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Research Article

Anticancer and Antioxidant Activity of *Morinda Citrifolia* Leaf Mediated Selenium Nanoparticles

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The goal of the present work is to fabricate selenium nanoparticles utilising *Morinda citrifolia* leaves extract via green approach. UV-Vis spectroscopy, FT-IR, and TEM were used to characterise the green nanoparticles. The size of generated SeNPs in *Morinda citrifolia* was anticipated to be 12-160 nm based on TEM images. The antioxidant activity of selenium NPs was discovered to be 66.7 to 83.7% of free radical inhibtion. When the concentration of nanoparticles rises, the viability of cancer cells decreases. It shows that biosynthesized selenium nanoparticles have anticancer properties that depend on the concentration. The brine shrimp lethality assay revealed that *Morinda citrifolia* mediated selenium nanoparticles have low cytotoxic effects.

1. Introduction

Nanotechnology addresses a progressive way for technological advancement that includes the administration of material at the nanometer scale (one billion times less than a meter) [1]. Nanotechnology implies any innovation on the nanoscale that has various applications in reality. Nanotechnology in a real sense incorporates the fabrication and utilization of chemical, physical, and biological systems at scales ranging from atoms to submicron measurements, and furthermore the combination of these subsequent nanomaterials into larger systems [2, 3].

Selenium (Se) is a fundamental trace component present in our body. Selenium is found in proteins as selenocysteine (Sec), which is referred to as selenoproteins. The presence of selenium in enzymes, which have a critical function in shielding the organism from the effects of oxidative stress, explains the element's importance in human nutrition [4, 5]. The presence of oxidoreductase in selenoproteins regulates redox equilibrium in the body. Because of the low toxicity of selenium nanoparticles (SeNPs), they have been investigated for variety of oxidative pressure as well as inflammatory diseases. Therefore, SeNPs pave a way to transmit various medications to the site of action (Amit [6]). The current study exposes the fabrication of SeNPs using *Morinda citrifolia* extract.

Fabrication of nanoparticles via green approach using medicianl plants extracts has recently gained popularity. Phytochemicals, in particular, constitute the backbone of plants, may readily produce nanoparticles with lower toxicity. As the benefits of *Morinda citrifolia L.* and its products become more widely known, researchers have begun to take an interest in them [7]. *M. citrifolia L.*, belongs to the family Rubiaceae spread all over in tropical Asia and Polynesia. It

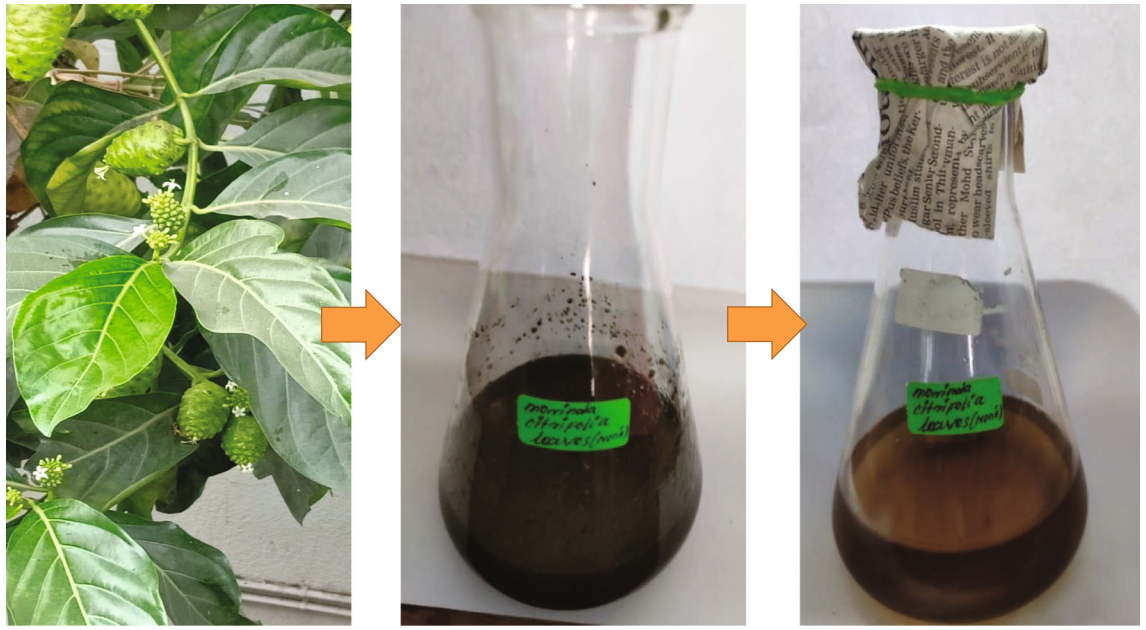

Figure 1: Preparation of *Morinda citrifolia* plant extract.

