

GREEN SYNTHESIS OF SELENIUM NANOPARTICLE USING COMBINATIONS OF LEAF EXTRACTS OF *WHITE ALLIUM CEPA L.* AND ITS BIOLOGICAL APPLICATIONS

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Abstract: The selenium nanoparticles (Se NPs) were formed by mixing of White *Allium cepa L.* peel extract and sodium hydroselenite (NaHSeO_3) solution. Se NPs were dispersed when this mixture was agitated. The goal of the investigation was to determine the phytochemicals in peel extract, green synthesis, characterization, and application. Large amounts of phytoconstituents were shown by screening analysis in the aqueous extract, and concurrently, the synthesized Se NPs, by Fourier Transform Infrared Spectroscopy (FTIR), confirmed that the functional groups linked to bioactive compounds were present. Positive results were reported for the aqueous extract's total flavonoid, phenolic, and tannin concentrations. According to UV-visible spectroscopic examination, the suspension solution validated the synthesis of Se NPs (210–280 nm). According to Field Emission Scanning Electron Microscope (FE-SEM), Se NPs were spherical and had diameters between 43.78 and 57.37 nm. The average particle size of Se NPs was found to be 22.8 nm by Atomic Force Microscopy (AFM) research. The amorphous form of Se NPs was demonstrated by X-ray diffraction (XRD) analysis. Gas chromatography and mass spectroscopy (GC-MS) analysis of the White *Allium cepa L.* extract revealed the presence of several phytochemical constituents, including siloxanes, hydrocarbons, aromatic compounds, and fatty acid derivatives, which may play an important role as reducing and stabilizing agents during nanoparticle synthesis. This study was designed to synthesize Se NPs from a safe substance (plant) and evaluate their biochemical and biological activity.

Keywords: nanotechnology, plants extract, selenium NPs, total antioxidant activity, white *Allium cepa L.*

1. Introduction

Green technologies are a relatively new trend that has grown in popularity over the past few decades. This is understandable given that their development aims to maintain Earth's natural resources. Green synthesis has received a lot of attention because it is environmentally and cost-effective [1]. The creation of nanomaterials can be accomplished reasonably easily and efficiently by reducing elemental Se to Se ions with the help of suitable reducers or stabilizers. The green synthesis technique uses a variety of biological agents and biomolecules that are involved in the production of nanoparticles to fuse NPs both internally and externally [2]. The importance of nanomaterials and nanotechnology in enhancing human existence as a result of scientific discoveries has led to an increase in interest in these topics, as seen by their diverse range of applications [3]. Because of their special chemical and physical characteristics, such as electrical and thermal conductivity and catalytic activity, nanomaterials have also become important in the advancement of technology [4]. The primary objective of nanomaterials is to produce components that are lighter, more compact, and perform better than traditional non-nanomaterials. Additionally, in order to investigate and create nanostructures, the science of nanomaterials integrates a number of basic and applied science fields [5, 6]. Selenium nanoparticles were created organically utilizing a number of ways, including *Bacillus sp.* Msh [7]. Bacteria, fungi, and plant extracts are the primary producers of biosynthesis, often known as biological or green synthesis. Because no hazardous waste is produced, it is less hazardous than chemical synthesis and is regarded as environmentally benign. However, variations in the composition of biological agents at different times may cause the faithfulness rate to differ between batches [8]. The onion (*Allium cepa L.*), a member of the *Allium* genus that is widely spread, is second only to tomatoes in terms of commercial importance. The plant comes in a variety of hues, including red, yellow, white, green, and purple, and each cultivar

