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Phytofabrication of Selenium-Silver Bimetallic Nanoparticles Using *Echinacea purpurea* Extract: Characterization and Antioxidant Activity

Echinacea purpurea Ekstraktı Kullanılarak Selenyum-Gümüş Bimetalik Nanopartiküllerin Fitofabrikasyonu: Karakterizasyon ve Antioksidan Aktivite

Berfin Rumeysa SARI [1*], **Çiğdem AYDIN ACAR** [1,2*]

[1] Burdur Mehmet Akif Ersoy University, Department of Health and Biomedical Sciences, Burdur, Türkiye
[2] Burdur Mehmet Akif Ersoy University, Bucak School of Health, Department of Nursing, Burdur, Türkiye

ABSTRACT

The phytofabrication of selenium-silver bimetallic nanoparticles (Se-Ag BNPs) utilizing *Echinacea purpurea* aqueous extract is investigated in this study. The synthesis process is characterized by a visible color change to dark reddish brown, a sign indicating successful nanoparticle formation. UV-visible spectrum analysis confirms the presence of SeNPs and AgNPs with absorption maxima at 268 nm and 325 nm, respectively. X-ray diffraction (XRD) patterns reveal the crystalline structure of the synthesized Se-Ag BNPs, exhibiting characteristic peaks consistent with metallic silver and selenium nanoparticles. Transmission electron microscopy (TEM) analysis showcases the diverse morphological structures of the Se-Ag BNPs, predominantly spherical but also featuring hexagonal and oval shapes. The average particle size is determined to be 33.38 nm, indicating uniformity and stability. Furthermore, the antioxidant properties of Se-Ag BNPs are evaluated through DPPH and ABTS radical scavenging assays, demonstrating dose-dependent scavenging capabilities with IC_{50} values of 264.78 µg/mL and 344.19 µg/mL, respectively. These findings underscore the potential of Se-Ag BNPs as effective antioxidants, offering promising applications in various fields such as biomedicine and environmental remediation. Comparisons with previous studies highlight the efficacy of the biosynthesis method using *Echinacea purpurea* extract in producing Se-Ag BNPs with superior antioxidant activity.

Keywords: Selenium, Silver, Bimetallic, Nanoparticles, *Echinacea purpurea*, Antioxidant

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ARAŞTIRMA MAKALESİ

*Corresponding author: cacar@mehmetakif.edu.tr

ÖZ

Bu çalışmada *Echinacea purpurea* sulu ekstraktı kullanılarak selenyum-gümüş bimetalik nanopartiküllerin (Se-Ag BNP'ler) fitofabrikasyonu araştırılmaktadır. Sentez süreci, başarılı nanoparçacık oluşumunu gösteren bir işaret olan koyu kırmızımsı kahverengiye gözle görülür bir renk değişimi ile karakterize edildi. UV-görünür spektrum analizi, sırasıyla 268 nm ve 325 nm'de maksimum absorpsiyonla SeNP'lerin ve AgNP'lerin varlığını doğruladı. X-ışını kırınımı (XRD) desenleri, sentezlenen Se-Ag BNP'lerin kristal yapısını ortaya çıkardı ve metalik gümüş-selenyum nanopartikülleri ile tutarlı karakteristik tepe noktaları sergiledi. Transmisyon elektron mikroskobu (TEM) analizi, Se-Ag BNP'lerin ağırlıklı olarak küresel olan ancak aynı zamanda altıgen ve oval şekillere sahip olan çeşitli morfolojik yapılarını sergilemektedir. Ortalama parçacık boyutunun 33,38 nm olduğu belirlendi, bu da tekdüzelik ve stabiliteyi göstermektedir. Ayrıca Se-Ag BNP'lerin antioksidan özellikleri, sırasıyla 264,78 µg/mL ve 344,19 µg/mL IC_{50} değerleriyle doza bağlı temizleme yeteneklerini gösteren DPPH ve ABTS radikal temizleme analizleri yoluyla değerlendirildi. Bu bulgular, biyotıp ve çevresel iyileştirme gibi çeşitli alanlarda umut verici uygulamalar sunan Se-Ag BNP'lerin etkili antioksidanlar olarak potansiyelini vurgulamaktadır. Önceki çalışmalarla yapılan karşılaştırmalar, üstün antioksidan aktiviteye sahip Se-Ag BNP'lerin üretiminde *Echinacea purpurea* ekstraktının kullanıldığı biyosentez yönteminin etkinliğini vurgulamaktadır.