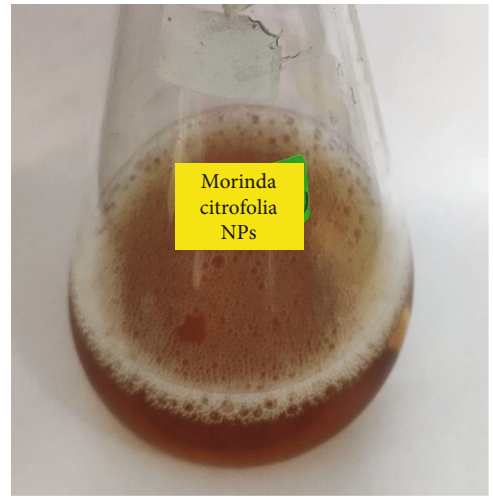

Figure 2: Green synthesis of selenium nanoparticles.

appears to have been highly valued therapeutically in the Tropical regions, and the plant is normally produced for its root system, leaves, and organic matter. These plants roots are an excellent source of anthraquinones, which are typically found as aglycones and, to a lesser extent, as glycosides. All components of the tree have been widely used medicinal services for the relief of arthritic as well as other pains, and for their healing properties [8].

2. Materials and Methods

2.1. Preparation of Extract. Morinda citrifolia leaves were obtained from Thiruparuthikundaram, Kancheepuram. The collected leaves were thoroughly washed under the tap water. For seven days, the leaves were dried in the shade at room temperature. The leaves of *Morinda citrifolia* separately grounded using mixier grinder into fine powder. 1gm of powdered leaves of *Morinda citrifolia* was added to 100 mL of distilled water and heated at a temperature of 60-70°C using a heating mantle. Finally, using Whatmann No. 1 filter paper, the mixture was filtered and the extract was stored for further use (Figure 1).

2.2. Synthesis of Selenium Nanoparticles. Sodium selenite (Na_2SeO_3), 30 mM dissolved in 50 mL of distilled water. To that, 50 mL of *Morinda citrifolia* leaf extract was slowly added. Then the reaction mixture was kept on a magnetic stirrer at 650-700 rpm for 48-72 hours.

2.3. PURIFICATION AND CHARACTERIZATION of NPs USING TEM &FT-IR. The collected NPs were kept for centrifugation at 8000 rpm for 10 min. The pellet obtained dried at 70°C in hot air oven for 12 h. The dried pellet was grinded

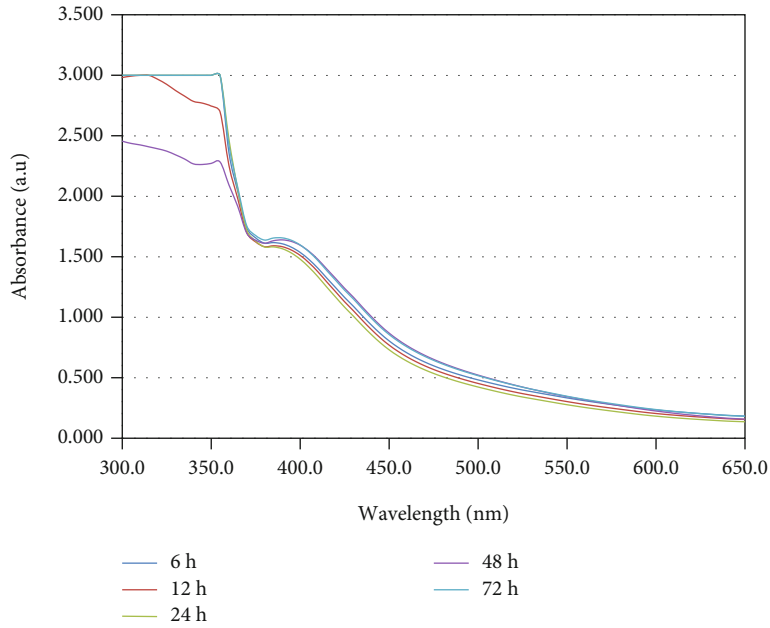

Figure 3: UV-Visible spectra of biosynthesized SeNPs.

using mortar and pestle and the powder was stored for further use. The TEM and FT-IR analysis was carried out using the powdered pellet.

