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Biosynthesis of Silver Nanoparticles using *Echinacea pallida* (Nutt.) Nutt. and Antioxidant Activity Thereof

[1]Esma Nur Gecer and [1,2]Ramazan Erenler[*]
[1]*Department of Chemistry, Faculty of Arts and Sciences, Tokat Gaziosmanpasa University, 60240 Tokat, Turkey.*
[2]*Faculty of Health Sciences, Igdir University, Igdir, Turkey.*
rerenler@gmail.com*

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Summary: In this work, silver nanoparticles (Ep-AgNPs) were synthesised using *Echinacea pallida* (Nutt.) Nutt. The dried leaves of *E. pallida* were collected and heated at 55 °C in deionized water, and filtered, and the leaf extract was treated with $AgNO_3$ to produce the Ep-AgNPs. The Ep-AgNPs were analyzed by Ultraviolet-visible (UV-Vis), Fourier transform infrared (FTIR), X-ray diffraction (XRD), and scanning electron microscope (SEM). The characteristic hydroxyl gave the peak at 3147 cm^{-1}. In UV-Vis analysis, observation of absorption band at 468 nm proved the achievement of Ep-AgNPs synthesis. SEM analysis presented the spherical shape of nanostructures with an average size of 77.82 nm. The face-centered crystal structure of Ep-AgNPs was revealed by the XRD analysis. The 1,1-diphenyl-2-picryl-hydrazyl radical ($DPPH^{\bullet}$) scavenging, ferric reducing antioxidant power (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) assays were employed for antioxidant study in which Ep-AgNPs exhibited excellent antioxidant effect. Ep-AgNPs displayed outstanding $DPPH^{\bullet}$ activity (6.34, IC_{50}, µg/ml) compared to the standard BHT (10.78, IC_{50}, µg/ml). The high activity was observed for $ABTS^{\bullet+}$ and reducing power assays as well. Hence, Ep-AgNPs could be a valuable material for the food and pharmaceutical industry.

Keywords: *Echinacea pallida,* Silver nanoparticles, Antioxidant, Spectroscopy, Green synthesis.

Introduction

Due to the large surface area to volume ratio, nanoparticles exhibit unique physical, chemical, and biological properties [1]. The research on nanoparticles has become the fundamental area for modern material sciences and medicine. Due to the great variety of properties such as optics, anticancer, antimicrobial, antioxidant, anticoagulant, thrombolytic, antidiabetic, and catalytic, the nanoparticles have gained great interest in electronics, biology, medicine, and pharmacy [2-6]. Metal nanoparticles with unique properties are being studied extensively for their potential applicability in various fields [7, 8]. Silver nanoparticles are highly stable and show toxic properties against bacteria, fungi, and viruses. Since Ag^+ ions obtained from silver nanoparticles have high bactericidal activity, they cause cell death by targeting the respiratory chain and cell division [9]. During nanoparticle synthesis, plant extracts play a critical role in their reducing and coating properties. Due to these properties in the scientific and technological field, it is the subject of intense scientific research. Since plants are cheap to obtain and scalable, they are more preferred than other biological methods for silver nanoparticle synthesis. Plants contain many antioxidants that act as reducing and coating agents [10]. However, metabolites that are of microbial and animal origins have also been used for the green synthesis of different metal nanoparticles for biomedical applications [11, 12].

Natural products attract attention in drug invention due to a large variety of biological activities [13-16]. *Echinacea* genus belonging to the Asteraceae family is an herbaceous and perennial plant with ornamental and medicinal value. The *Echinacea* genus has 11 species, among them, *E. purpurea*, *E. angustifolia* and *E. pallida* have medicinal and commercial importance. These three *Echinacea* species are widely grown in the United States, Canada, and Europe, especially Germany. *E. pallida* is phytochemically unique in terms of phenolic content and alkalamides, thus possessing important biological activities such as stimulating/strengthening the immune system and antioxidant, wound healing, and antimicrobial effects [17, 18]. The biologically active components of *Echinacea* are alkamides, [11]polyacetylenes, and phenolic acids such as caftaric acid, caffeic acid, and chlorogenic acid [19]. Their chemical contents may differ in various conditions such as plant species, plant part, climate, soil, and altitude [20].