has a unique flavor. *White Allium cepa L.* is a rich source of a variety of phytochemicals, including flavonoids, phenolic acids, fatty acids, organo-sulfur compounds, and saponins, all of which have positive health-promoting properties [9]. Antioxidants are compounds and agents that either stabilize or destroy free radicals. Biologically active antioxidants have the ability to efficiently scavenge free radicals and bind redox metals at physiologically relevant amounts [10]. Comparable antioxidant capacity has been demonstrated for a range of new compounds, which have been tested for their radical-scavenging activity [11]. However, this definition was eventually expanded to encompass compounds that repair the oxidative damage to the system as well as any agent that delays, stops, or eliminates oxidative damage to a target molecule [12]. An imbalance in the oxidant-antioxidant balance results in oxidative/nitrosative stress, which raises the generation of reactive oxygen and/or RNS. Reducing hydroperoxides into stable molecules and delaying oxidation reactions are two benefits of effective antioxidants [13]. The two main strategies the body employs to guard against oxidative damage are the removal of free radicals and their scavenging by enzymes and electron donors. [14]. *White Allium cepa L.* contains high amounts of phenolic compounds and flavonoids like Quercetin and Kaempferol with potent antioxidant properties. They neutralize reactive oxygen species (ROS) [15], transfer hydrogen and electrons, and bind metal ions, which helps to limit oxidative stress and free radical generation. They can also increase the activity of antioxidant enzymes, such as superoxide dismutase and catalase, which helps prevent degenerative diseases [16, 17]. According to certain research, the development of intrauterine diseases is significantly influenced by elevated levels of ORS and inflammatory biomarkers [18, 19]. Furthermore, a breakdown in hormone synthesis happens when reproductive organs experience severe oxidative stress, which may lead to infertility [19, 20]. Nutrition is fundamental to disease prevention and the enhancement of overall health, as it provides essential macro- and micronutrients necessary for normal cellular function. Recent research further indicates that adherence to the Mediterranean diet correlates with improved fertility parameters in both males and females, primarily through the reduction of oxidative damage to sperm and oocyte DNA [21]. The majority of *White Allium cepa L.* are common vegetables that are high in phenolics. According to reports [22]. The largest class of phenolic chemicals is flavonoids. *White Allium cepa L.* contain at least 25 distinct flavonols that have been identified [23, 24]. *White Allium cepa L.* contains six main flavonol compounds: (i) isorhamnetin, one of the methyl ethers of quercetin; (ii) quercetin monoglucoside; (iii) quercetin diglucosides; (iv) rutin; (v) isorhamnetin monoglucoside; and (vi) kaempferol. Although some varieties of *White Allium cepa L.* contain kaempferol, it is significantly less frequent than quercetin [25, 26]. So, the novel objectives of this study were to investigate the green synthesis of selenium nanoparticles using *White Allium cepa L.* extract, as well as study the effect antioxidant capacity with different levels in infertile Iraqi women.

2. Materials and methods

2.1. Collection of plant and chemicals. *White Allium cepa L.* fresh leaves were gathered and bought commercially at a nearby market. After being cleansed with deionized water, the leaves were sliced into tiny pieces. For later usage, allow these small pieces of *White Allium cepa L.* leaves extract to dry at room temperature [27]. The chemicals, which were used in this study, were obtained from Merck (USA). Naxifer™ kit (Iran).

2.2. Biosynthesis of SeNPs using green synthesis. Five grams of the ground *White Allium cepa L.* peels were put in a beaker with 200 milliliters of deionized water and heated to 50 degrees Celsius for ten minutes. The mixture was then swirled for thirty minutes. 0.5 grams of sodium hydroselenite (NaHSeO_3) were combined with 50 milliliters of deionized water in a separate beaker, and the mixture was swirled for half an hour. A single beaker was then filled with the contents of both beakers, and it was swirled for an hour. In order to extract the precipitate, the mixture was centrifuged at 4000 rpm. After that, the material was dried in an oven for a whole day at 100 °C. The spectra of the solution was then recorded using the UV-Vis spectrometer after the Se-*White Allium cepa L.* composite was ground [28]. For FTIR, XRD, and AFM, FE-SEM and GC-mass

spectroscopy studies. For total antioxidant capacity, the BIOBASE AO-EL10A Elisa Microplate Reader method was used according to standardized protocol.

2.3. Clinical application

2.3.1 Collection of blood specimens. Three healthy blood volunteers (n = 3) and sixteen infertile females (n = 16) were recruited for the total antioxidant test. Sodium citrate tubes were used to collect a 5 mL sample of peripheral blood. Women between the ages of 18 and 45 who are blood infertile and do not take medication for conditions such as diabetes mellitus, hypertension, heart disease, or vitamin or mineral supplements within 14 days before blood donation.