2.4. Antioxidant Assay. The DPPH assay of free radical scavenging using *Morinda citrifolia* leaves extract mediated SeNPs was carried out by the procedure reported in (Rajeshkumar et al. [9]). Various concentrations (10, 20, 30, 40 and 50 μg/mL) of *Morinda citrifolia* extract synthesized selenium nanoparticles was added to 1 mL of DPPH and 450 μl of TrisHCl buffer was added and kept in incubation for 30 mins. The free radical scavenging was analysed by measuring absorbance at 517 nm. As a control, BHT was used. Ascorbic acid was employed as a reference substance. The inhibition percentage was determined from the following equation,

$$\%\text{inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \text{x}100 \quad (1)$$

2.5. Anticancer Activity

2.5.1. MTT Assay. MTT assay is called as (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide. Mossman proposed the MTT assay for the first time in 1982. MTT is broken in live cells by mitochondrial dehydrogenase, resulting in the quantifiable purple product formazan. The quantity of formazan generated is related to the number of live cells and inversely related to the extent of cytotoxicity. The wells should be washed twice or three times with MEM (w/o) FCS. Add 200 μL of MTT with a concentration of 5 mg/mL, followed by incubation for 6-7 hrs. Following incubation, DMSO (1 ml) was added to the wells and stir with a pipette for 45 seconds. If live cells contain formazan

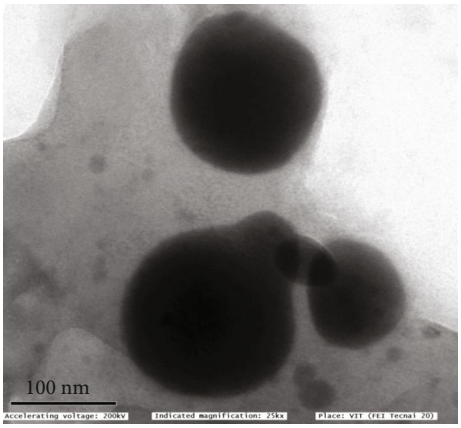

Figure 4: Transmission electron microscopic image of SeNPs synthesized using *Morinda citrifolia*.

crystals following the addition of solublizing reagent (DMSO), the purple colour development occurs. The solution was tested using the UV-visible spectroscopy and the optical density at 595 nm measured using DMSO as control. The percentage of cell viability was calculated using the below equation.

$$\text{Cell viability}(\%) = \frac{\text{Mean OD}}{\text{Control OD}} \text{ x } 100 \quad (2)$$

2.6. Cytotoxicity Activity. Weighing and dissolving 2 g of iodine-free salt in 200 mL of purified water. 10-12 ml saline water filled in 6 well ELISA plates. 10 nauplii were added to the wells (5, 10,15, 20, 25 μL & control). The nanoparticles were then introduced at the desired concentration level and incubated the plates for 24 h.