Anahtar Kelimeler: Selenyum, Gümüş, Bimetalik, Nanopartiküller, Ekinezya, Antioksidan

1. Introduction

Nanotechnology is the process of creating new materials and devices with distinctive properties by moulding individual atoms, molecules, molecular clusters, or surfaces into specific shapes (1,2). The domain of nanotechnology has undergone exponential advancement in recent years, culminating in the pervasive integration of nanoparticle constituents within a myriad of products across diverse applications (3,4). Nowadays, nanotechnology is advancing at a breakneck speed and has a lot of potential advantages for human health. These materials' unique physical features and enhanced strength, durability, flexibility, and performance have been used in a wide range of industries and treatment modalities, such as prognostic visual monitoring of therapy, targeted drug delivery, and tumour detection (5). The speed at which nanotechnology is developing has demonstrated how heavily it depends on contemporary science and technology. In order to create novel materials with special features, modern nanotechnology makes use of developments in chemistry, physics, biology, medicine, materials science, biotechnology, and electronics. Because of this, there has been a lot of interest in creating nanoparticles (NPs) with unique properties for a range of uses. Particles with a diameter of 100 nm or less are referred to as nanoparticles. NPs differ from larger particles in the macro-scale size in that they have unique characteristics. Because of their reduced size range, nanoparticles have superior physical, chemical, and biological properties than bigger particles generated from the same precursor (6-8).

Traditionally, physical or chemical synthesis techniques are used to create nanomaterials. Regrettably, the toxic wastes produced by these processes had detrimental effects on ecosystems and human health (9). A practical and more sustainable option is the use of biological synthesis (green synthesis, green chemistry) for the creation of nanoparticles (Figure 1). When compared to traditional methods, they provide the advantages of being more economical, environmentally friendly, and energy-efficient. Biosynthesis is becoming increasingly important in nanotechnology since it is environmentally benign and straightforward (10-12). One of the most widely used materials in biosynthesis is metal nanoparticles, and the amount of research on this topic is increasing yearly (13). Plants, bacteria, algae, and fungi have been employed in the process of biological synthesis. However, a variety of NPs have been synthesised using plant materials, such as leaves, fruits, roots, stems, and seeds. Compared with bacterial and fungal-mediated synthesis, the use of plant extracts is one of the favorable techniques for producing metal nanoparticles. Additionally, a variety of phytochemicals found in their extract may serve as organic stabilising and/or reducing agents to aid in the formation of NPs (14,15).

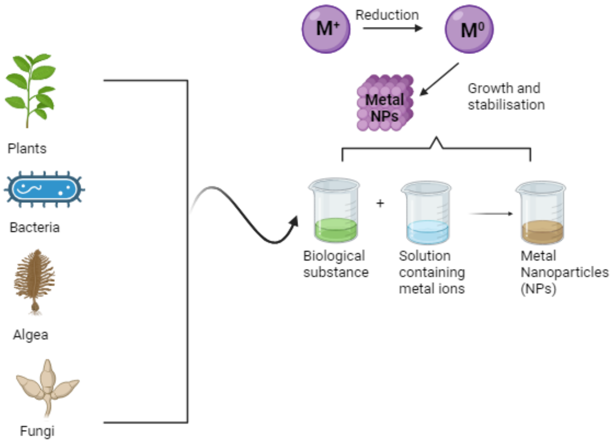

Figure 1: Schematic presentation of nanoparticles biosynthesis

Due to their distinctive qualities and the wide range of uses they have in optoelectronics, biological sensors, biological imaging, catalytic processes, antibacterial, and other biological domains, various metallic nanoparticles have been developed and identified over the past several decades (15). Bimetallic nanoparticles (BNPs) are one of the various nanoparticle types. Two differently shaped metal nanoparticles combine to form bimetallic nanoparticles. Metal atoms can occur in a variety of ways, including crown-jewel, hollow, heterostructure, alloyed, porous structures cluster-in-cluster, random alloy, and core shell formations. The distinct geometrical structure and mixing pattern of bimetallic nanoparticles enhance nanoparticles utility (16-18). The greatly enhanced optical, catalytic, and biological characteristics of bimetallic nanoparticles (BNPs) make them superior to monometallic NPs (MNPs) (19,20). Bimetallic nanoparticles have garnered a lot of interest lately from scientists because of their unique optical, magnetic, electrical, and catalytic characteristics (21,22). Bimetallic nanoparticles, one type of nanomaterial, are attracting a lot of interest in biomedicine due to its many uses, including sensing, catalysis, antioxidant, antibacterial, anticancer, and anti-diabetic effects (23). Many types of bimetallic nanoparticles including magnetic and noble elements have been synthesised with varied forms or geometry layouts (24).