2.3.2. Preparation of Samples. For 20 minutes, the blood samples were centrifuged at 4000 x g. The extracted plasma was subjected to various concentrations of white *Allium cepa* L. aqueous extract or SeNPs (20, 40, 60, 80, and 100 µg/mL) with a ratio of 1:10 (plasma: female infertility) and (plasma: Se NPs) and incubated for 30 minutes at 50°C. For **Ethics Declaration**, this study was conducted using human serum samples provided by the *Women's Obstetrics and Gynecology Hospital*. Ethical approval for the use of these samples was obtained from the hospital's ethics committee under approval number 2159, dated 29 July 2025. All procedures were performed in accordance with institutional guidelines and ethical standards.

2.4. Statistical analysis. The program SPSS 26.0 (SPSS Inc., Chicago, IL, USA) was utilized to perform one-way variance analysis (ANOVA) on biological activities and semi-quantitation data. A p-value of less than 0.01 indicated a significant result 0.05 was considered significant [27, 29]

3. Results and Discussion

3.1. UV-Visible spectroscopy. The UV-visible spectra of selenium nanoparticles (SeNPs) produced using green synthesis. High absorption is seen in the UV area (210–280 nm) in Fig. 1, which is followed by a gradual decrease in absorbance toward the visible region. This activity is caused by phytochemicals from the plant extract that act as stabilizing and reducing agents as well as electronic transitions of the selenium core. The absence of a distinct surface plasmon resonance (SPR) band in the visible range is a characteristic of non-metallic SeNPs, indicating the formation of small, uniformly distributed nanoparticles with strong colloidal stability [30].

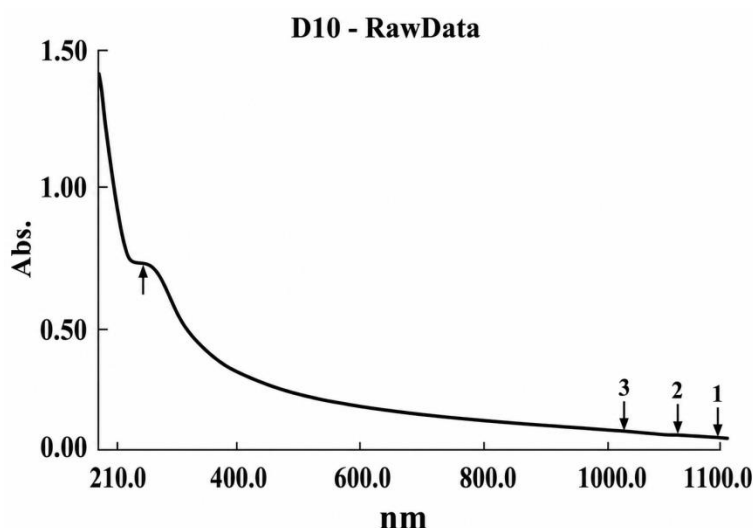


Fig. 1. Uv-Visible analysis of selenium nanoparticles (SeNPs) produced using *White Allium cepa* L. peel extract

3.2. Qualitative phytochemical. Qualitative phytochemical analysis was used to investigate the chemical components of the white leaf extract of *white Allium cepa* L. The Department of Chemistry at Karbala University conducted the analysis. College of Education for Pure Sciences, Karbala, Iraq. A phytochemical investigation of *white Allium cepa* L. cultivar's peel extract. To

achieve effective and sensitive compound detection, the compounds were ionized using both positive and negative electrospray ionization (ESI) modes. Alkaloids and terpenoids have identifiable peaks in the negative-ion mode as opposed to the positive-ion mode, especially in the phenol, flavonoid, saponin, tannin, and glycoide elution region. Additionally, it showed lower noise and more intense $[M-H]^-$ ions, which led to improved sensitivity and greater signal-to-noise ratios. The chemicals that were found were displayed in Table 1.