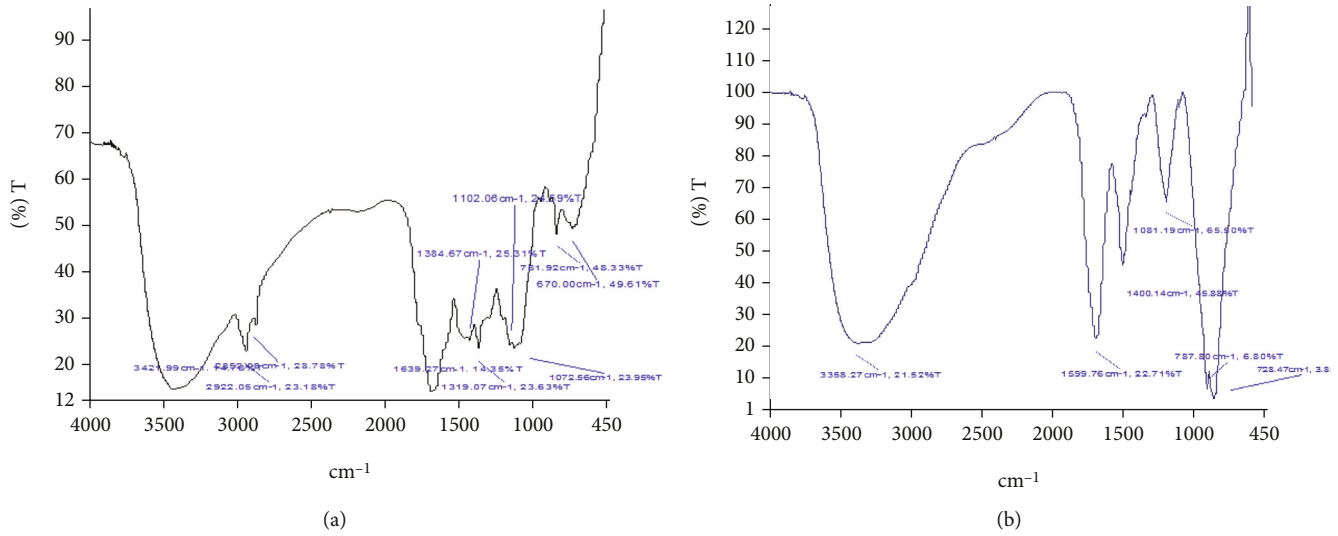

Figure 5: Fourier Transform Infra-red spectrum of (a) *Morinda citrifolia* leaves extract (b) *Morinda citrifolia* leaves extract mediated SeNPs.

Table 1: Functional groups present in the reducing agent and nanoparticles.

Morinda citrifolia			SeNPs		
Functional group	Peaks	Types of vibration	Functional group	Peaks	Types of vibration
Phenols and alcohols	3427.99	Hydrogen bonded O-H stretch	Phenols and alcohols	3358.27	Hydrogen bonded O-H stretch
Amide group	1639.27	N-H bend	Amines primary	1599.76	N-H bend
Nitro group	1319.09	N=O bend	Nitro groups	1400.16	N=O bend
Nitro group	1384.67	N=O bend	Ethers	1081.19	C-O stretch
Ethers	1102.06	C-O stretch	Esters	787.27	C-O stretch

After incubation, the plates were counted for the presence of live nauplii and the number was estimated using the following formula:

$$\text{number of dead nauplii/number of dead nauplii} + \text{number of live nauplii} \times 100 \quad (3)$$

3. Results and Discussion

3.1. Visual Observation. The formation of metal nanoparticles upon the addition the plant extract is accompanied by the colour change of the solution. As depicted in Figure 2 the *Morinda citrifolia* mediated selenium nanoparticles gradually changes its colour to light brown after 28 h and pale brown colour after 31 h and finally brownish red colour was obtained after 56 h.

3.2. UV-Visible Spectroscopy Analysis. The formation of SeNPs by using *Morinda citrifolia* leaf extract was preliminarily confirmed by UV-visible spectroscopy (Figure 3). The readings were recorded at specific time intervals such as 6, 12, 24, 48 and 72 h. SeNPs exhibited an absorption peak at 390 nm in its UV spectrum.

3.3. Transmission Electron Microscope. The morphological characteristics of biosynthesized SeNPs observed through

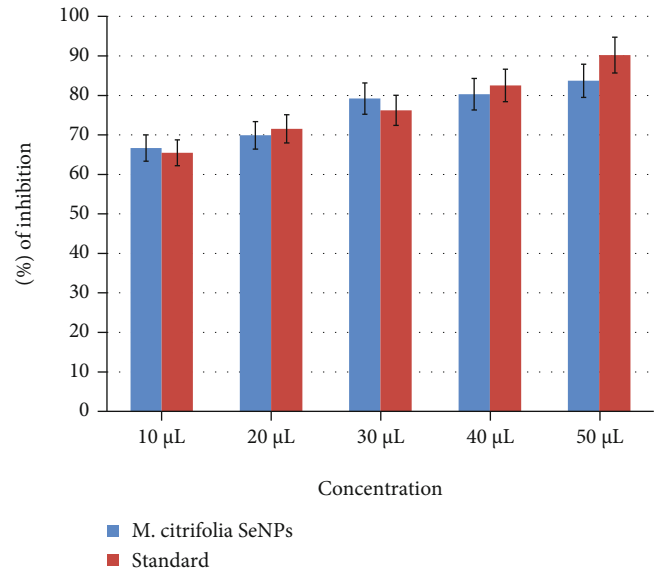

Figure 6: Free radical scavenging activity of *Morinda citrifolia* leaves extract mediated SeNPs.

