A$ was not able to reduce depression as exhibited by no significant change in immobility time as compared to control. The lowest immobility time was depicted by $SeNP_{E4}$ (1.115 ± 0.0213 min). The higher concentration of phytochemicals in the $SeNP_{E4}$ as compared to the other SeNPs could be responsible for the higher antidepressant action.

Table 2. Effect of Se NPs on immobility time in mice

Treatment Group	Total Immobility duration (min)*
Control (Normal Saline)	3.400 ± 0.04450
Se NP_A (0.05 mL, i.p)	3.225 ± 0.05685
Se NP_{E1} (0.05 mL, i.p)	2.373 ± 0.03774
Se NP_{AE2} (0.05 mL, i.p)	1.750 ± 0.1204
Se NP_{E3} (0.05 mL, i.p)	1.232 ± 0.02286
Se NP_{E4} (0.05 mL, i.p)	1.115 ± 0.02125

*Reported as mean ± standard error of mean; n=6

The data of immobility time was subjected to statistical treatment using GraphPad Prism software and it was found that except $SeNP_A$ all other SeNPs exhibited a significant improvement in swimming ($p<0.001$) as revealed by a reduced immobility time (Figure 9).

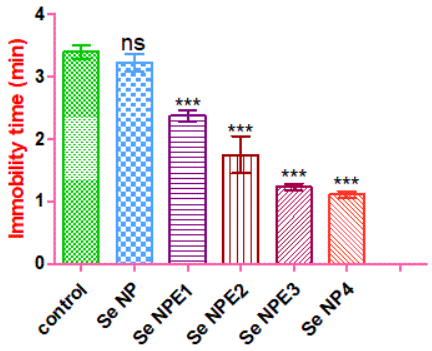

Figure 9. Immobility time in mice on treatment with SeNPs

Tinospora cordifolia aqueous extracts have been reported to exhibit antidepressant action by virtue of the presence of phenolic compounds in it [24]. Hence it was evident that a higher amount of extract used for the synthesis of SeNPs resulted in a better antidepressant action in comparison to lower amounts.

3. MATERIAL AND METHODS

3.1. Collection and preparation of plant material

The leaves of *Tinospora cordifolia* were collected from the Shubham plant nursery at Bhopal. The leaves were washed with distilled water and shade dried. The dried leaves were coarsely powdered using slow speed grinder and packed in airtight container until used.

3.2 Extraction of *Tinospora cordifolia* leaf

The powdered leaf material was extracted using distilled water as the extraction solvent. Briefly, 50 g of leaf powder was taken in a glass jar and to it was added 100 mL of distilled water. The jar was capped and shaken intermittently for first 6 hours. The jar was allowed to stand overnight undisturbed to allow for complete extraction of the soluble components. The menstruum was filtered using muslin cloth and stored at 4°C and used for further studies.

3.3 Total phenolic content determination [25]

0.5 mL of the leaf extract was diluted thrice with equal quantity of distilled water. One ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Then 1.5 ml Folin-Ciocalteu reagent was added and allowed to incubate at room temperature for 5 min. Four ml of 20% (w/v) Na_2CO_3 was added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the sample was measured at 765 nm against blank, i.e., distilled water. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. The total phenolic content was reported as gallic acid equivalent (GAE/mg).

3.4 Preparation of Selenium Nanoparticles (SeNP) [6, 13]

To make solutions, the appropriate concentrations of ascorbic acid (100 mM) and sodium selenite (25 mM) were dissolved in distilled water. Selenium nanoparticles were produced by reducing the sodium selenite with either plant extract or ascorbic acid. In summary, three milliliters of ascorbic acid solution, three milliliters of sodium selenite solution and ten grams of polyvinyl alcohol (PVA) were diluted in a beaker using nine milliliters of distilled water. One milligram of NaOH solution was added to this solution in order to slightly raise its pH. As the solution got closer to an alkaline pH, its color started to change from colorless to red. For thirty minutes, the solution was swirled to complete the reduction process. Ten minutes of 10,000 rpm centrifugation were used for the Se NP solution. The supernatant, which contained too much PVA, was discarded, and the sediment was once more mixed in 5 milliliters of distilled water. For preparing the Se NP using plant extract and to study the effect of extract concentration on Se NP synthesis, four formulations were prepared by taking 1, 2, 3 & 4 of the leaf extract solution in place of ascorbic acid (Table 3).