Table 1. The metabolites were found in *White Allium cepa L.* cultivars that underwent qualitative phytochemical analysis in both positive and negative ionization techniques

Phytochemicals	Se- <i>Allium cepa L.</i>
Phenols	+
Flavonoids	+
Alkaloids	-
Saponin	+
Tannin	+
Glycosides	+
Terpenoid	-

3.3. Fourier Transform Infrared Spectroscopy (FTIR). FTIR analysis of selenium nanoparticles (SeNPs) produced using *White Allium cepa L.* peel extract revealed distinctive absorption bands corresponding to functional groups involved in nanoparticle production and stability [31]. The broad band at about 3311 cm^{-1} is caused by the O–H stretching vibrations of phenolic compounds, indicating that they serve as capping agents. Peaks at 2922 and 2850 cm^{-1} represent aliphatic C–H stretching vibrations. The band at 1725 cm^{-1} is attributed to C=O stretching of carbonyl groups, while the peak at 1616 cm^{-1} is linked to aromatic C=C or amide I vibrations. Phenolic and polysaccharide C–O stretching is associated with the strong band at 1018 cm^{-1} . Additional bands at 888 and 644 cm^{-1} , which are due to Se–O or Se–Se vibrations, confirm the formation of SeNPs (Fig. 2).

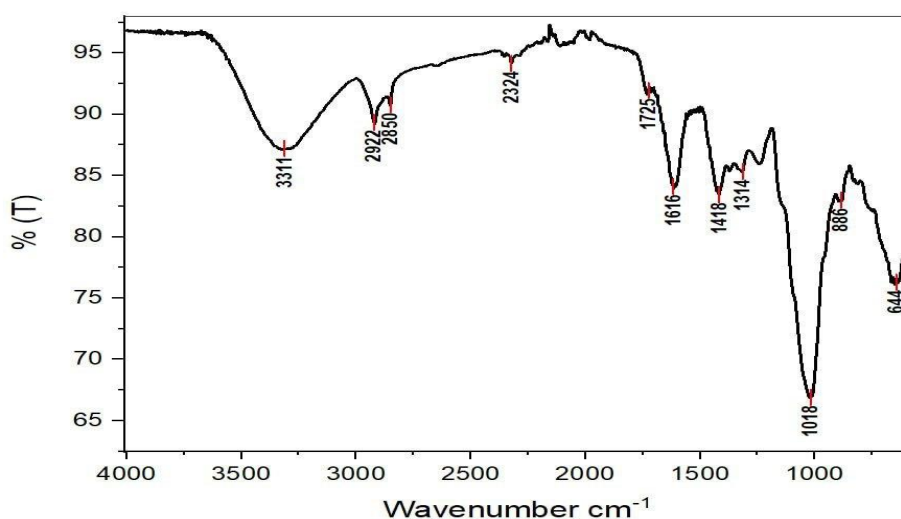


Fig. 2. FTIR analysis of selenium nanoparticles (SeNPs) produced using *White Allium cepa L* peel extract

3.4. Field Emission Scanning Electron Microscope (FESEM). The FESEM micrograph indicates the successful production of selenium nanoparticles (SeNPs) with mostly spherical to quasi-spherical morphology. Because phytochemical capping agents are present in green-synthesised nanoparticles, the particles are often well distributed with a small amount of

aggregation. The creation of nanoparticles within the nanometer scale is confirmed by the measured particle sizes, which vary roughly from 43.78 to 57.57 nm. The very homogeneous particle size distribution suggests that the bioactive substances in the *White Allium cepa L.* peel extract successfully stabilize SeNPs [32] (Fig. 3).