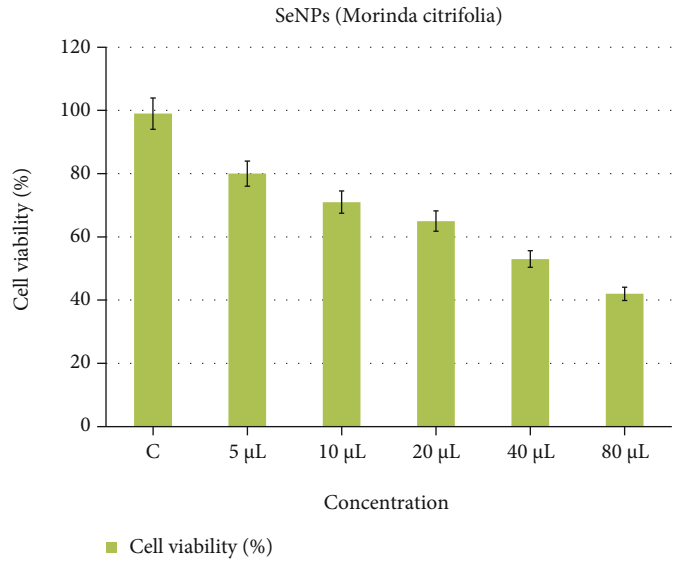

Figure 7: Anticancer activity of *Morinda citrifolia* mediated selenium nanoparticles against Hep G2 cell line.

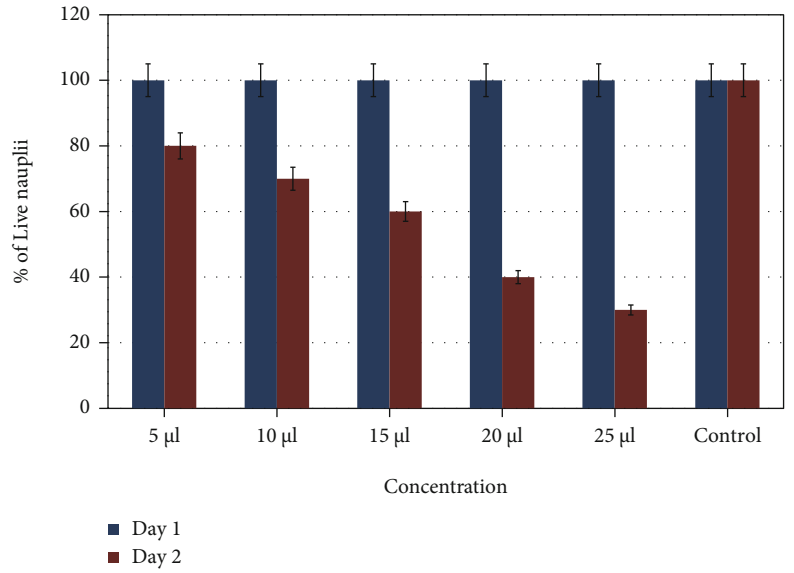

Figure 8: Brine shrimp lethality assay of SeNPs synthesized using *Morinda citrifolia.*

Transmission Electron Microscopy (TEM). Figure 4. shows the spherical morphology of SeNPs with the size ranges from 120-160 nm.

3.4. Ft-IR. Substance specific vibrations of the molecules led to the specific signals obtained by the FT-IR spectroscopy. Some of the absorption peaks at 3427.99 and 1639.27 cm^{-1} represents stretching Hydrogen bonded O-H phenols, alcohol and N-H bend amide functional groups which was depicted in Figure 5(a) and Table 1. And peaks at 1319.09 and 1384.67 cm^{-1} indicates the N=O bend nitro group. The peaks at 1102.06 cm^{-1} corresponds the stretching C-O ether functional group.

As depicted in Figure 5(b), the FT-IR spectra of green synthesized SeNPs showed peak at 3358.27 cm^{-1}, which indicated the presence of stretching Hydrogen bonded O-H phenols and alcohol groups. The band at 1599.76 cm^{-1} indicated N-H Bend primary amine functional group. The peaks obtained at 1400.16 and 1081.19 cm^{-1} indicated the N=O bend nitro group and C-O stretch ether functional groups. The bands at 787.27 cm^{-1} indicates the C-O stretching of esters.