Among the metal nanoparticles, silver (Ag), selenium (Se), and their bimetallic alloy NPs need to gain prominence by advancing optical, physical, chemical, catalytic, photothermal, and electrical contributions for a range of uses (25). Silver nanoparticles (AgNPs) have been extensively researched due to their superior physical, chemical, and biological features, and AgNPs showed better efficacy due to their larger capacity and higher surface area (area-to-volume ratio) compared to bulk silver (26,27). Silver nanoparticles have substantial biological capabilities, including antibacterial, anti-inflammatory, antidiabetic, antioxidant, anticancer, and antiviral activities (28,29). Selenium is a vital trace element that keeps a number of deadly or degenerative diseases at bay and is necessary for both human and animal immune systems to operate normally. According to reports, selenium is a crucial component that protects human cells and tissues from oxidative damage and stress by functioning as a cofactor and coenzyme at the catalytic-active sites of several selenoproteins and enzymes (30,31). Selenium nanoparticles (SeNPs) have drawn a lot of interest due to their distinct biological properties and minimal toxicity (32).

Oxidation is necessary for the human body's biochemical processes that produce energy to continue. Reactive oxygen species (ROS) are created during metabolic processes within cells. Lower levels of ROS are helpful for cellular formation, metabolism, growth, and signalling; nevertheless, an imbalance between their production and accumulation led to oxidative stress (33,34). The antioxidant qualities of silver and selenium nanoparticles make them important for health in a variety of biological and biochemical processes. However, the characteristics and biological activities of these nanoparticles may vary due to differences in the metabolite compositions and contents in the plant extracts utilised during the reduction process, requiring additional research on powerful AgNPs and SeNPs employing a larger variety of plants (35,36).

Echinacea purpurea (Echinacea flower) is an indigenous plant found in the Great Plains of the United States and the Canadian prairies. *Echinacea purpurea* is traditionally used to treat inflammatory disorders (37,38). In North America and Europe, *E. purpurea* is a popular medicinal plant used for treating and preventing upper respiratory tract infections. The bioactive components in Echinacea preparations, such as alkamides, derivatives of caffeic acid, and polysaccharides, are responsible for their pharmacological actions (such as immunomodulatory activity, antifungal and antibacterial activities, and antioxidant property) (39). Studies have emphasized that the plant contains rich phenolic compounds, flavonoids and polyphenols. These components reduce oxidative stress by neutralizing free radicals and prevent cellular damage. Research shows that Echinacea supports antioxidant properties as well as anti-inflammatory effects. These properties, combined with Echinacea purpurea's ability to strengthen the immune system, produce positive effects on overall health (40, 41). Therefore, *E. purpurea* has an important place as a natural source of antioxidants (42). In this study, *E. purpurea* extract was preferred because of its antioxidant properties and various biomolecules (phenols, flavonoids, etc.) that it naturally possesses. These components contribute to the reduction and stabilization processes in the synthesis of nanoparticles.

The aim of this study is to synthesize selenium-silver bimetallic nanoparticles (Se-Ag BNPs) using the aqueous extract of *Echinacea purpurea* and to characterize the resulting nanoparticles while evaluating their antioxidant activities. The role of the aqueous extract as an environmentally friendly and cost-effective resource in nanoparticle synthesis will be emphasized. Additionally, the effectiveness of the obtained Se-Ag BNPs demonstrated through various antioxidant assays will highlight the advantages of this biosynthesis method, providing an important foundation for future research.

2. Material and Method

Preparation of *Echinacea Purpurea* Extract

A dried *Echinacea purpurea* plant was purchased for the biosynthesis procedure from a local market. 5g was weighed, and 100 millilitres of distilled water (dH_2O) were used to dissolve it. A microwave oven (900 W) was used to boil the mixture for 1 minutes. Time was allowed for the plant extract to cool down. Once the plant extract was chilled, it was filtered through Whatman No. 1 filter paper to produce *Echinacea purpurea* plant extract. For a minute, the extract was centrifuged at 1500 rpm. To be used later, the *Echinacea purpurea* extract was transferred to a sterile tube and kept at +4°C. *Echinacea purpurea* extract was carried out according to the method used by Ceylan et al. (2024) (43).