Table 3. Quantity used for synthesis of Se NPs

Formulation	Extract (mL)	Ascorbic acid (mL)	Distilled Water (mL)
Se NP_A	-	3.0	9.0
Se NP_{E1}	1.0	-	9.0
Se NP_{E2}	2.0	-	9.0
Se NP_{E3}	3.0	-	9.0
Se NP_{E4}	4.0	-	9.0

3.5. Characterization of SeNP

3.5.1. Spectrophotometric characterization

The preparation of SeNP was confirmed by measuring the absorption maxima of the SeNP solution in reference to selenium nitrite solution from 300-850 nm using UV-Visible

How to cite this article: Chaurasia R, Gupta SP. Formulation, characterization and antidepressant evaluation of phyto-assisted selenium nanoparticles synthesized using Tinospora cordifolia aqueous extract. J Res Pharm. 2025; 29(1): 352-359.

https://dx.doi.org/10.29228/jrp.2022.00
ISSN: 2630-6344

3.5.2. FT-IR spectral study

The prepared SeNP was subjected to FTIR analysis in order to look into the functional groups that were part of the preparation. The spectrum that was recorded ranged from 400 to 4000 cm^{-1}.

3.5.3. Particle Size determination

The particle size of SeNP was measured using a particle size analyzer and the differential light scattering (DLS) principle. Following the creation of a diluted suspension of SeNP in distilled water, the particle size and distribution were assessed.

3.5.4. Surface characterization

The surface morphology of the prepared SeNP was analyzed using scanning electron microscopy (SEM).

3.6. Antidepressant action [26]

Male mice weighing 20–25 g were used in the experiment. The protocol of the present work was approved by Institutional Animal Ethical Committee (IAEC). In the institute's animal house, the animals were kept in groups in 38x23x10 cm poly acrylic cages. For a week prior to and during the experiments, a maximum of four animals per cage were housed and maintained under standard laboratory conditions. The temperature was 27±2°C, the relative humidity (RH) was 44-56%, and there was a natural light and dark cycle of 14 hours of light and 10 hours of darkness. The animals were allowed unlimited access to Golden Feeds, tap water, and the normal diet of India. The antidepressant activity of the Se NP was assessed using Forced Swim Test method. Se NP and fluoxetine were injected intraperitoneally into each mouse thirty minutes prior to the test. The drugs had been dissolved and distributed in DMSO at a standard volume of 0.05 mL per 20 g body weight. To measure the effects of the test drug, mice were placed individually in a glass cylinder (25 cm high by 10 cm diameter) filled with water (22–25°C) up to a height of 10 cm. Each mouse was allowed six minutes to swim during the test, and during the final four minutes, the duration of the mouse's immobility was tracked and documented. The mouse's time floating in the water with no force applied and moving only enough to keep its head above the surface was called the immobility period. Once the animals were dried using a tower, they were returned to their enclosures.

4. CONCLUSION

In this study, SeNPs was rapidly synthesized from *Tinospora cordifolia* leaf aqueous extract and was characterized by UV–Vis spectrophotometer, FT-IR, SEM and DLS analysis. Rapidly synthesized Se NPs demonstrated significant antidepressant action = in forced swim test in mice. In terms of the synthesis of SeNPs, the leaf extract turned out to be the most affordable, energy-efficient, safe, and friendly to the environment and human health. This environmentally friendly method may be used in environmental and biomedical settings in the near future.

Acknowledgements: The authors are thankful to RB Science Research Lab, Bhopal, India for performing few studies related to SeNPs.