3.5. Antioxidant Activity. Antioxidants acts as a defense mechanism to prevent the body from experiencing alleviating chronic diseases by reducing the cellular oxidative damage caused by the free radicals [10]. The antioxidant

activity of *Morinda citrifolia* mediated selenium nanoparticles was analysed by adopting DPPH method. As shown in Figure 6, the selenium nanoparticles showed significant free radical inhibition higher at 50 μL concentration in dose dependent manner. At 10 μL concentration the selenium nanoparticles showed free radical inhibition around 66.7% and 83.7% was obtained for 50 μL concentration.

3.6. Anticancer Activity. Figure 7 shows the Screening of biosynthesized SeNPs results in potential anticancer activity against HepG2. Different concentrations of selenium nanoparticles (5, 10, 20, 40, 80 μL) was used in this study. The cytotoxicity analysis of the SeNPs shown a concentration dependent response and higher cytotoxicity increament observed at higher concentration (80 μL).

3.7. Cytotoxic Effect of Brine Shrimp Lethality Assay. The cytotoxicity of green synthesized selenium nanoparticles was tested by adopting brine shrimp lethality assay. At day 2, the percentage of live nauplii at lower concentration (5 μl and 10 μl) was found to be 70% & 80%. As shown in Figure 8, only 30% of nauplii was alive at higher concentration (25 μL). Therefore, the cytotoxic results predicted low toxicity of selenium nanoparticles.

4. Discussion

The microbial synthesis of SeNPs using non-pathogenic bacterium *Zooglea ramigera* obtained trigonal selenium nanorods [11]. In previous research work, broccoli mediated selenium nanoparticles, FT-IR analysis showed peaks at 3235, 1595, 1407, and 1099 cm-1 indicates the presence of O-H stretch, N-H bend, C-F stretch and C-O stretch of aliphatic ether, respectively. SeNPs (64 g/mL) exhibited complete inhibitory action against Streptococcus agalactiae, *Escherichia fergusonii,* and *Pseudomonas aeruginosa* and could be used in replacement of antibiotics for treating cutaneous infections caused by bacteria [12]. Biosynthesis of SeNPs syntheisized using *Clausea dentata* exhibited strong larvicidal activity with increased concentration against the fourth instar larvae stage of *Culex quinquefasciatus, Aedes Aegypti, and Anopheles stephensi* [13]. SeNPs with doxorubicin combination exhibited excellent anti-cancer effect and it was found that SeNPs induced MCF-7cell death through apoptosis [14]. The microbial synthesis of SeNPs by using *Bacillus species* against *Candida albicans* and *Aspergillus fumigatus* exhibited good antifungal activity (Mojtaba Shakibaie et al., 2015). SeNPS were biosynthesized using *Azoarcus* sp. which transformed the selenite to Se and generated spherical SeNPs. Dried *Vitis vinifera* (raisin) extract synthesized uniformly shaped and biopolymer (lignin)-capped selenium nanoballs [15]. The biogenic fabrication of SeNPs via the flower extract of *Bougainvillea spectabilis* resulted in stable hollow SeNPs with an avergae size of 24.24 ± 2.95 nm [16]. The cytotoxic behaviour of SeNPs in combination with X-ray was demonstrated in treating lung cancer cell lines. The participation of SeNPs in caspase-3 activation and its downstream target showed that lung cancer cells undergo apoptosis [17–22]).

5. Conclusion

Using the leaf extract of *Morinda citrifolia*, a simple approach was explored to develop a green, eco-friendly manner to synthesize SeNPs. The production of SeNPs was validated by the brownish red color obtained after adding sodium selenite to Morinda citrifolia leaf extract. UV-Vis spectroscopy, FT-IR, and TEM were also used to confirm the SeNPs, which were found to be 120-160 nm in size in Morinda citrifolia. In *Morinda citrifolia*, selenium NPs was shown to have an antioxidant activity of 66.7 percent to 83.7 percent of free radicals. The viability of tumour cells lowered, while increasing the concentration of nanoparticles. It proves that selenium nanoparticles mediated *Morinda citrifolia* have anticancer properties. In the brine shrimp lethality assay, selenium nanoparticles produce less harmful findings. The findings of this study imply that selenium nanoparticles mediated by *Morinda citrifolia* can be employed in biomedical applications in the future.

Data Availability

The data used in this study is done by the authors.

Conflicts of Interest

The authors declare, there is no conflict of interest.

Authors' Contributions

SR designed, SR, MN, carried out research, and SR, MN, MT, KA and SB wrote and corrected the manuscript.

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