Synthesis of Bimetallic Nanoparticles From *Echinacea Purpurea* Extract

Se-Ag BNPs were produced by applying the straightforward method that Hashem et al. (25). 20 mL of sodium selenite (Na_2SeO_3) (Bostonchem, Boston, MA, Cas #10102-18-8) and 60 mL of plant extract were mixed, then 2 ml of L-ascorbic acid (Carlo Erba, France, Cas#50-81-7) was added, after that 20 mL of silver nitrate ($AgNO_3$), and 60 mL of plant extract. The solution was vigorously agitated at 60°C. The pH was then adjusted by adding 1M NaOH. During the technique, the completed liquid was magnetically agitated for two hours at 60°C, resulting in a colour change. Following the colour change, the solution was centrifuged at 10,000 rpm for 15 minutes to separate the precipitate, and the procedure was repeated with distilled water. After washing with methanol, the Se-Ag BNPs were dried overnight at 40°C and ground into powder.

Characterization of Se-Ag BNPs

Visual examination was the initial characterization. The visual examination of the reduction of metal ions was conducted by observing the colour change of Se-Ag BNPs in the reaction media. The biosynthesized Se-Ag BNPs were characterised using UV-Vis spectroscopy (T60, PG Instruments Ltd., Japan). The spectra were measured between 200 and 600 nm in order to determine the maximal surface plasmon resonance. The surface shape, edge size and dispersion of the biosynthesised Se-Ag BNPs were investigated by transmission electron microscopy (TEM) (JEOL JEM-1400 Plus) analysis. TEM analysis was conducted at Çanakkale Onsekiz Mart University Science and Technology Application and Research Center (ÇOBİLTUM). Crystallographic characterization of the purified Se-Ag BNPs was performed by X-ray diffraction (XRD) (Bruker AXS D8 Advance) analysis. XRD analysis was carried out at Burdur Mehmet Akif Ersoy University Scientific and Technology Application and Research Center (Biltekmer).

Antioxidant Activity

1-Diphenyl-1-2-picrylhydrazyl (DPPH) scavenging assay

Methanol was used to serially dilute each sample. Se-AgNPs and *Echinacea purpurea* plant extract samples were produced separately and added in varying doses (0-5000 µg/mL) to a DPPH (abcr GmbH, Germany, Cas# 1898-66-4) solution (0.1 mM) in methanol (1:1 ratio). The final combination was allowed to stand at room temperature for 30 min in the dark. Using a spectrophotometer (T60 UV-PG Instruments), spectrophotometric measurements were performed on control test samples at 517 nm. A positive control was ascorbic acid. Every measurement was made three times. The percentage scavenging activity was calculated using the following formula (44):

$$\%\,Inhibition = \ (\,Control - Sample)/Control * 100$$

%Inhibition: Percentage of the radical scavenging activity, Control: Absorbance of the control sample (DPPH solution without test sample), Sample: Absorbance of the test sample (DPPH solution with test compound)

2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay

ABTS* radicals were prepared by the reaction using a 1:1 (v/v) ratio. 7 mM ABTS (PanReac Applichem GmbH, Darmstadt, Germany, Cas no#30931-67-0) and 2.5 mM potassium persulfate ($K_2S_2O_8$) were mixed and incubated for 16 hours at room temperature in darkness. Methanol was added to this solution until it reached an absorbance of 0.70 at 734 nm. Se-AgNPs and *Echinacea purpurea* plant extract were prepared by serial dilution with methanol at different concentrations (0–5000 µg/mL). 80 µL of sample and 0.92 mL of ABTS* solution were combined to create the test mixture, which was then incubated for 30 minutes in the dark. Ascorbic acid was used as a positive control. The absorbance of samples was measured at 734 nm wavelength using a spectrophotometer (T60 UV-PG Instruments) (45). The % inhibition was calculated using the following formula:

$$\%\,Inhibition = \ (\,Control - Sample)/Control * 100$$

%Inhibition: Percentage of the radical scavenging activity, Control: Absorbance of the control sample (ABTS solution without test sample), Sample: Absorbance of the test sample (ABTS solution with test compound)

Statistical Analysis

All experiments were performed in at least triplicate and the results were expressed as mean ± standard deviation (SD). Half maximum inhibitor concentration (IC_{50}) was calculated using GraphPad Prism Software.