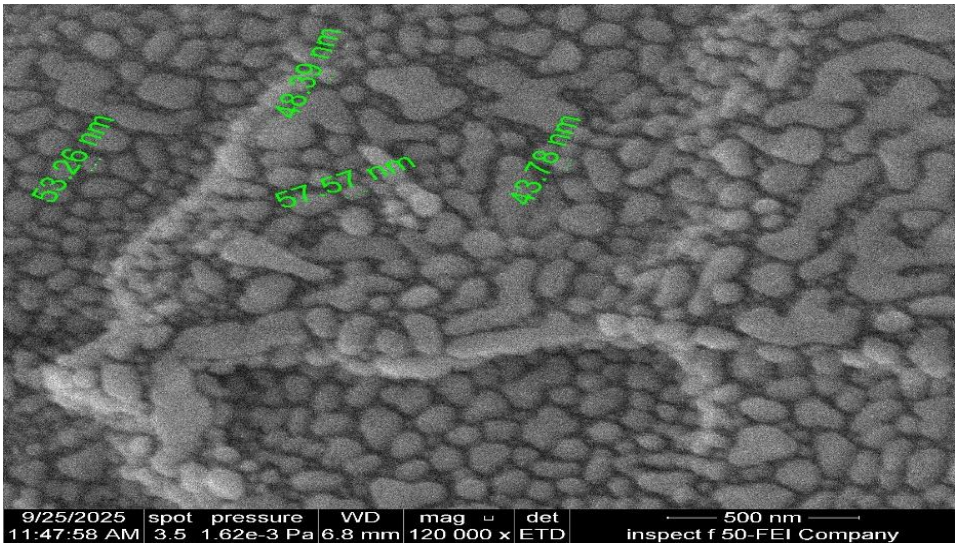


Fig. 3. FESEM micrograph of Se-*White Allium cepa L.* nanoparticles

3.5. Atomic force microscopy (AFM). In Fig. 4, selenium nanoparticles' surface morphology made with *White Allium cepa L.* peel extract was examined using atomic force microscopy (AFM). The surface of the 2D deflection image is uneven and rough, suggesting that the nanoparticles are not evenly distributed and have slightly aggregated.

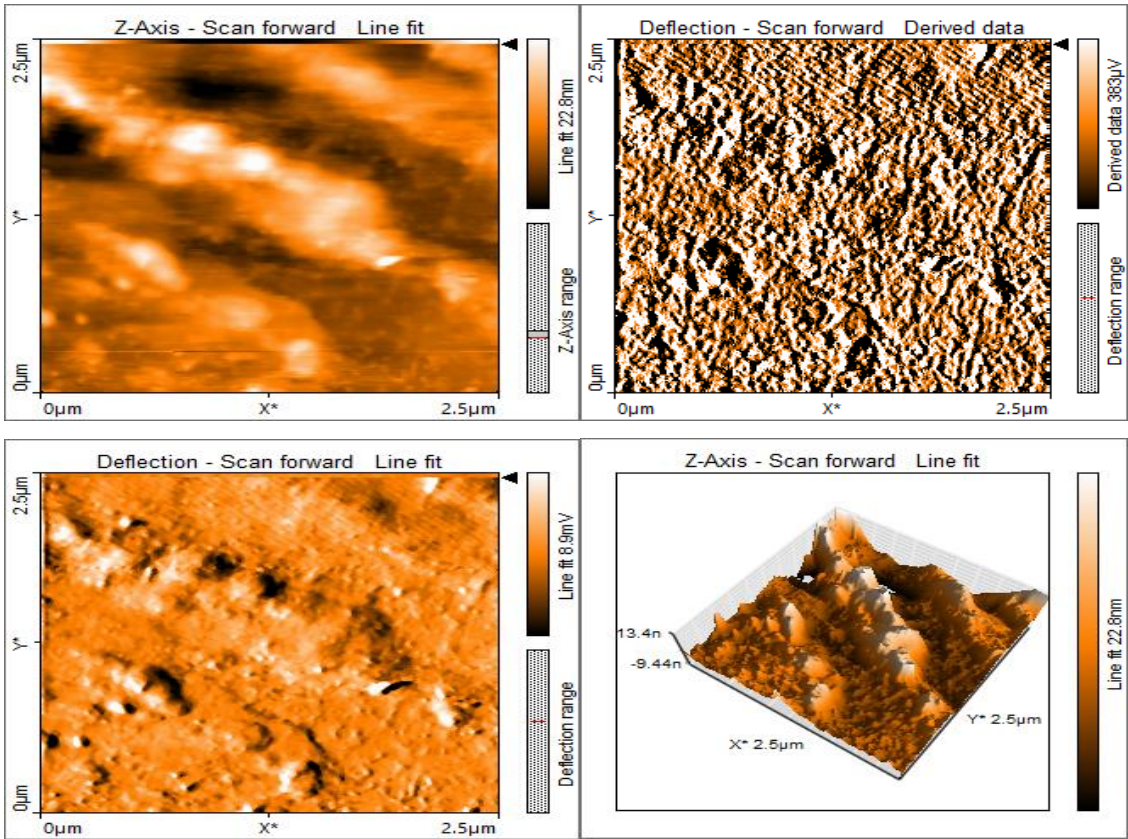


Fig. 4. AFM of selenium nanoparticles by phytochemical substances present in *White Allium cepa L.*