Author contributions: Concept – S.P.G.; Design – R.C., S.P.G; Supervision – S.P.G.; Resources – R.C., S.P.G.; Materials – R.C.; Data Collection and/or Processing – R.C.; Analysis and/or Interpretation – R.C., S.P.G.; Literature Search – R.C.; Writing – R.C., S.P.G.; Critical Reviews – S.P.G..

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Bisht N, Phalswal P, Khanna PK. Selenium nanoparticles: A review on synthesis and biomedical applications. Mater Adv. 2022; 3: 1415-1431. https://doi.org/10.1039/D1MA00639H.

[2] Nile SH, Thombre D, Shelar A, Gosavi K, Sangshetti J, Zhang W, Sieniawska E, Patil R, Kai G. Antifungal properties of biogenic selenium nanoparticles functionalized with nystatin for the ınhibition of *Candida albicans* biofilm formation. Molecules. 2023; 28(4): 1836. https://doi.org/10.3390/molecules28041836.

[3] Escobar-Ramírez MC, Castañeda-Ovando A, Pérez-Escalante E, Rodríguez-Serrano GM, Ramírez-Moreno E, Quintero-Lira A, Contreras-López E, Añorve-Morga J, Jaimez-Ordaz J, González-Olivares LG. Antimicrobial activity of Se-nanoparticles from bacterial biotransformation. Fermentation. 2022; 7: 130. https://doi.org/10.3390/fermentation7030130.