3. Results and Discussion

Phytofabrication of Se-Ag BNPs

Echinacea purpurea aqueous extract was used for the biosynthesis of Se-Ag BNPs (Figure 2). The earliest observation of Se-Ag BNP production is a visible colour change. After bimetallic nanoparticles were synthesised, the solution's colour transitioned to dark reddish brown (Figure 3a). Hashem et al. confirmed the formation of bimetallic nanoparticles with color change consistent with our study (25).

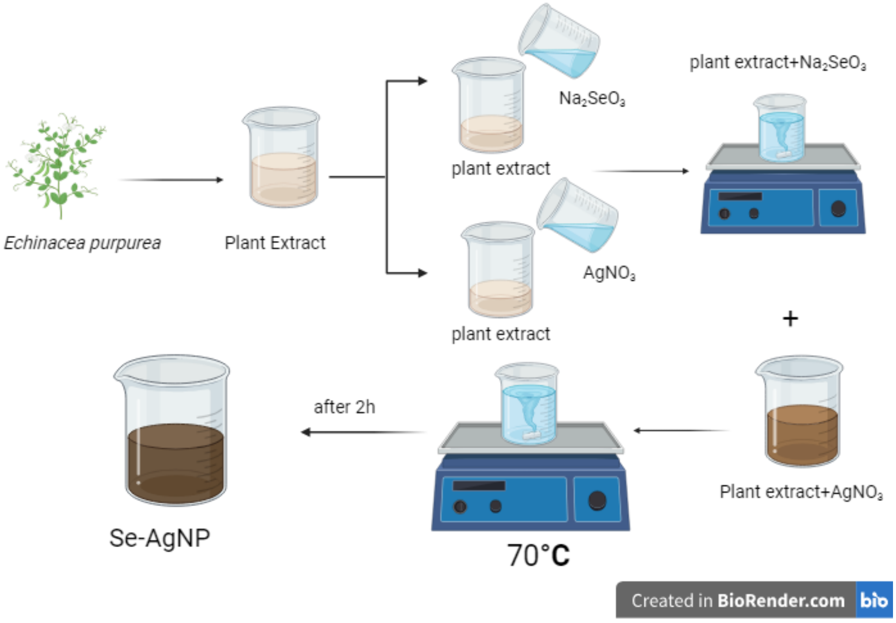

Figure 2: Biosynthesis of selenium-silver bimetallic nanoparticles (Se-Ag BNP) using *Echinacea purpurea* extract

Characterization of Se-Ag BNPs

Se-Ag BNPs stability and formation were assessed using a UV-visible spectrum. AgNPs are viewed as brown in many situations because they absorb radiation in the 350–450 nm visible range of the electromagnetic spectrum due to the stimulation of surface plasmon oscillations (46). The crystalline structure of SeNPs results in absorption maxima at 200 to 300 nm. As a result, the detection of the peak between 200 and 300 nm confirms the presence of SeNPs (47,48). In this study, it was determined that the UV–vis absorption spectrum showed absorption maximum at 325 nm, indicating the presence of AgNPs, and a shoulder peak at 268 nm, indicating the presence of SeNPs (Figure 3b).

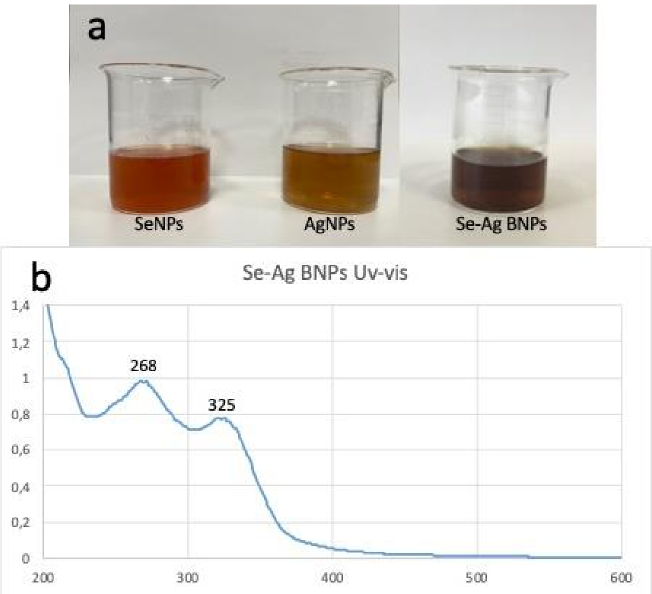

Figure 3: Color change indicating the formation of Se-Ag BNPs (a) UV-Vis spectrum of Se-Ag BNPs synthesized using *Echinacea purpurea* plant extract (b)

XRD analysis was used to examine the produced Se-Ag BNPs phase and crystal structure. The XRD pattern of the synthesized NPs is shown in Figure 4. The pattern makes it evident that neither of the initial precursors' characteristic peaks—sodium selenite nor silver nitrate—is present. XRD measurements were confirmed the synthesis of the nano-complex (bimetallic Ag-Se BNPs) (49).