The 3D height profile shows the presence of nanoscale peaks and valleys over the scanned area ($2.5 \times 2.5 \mu\text{m}$), showing moderate surface roughness. Atomic Force Microscopy analysis revealed Se NPs had an average particle size of 22.8 nm. This morphology shows effective bio-capping and stability of selenium nanoparticles by phytochemical substances present in *White Allium cepa L* [31].

3.6. X-ray diffraction analysis (XRD). The crystalline structure of selenium nanoparticles made using *White Allium cepa L.* peel extract was investigated using XRD analysis. The characteristic planes of crystalline selenium are responsible for the notable diffraction peaks shown in the XRD pattern at 2θ values of 17.6° , 23.9° , 30.1° , 41.6° , 44.0° , 52.0° , 56.1° , and 61.8° . The crystalline character of the generated nanoparticles is confirmed by the presence of strong and forceful peaks. Additionally, the absence of other contaminating peaks indicates the high purity of the selenium nanoparticles. The slight peak broadening suggests the creation of nanoparticles [33]. The results reveal that the *White Allium cepa L.* peel extract effectively assisted the green production and stability of crystalline selenium nanoparticles [34]. As seen in Fig. 5.

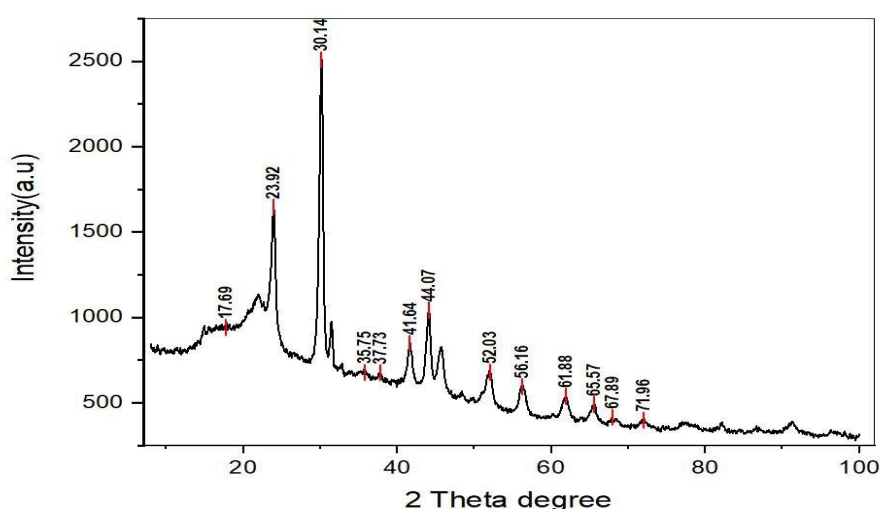
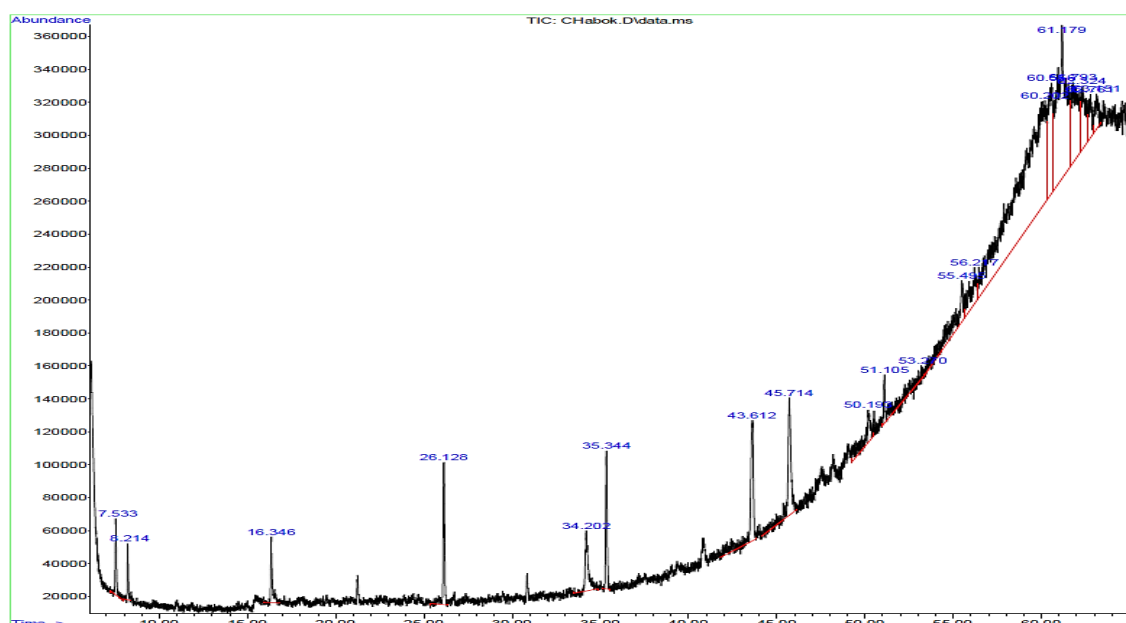