[4] Pyrzynska K, Sentkowska A. Biosynthesis of selenium nanoparticles using plant extracts. J Nanostruct Chem. 2022; 12: 467-480. https://doi.org/10.1007/s40097-021-00435-4.
[5] Shahabadi N, Zendehcheshm S, Khademi F. Selenium nanoparticles: Synthesis, in-vitro cytotoxicity, antioxidant activity and interaction studies with ct-DNA and HSA, HHb and Cyt c serum proteins. Biotechnol Rep. 2021; 30: e00615. https://doi.org/10.1016/j.btre.2021.e00615.
[6] Cittrarasu V , Kaliannan D, Dharman K, Maluventhen V, Easwaran M, Liu WC, Balasubramanian B, Arumugam M. Green synthesis of selenium nanoparticles mediated from *Ceropegia bulbosa* Roxb extract and its cytotoxicity, antimicrobial, mosquitocidal and photocatalytic activities. Sci Rep. 2021; 11: 1032. https://doi.org/10.1038/s41598-020-80327-9.
[7] Alipour S, Kalari S, Morowvat MH, Sabahi Z, Dehshahri A. Green synthesis of selenium nanoparticles by *Cyanobacterium spirulinaplatensis* (abdf2224): Cultivation condition quality controls. BioMed Res Int. 2020; 6635297. https://doi.org/10.1155/2021/6635297.
[8] Vahdati M, Moghadam VT. Synthesis and characterization of Selenium nanoparticles-lysozyme nanohybrid system with synergistic antibacterial properties. Sci Rep. 2020; 10: 510. https://doi.org/10.1038/s41598-019-57333-7.
[9] Boroumand S, Safari M, Shaabani E, Shirzad M, Faridi-Maji R. Selenium nanoparticles: synthesis, characterization and study of their cytotoxicity, antioxidant and antibacterial activity. Mater Res Express. 2019; 6: 0850d8. https://doi.org/10.1088/2053-1591/ab2558.
[10] Shubharani R, Mahesh M, YoganandaMurth VN. Biosynthesis and characterization, antioxidant and antimicrobial activities of selenium nanoparticles from ethanol extract of bee propolis. J Nanomed Nanotechnol. 2019; 10: 1. https://doi.org/10.4172/2157-7439.1000522.
[11] Verma P, Maheshwari SK. Preparation of sliver and selenium nanoparticles and ıts characterization by dynamic light scattering and scanning electron microscopy. J Microsc Ultrastruct. 2018; 6(4): 182-187. https://doi.org/10.4103/jmau.jmau_3_18.
[12] Tugarova AV, Mamchenkova PV, Dyatlova YA, Kamnev AA. FTIR and Raman spectroscopic studies of selenium nanoparticles synthesised by the bacterium *Azospirillum thiophilum*. SpectrochimicaActa A Mol Biomol Spectrosc. 2018; 192: 458-463. https://doi.org/10.1016/j.saa.2017.11.050.
[13] Nguyen THD, Vardhanabhuti B, Lin M, Mustapha A. Antibacterial properties of selenium nanoparticles and their toxicity to Caco-2 cells. Food Control. 2017; 77: 17-24. https://doi.org/10.1016/j.foodcont.2017.01.018.
[14] Yazdi MH, Mahdavi M, Faghfuri E, Faramarzi MA, Sepehrizadeh Z, Hassan ZM, Gholami M, Shahverdi AR. Th1 ımmune response ınduction by biogenic selenium nanoparticles in mice with breast cancer: preliminary vaccine model. Iran J Biotech. 2015; 13(2): e1056. https://doi.org/10.15171/ijb.1056.
[15] Sharma G, Sharma AR, Bhavesh R, Park J, Ganbold B, Nam J-S, Lee S-S. Biomolecule-mediated synthesis of selenium nanoparticles using dried *Vitis vinifera* (Raisin) extract. Molecules. 2014; 19(3): 2761-2770. https://doi.org/10.3390/molecules19032761.
[16] Prasad KS, Selvaraj K. Biogenic synthesis of selenium nanoparticles and their effect on As(III)-ınduced toxicity on human lymphocytes. Biol Trace Elem Res. 2014; 157(3): 275-283. https://doi.org/10.1007/s12011-014-9891-0.
[17] Yazdi MH, Mahdavi M, Setayesh N, Esfandyar M, Shahverdi AR. Selenium nanoparticle-enriched *Lactobacillus brevis* causes more efficient immune responses in vivo and reduces the liver metastasis in metastatic form of mouse breast cancer. DARU J Pharm Sci 2013; 21: 33-41. https://doi.org/10.1186/2008-2231-21-33.
[18] Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon 2019; 5(9): e02437. https://doi.org/10.1016/j.heliyon.2019.e02437.
[19] Mikhailova EO. Selenium nanoparticles: Green synthesis and biomedical applications. Molecules 2023; 28(24): 8125. https://doi.org/10.3390/molecules28248125.
[20] Nazir I, Chauhan RS. Qualitative phytochemical analysis of *Tinospora cordifolia* and *Withania somnifera*. The Pharm Innov J. 2018; 7(10): 333-336.
[21] Premnath R, Lakshmidevi N. Studies on Anti-oxidant activity of *Tinospora cordifolia* (Miers.) leaves using in vitro models. J Am Sci. 2010; 6(10): 736-743.
[22] Sentkowska A, Pyrzynska K. The ınfluence of synthesis conditions on the antioxidant activity of selenium nanoparticles. Molecules. 2022; 27: 2486. https://doi.org/10.3390/molecules27082486.
[23] Lin ZH, Chris-Wang CR. Evidence on the size-dependent absorption spectral evolution of selenium nanoparticles. Mater Chem Phys. 2005; 92: 591–594. https://doi.org/10.1016/j.matchemphys.2005.02.023.
[24] Mutalik M, Mutalik M. *Tinospora cordifolia*: Role in depression, cognition and memory. Aust J Med Herbalism. 2011; 23(4): 168-173
[25] Singh J, Narwaria US. Evaluation of anti-inflammatory action of *Melaleuca bracteata* F. Muell. leaf extract. J Pharmacol Biomed. 2021; 5(3):319-325.
[26] Malviya M, Mishra B, Korde B. Antidepressant molecules: Synthesis and evaluation of novel azetidinone compounds. J Pharmacol Biomed. 2021; 5(2): 286-294.