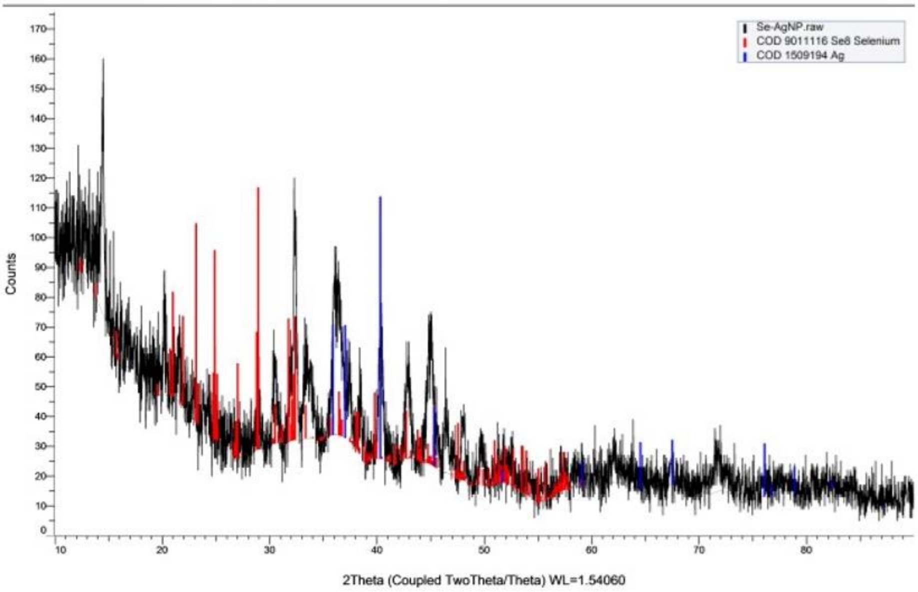

Figure 4: XRD patterns of Se-Ag BNPs synthesized using *Echinacea purpurea* plant extract

Figure 5 shows the morphological structure of the synthesized Se-Ag bimetallic nanoparticles. Transmission electron microscopy analysis revealed that the nanoparticles were of various shapes and sizes. The main shape observed was generally spherical, although in some cases hexagonal-like and oval structures were also observed. These different shapes and sizes are thought to be due to various interactions during the synthesis process. The synthesized bimetallic Ag-Se BNPs, the particle sizes ranged from 13.69 nm to 56.42 nm with an average size as 33.38 nm. Data from TEM images show that these nanoparticles are generally more uniform and spherical. These findings indicate that the nanoparticles obtained by mixing Se and Ag elements in a certain ratio have a stable morphology (15, 25). Consistent with our findings, Ag_2Se nanoparticles were produced using *P. aeruginosa*, and their diameters were found to be 30-40 nm microsphere-like structures (50). Olawale et al. (2021) synthesized near-spherical, pentagonal, and hexagonal shapes Ag@Se NPs with an average size of 33.1 nm from *Ocimum tenuiflorum L.* (51)*.* Similarly, Hashem et al. (2023) Bimetallic Se-Ag NPs produced by watermelon rind had spherical and oval shapes and the average size was 24.5 nm (25).