Fig. 5. XRD of selenium nanoparticles using green method

3.7. GC-Mass Spectroscopy. The white onion peel extract used for the synthesis of SeNPs was subjected to GC-MS. A number of peaks were observed in the chromatogram, representing different phytochemicals (Fig. 6). The most prominent compounds identified were Octasiloxane, Tetradecane, Eicosane, Hexadecane, Dodecane, Toluene and Decane [35]. The major peak was found at 60.200 min with an area percentage of 36.60%, which was identified as octasiloxane, and other siloxane compounds were also present at other retention times, suggesting their presence in high quantities in the extract [36]. The alkanes (dodecane, tetradecane, hexadecane, and eicosane) may help to stabilize the nanoparticles by preventing aggregation and enhancing colloidal stability [37]. Other compounds like toluene and i-propyl 11-octadecenoate may play a role in reduction and stabilization [38]. In conclusion, the GC-MS analysis identified several phytochemicals in white onion peel extract that may play a role as reducing and/or stabilizing agents in the green synthesis of SeNPs [39].

3.8. Biological Application. Total antioxidant capacity. The total antioxidant capacity (TAC) of the healthy group (No=3) was 93.71 ± 5.22 , which is the physiological baseline of the body's antioxidant equilibrium (Table 2). At this stage, free radicals are effectively neutralized by antioxidant defense systems without experiencing oxidative stress [40]. TAC was considerably higher than control ($P = 0.009$) in infertile women (No= 16) before receiving SeNPs, indicating a compensatory response to elevated oxidative stress. To combat too many free radicals, the body increases endogenous antioxidants like glutathione and superoxide dismutase [41].



20 µg/mL of SeNPs: TAC did not differ significantly ($P = 0.76$). It's possible that this modest dosage falls below the threshold required by the body to trigger antioxidant defense mechanisms [42]. The greatest statistically significant increase in total antioxidant capacity was seen at a dose of 40 µg/mL ($P = 0.008$). Since it increases the activity of selenoprotein enzymes like glutathione peroxidase, lowers free radical levels, and improves oxidative balance without causing toxicity, this dose seems to be ideal. *60 µg/mL of selenium nanoparticles:* at 60 µg/mL, there was also a statistically significant increase in total antioxidant capacity ($P = 0.039$), however it was not as noticeable as at 40 µg/mL. This suggests either a relative decrease in efficiency above the ideal dosage or persistent antioxidant activity with the start of cellular system saturation [42-46]. *80 and 100 µg/mL of Se NPs:* TAC did not significantly change. The overall response may be weakened by high doses that function as pro-oxidants, partially blocking cellular antioxidant mechanisms [41].

Table 2. The total antioxidant capacity of the healthy, infertile women, and Se NPs groups

Total Anti-Oxidant	Mean $\pm$ SD	P-value
Healthy Group	93.71* $\pm$ 5.22	< 0.00
Infertile women	109.7 $\pm$ 18.74	0.009
Se NPs (20 μ g/mL)	95.71 $\pm$ 19.94	0.76
Se NPs (40 μ g/mL)	112.61* $\pm$ 14.31	0.008
Se NPs (60 μ g/mL)	102.32* $\pm$ 8.87	0.039
Se NPs (80 μ g/mL)	103.24 $\pm$ 28.38	0.11
Se NPs (100 μ g/mL)	105.29 $\pm$ 34.89	0.08
Σ Mean Con. Se NPs	94.25* $\pm$ 12.23	0.0013

Total antioxidant levels were 17% higher in the infertile women (109.7 ± 18.74) than in the healthy group (93.71 ± 5.22). Following treatment with *White Allium cepa L.* extract-produced selenium nanoparticles, the antioxidant level dropped to 94.25 ± 12.23 , a fall of almost 14% as compared to the infertile group prior to treatment. Notably, following the delivery of SeNPs, the antioxidant level approached that of the healthy group. This difference was statistically significant ($P = 0.013$), implying that selenium nanoparticles could serve an essential regulatory role in maintaining redox balance. The observed decrease in total antioxidant capacity following therapy may be attributed to reduced oxidative stress and the restoration of physiological antioxidant

balance. These findings are consistent with prior research, which has shown that selenium nanoparticles have high antioxidant effects and may modify oxidative stress in biological systems. This observation is consistent with previous studies that established the significance of selenium nanoparticles in controlling oxidative stress and boosting cellular antioxidant defense mechanisms [46, 47].

To evaluate the impact of selenium nanoparticle treatment, the total antioxidant capacity was measured in healthy women, infertile women, and both primary and secondary infertile women. The results showed a significant difference in total antioxidant capacity (FRAP) between the studied groups ($P = 0.001$). The healthy control group had the lowest mean antioxidant level (93.71 ± 5.22), while infertile women had a higher value (109.7 ± 9.6). This rise may be a compensatory response to increased oxidative stress, which is usually associated with infertility. Following treatment with selenium nanoparticles (Se NPs), antioxidant capacity increased much more. After Se NP treatment, primary infertile women had the highest antioxidant levels (121.88 ± 23.76), indicating a significant improvement in their antioxidant defense system. Secondary infertile women treated with Se NP showed higher antioxidant levels (111.13 ± 16.07) compared to control and untreated infertile groups. These results imply that the systemic antioxidant status may be greatly enhanced by selenium nanoparticles made from *White Allium cepa L.* extract. Antioxidant enzymes like glutathione peroxidase, which shield cells from oxidative damage, are known to include selenium. Consequently, the observed rise in total antioxidant capacity following therapy may suggest that Se NPs have a positive impact on lowering oxidative stress linked to infertility. These results support the results of previous research. [27, 47-48].

Conclusion

The aqueous *white Allium cepa L.* leaf (onion) extract is a good source for synthesizing SeNPs and is considered a good antioxidant activity. UV-Vis, FTIR, FE-SEM, AFM, XRD, and GC-MS investigations verified the produced SeNPs. nanoscale size, spherical shape, and crystalline structure. In addition, phytochemicals of plants are responsible for the reducing and stabilizing of nanoparticles. Administering SeNPs to infertile women also increased antioxidant status and reproductive hormonal balance. Therefore, enhancing the characteristics of SeNPs with bio-compounds that have therapeutic potential can be a significant step toward creating safe, environmentally friendly medications for a variety of illnesses.

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Conflicts of Interest

The author declares no conflict of interest.

Ethics Declaration:

This study was conducted using human serum samples provided by the *Women Obstetrics and Gynecology Hospital*. Ethical approval for the use of these samples was obtained from the hospital's ethics committee under approval number 2159, dated 29 July 2025. All procedures were performed in accordance with institutional guidelines and ethical standards.

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