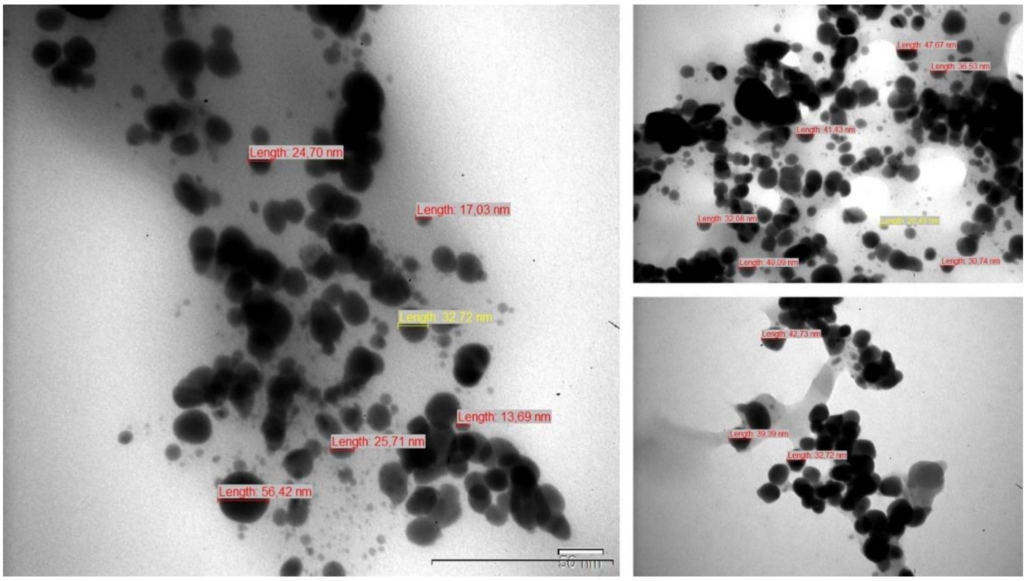

Figure 5: TEM images of Se-Ag bimetallic nanoparticles

Antioxidant Capacity of Se-Ag BNPs

Se-Ag BNPs were tested for their antioxidant capacity using DPPH and ABTS radical scavenging assays. DPPH reducing capacity of Se-Ag BNPs was evaluated on the basis of color change. It was found that Se-Ag BNPs scavenged DPPH radicals at dose-dependently increasing concentrations of 100, 250, 500, 1000, 2000, 3000 and 5000 µg/mL (Figure 6a). While the biosynthesized Se-Ag BNPs showed DPPH scavenging ability of 36.58% at low concentrations of 100 µg/mL, their scavenging capacity increased to 93.40% at high concentrations of 5000 µg/mL. At the same time, the biosynthesized Se-Ag BNPs were tested for their capacity to scavenge ABTS radicals at dosages of 100-5000 µg/mL. The data clearly showed that Se-Ag BNPs have dose-dependent ABTS radical scavenging properties (Figure 6a). Moreover, at low concentrations of 100 µg/mL, Se-Ag BNPs showed 18.85% scavenging capacity against ABTS. At a concentration of 5000 µg/mL, Se-Ag BNPs were found to have an increased ABTS radical scavenging percentage of 97.92%. The results also showed that the IC_{50} values of Se-Ag BNPs recorded for DPPH and ABTS were 264.78 µg/mL and 344.19 µg/mL, respectively (Figure 6b). A recently reported study showed that Ag-Se BNPs formed from Jerusalem Thorn seed and plant extract showed lower DPPH and ABTS scavenging activity. The IC_{50} value determined by DPPH for Ag@Se-P nanoparticles was 33.85 g/mL (49). Bimetallic Ag-Se NPs using gamma irradiation and a bacterial filter of *Bacillus paramycoides* were reported to have similarly dose-dependent antioxidant activity (IC_{50}: 98.52 µg mL^{-1}) (15).

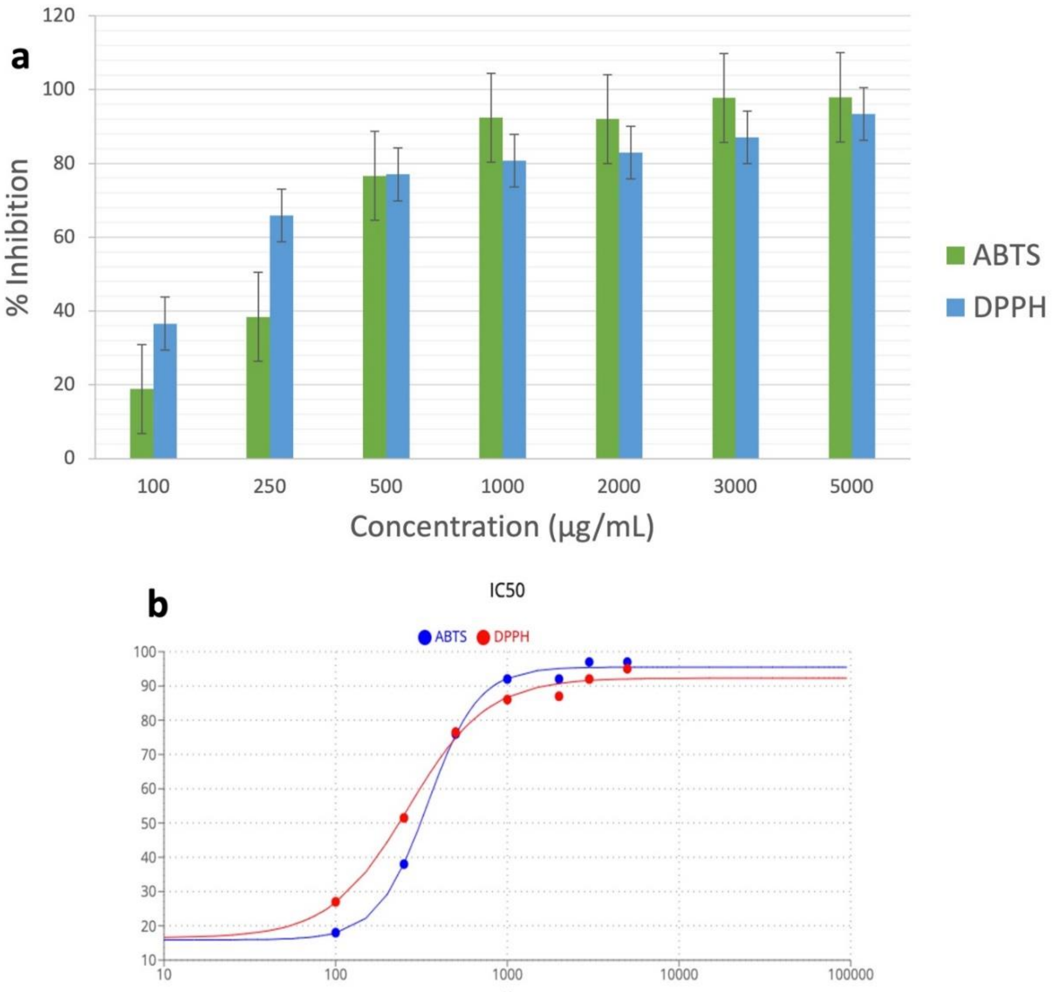

Figure 6: DPPH and ABTS radical scavenging activity of biosynthesized Se-Ag BNPs (a) IC 50 values for DPPH and ABTS results (b)

According to the results obtained from the evaluation of the antioxidant activities of the synthesized Se-Ag BNPs, they showed promising antioxidant potential in the present study. The ability of metal nanoparticles to neutralise free radicals has been extensively documented in the literature. The antioxidant effect of metal NPs was mainly due to the movement of electrons, which neutralised DPPH and prevented it from producing free radicals. In addition, the high surface-to-volume ratio may enhance the antioxidant activity of metal nanoparticles (15,52,53).

4. Conclusion

This study successfully demonstrates the phytofabrication of selenium-silver bimetallic nanoparticles (Se-Ag BNPs) using Echinacea purpurea aqueous extract. The observed color change and subsequent characterization through UV-visible spectroscopy, X-ray diffraction, and transmission electron microscopy confirm the successful synthesis of Se-Ag BNPs with uniform morphology and crystalline structure.

The antioxidant capacity of Se-Ag BNPs was evaluated using DPPH and ABTS radical scavenging assays, demonstrating dose-dependent scavenging abilities against both radicals. These findings suggest the potential of Se-Ag BNPs as effective antioxidants. The observed antioxidant activity of Se-Ag BNPs underscores their potential as a novel source of antioxidants. The documented ability of metal nanoparticles to neutralize free radicals, coupled with the high surface-to-volume ratio of Se-Ag BNPs, further supports their efficacy as antioxidants. These promising results warrant further investigation into the biomedical and industrial applications of bimetallic Se-Ag BNPs as potent antioxidants.

Future research should focus on optimizing the synthesis parameters of Se-Ag BNPs by varying the concentrations of Echinacea purpurea extract and reaction conditions to enhance particle size and stability. Additionally, conducting *in vivo* studies will be crucial to assess the biocompatibility, bioavailability, and therapeutic efficacy of these nanoparticles. Exploring their integration into drug delivery systems could target specific diseases, while investigating their effectiveness in environmental remediation will provide insights into their potential for removing heavy metals and pollutants from wastewater. Finally, conducting mechanistic studies to understand the antioxidant mechanisms of Se-Ag BNPs will further elucidate their biochemical interactions and health benefits, ultimately advancing their applicability across various fields.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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