[*]**To whom all correspondence should be addressed.**

Free radicals are toxic substances that are products of normal aerobic metabolic processes in the body. The antioxidant is defined as a molecule that can prevent or destroy the formation of free radicals [21]. The physiological role of compounds with antioxidant properties; they prevent damage to cellular components by neutralizing chemical reactions involving free radicals. The human being has various defense mechanisms against free radicals and reactive oxygen species. However, these mechanisms are insufficient against free radicals in some conditions such as cigarette smoking, alcohol, and bad living condition. Hence it is essential to take the antioxidant from foods, food supplements, and beverages [22-24]. Antioxidants can be divided into synthetic and natural products. Natural antioxidants are preferred to synthetic ones due to their higher safety [25-27]. Silver nanoparticles synthesized using some medicinal plants displayed antioxidant activity [28, 29]. Moreover, nanoparticles synthesized from curcumin exhibited anticancer activity [30].

Herein, green synthesis of Ep-AgNPs was carried out, and investigated their antioxidant activity.

Experimental

Chemicals

The chemicals, reagents, and solvents such as silver nitrate ($AgNO_3$), MeOH, butylated hdyroxyanisole (BHA), butylated hydroxytoluene (BHT), Trolox, ferrous chloride, DPPH, trichloroacetic acid, ABTS were bought from Sigma-Aldrich Chemicals, Germany.

Plant materials

Echinacea pallida (Nutt.) Nutt. was cultivated in the Aromatic and Medicinal Plant Field of Tokat Gaziosmanpasa University.

Synthesis of silver nanoparticles

E. pallida dried leaves (20 g) and deionized water (150 mL) were added to a round bottom flask (1.0 L), and the mixture was extracted at 55 °C for 3 h. After filtration with Whatman filter paper (Grade 1), the solid material was removed, and the extract solution (100 mL) was treated with silver nitrate deionized water solution (0.024 M, 200 ml) at 65 °C for 2 h. The mixture was cooled to ambient temperature, then the mixture was centrifuged at 15000 rpm for 15 min to yield the Ep-AgNPs washed thoroughly with deionized water. The desired Ep-AgNPs were dried by lyophilization [29].

Characterization of silver nanoparticles

Hitachi U-2900 spectrophotometer was employed for UV-Vis measurement in the range of 200 to 800 nm. XRD analysis was executed to present the crystalline nature of Ep-AgNPs. Empyrean, Malvern Panalytical diffractometer was used for the XRD pattern. The operation voltage was set as 45 kV and 40 mA. Dynamic light scattering (DelsaNano C instrument) was used to calculate the particle size. The morphology of synthesized Ep-AgNPs was observed on SEM. The elemental analysis was carried out by an EDAX detector and EDX. Quanta 450 FEG and Quanta Feg450 were used for surface, point analysis, and SEM analysis, respectively. Fourier transform infrared (FT/IR-4700 Jasco) spectrometer was used to present the functional groups of the natural compounds.

Antioxidant activity

DPPH free radical scavenging effect

1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging assay was used. Initially, stock solutions of Ep-AgNPs (1.0 mg/mL) were prepared. Various concentrations of Ep-AgNPs (2-40 µg/mL, 3.0 mL) were added to the DPPH solution in ethanol (1.0 mL, 0.26 mM). BHA, BHT, and Trolox were used as the standards. The mixture was incubated at ambient temperature for 30 min, then absorbance was recorded at 517 nm. The results were indicated as IC50 which represented the scavenging 50% of DPPH free radicals [31].

$ABTS^{\bullet+}$ radical cation scavenging assay

Initially, Ep-AgNPs stock solution (0.25 mg/mL, in DMSO) was prepared, and phosphate buffer (pH 7.4, 0.1 mM) was produced then the treatment was executed in this buffer solution. $ABTS^{\bullet+}$ radical cation solution was prepared by treatment of ABTS (2.0 M, 100 mL) with sodium persulfate (2.45 mM, 200 mL) at dark for 6 hours at room temperature. The different concentration of Ep-AgNPs (2-40 µg/mL) was reacted with $ABTS^{\bullet+}$ solution. The spectroscopic measurement was executed at 734 nm. The results were calculated concerning to the Trolox equivalent (TE) [32].

Reducing power

Ep-AgNPs solution (100 µL, 40-150 µg/mL) was mixed with phosphate buffer (1.15 mL, 0.20 M, pH 6.7), potassium ferric cyanide (1.0%, 1.25 mL) then the mixture was incubated for 30 min at 50°C. Trifluoroacetic acid (1. 25 mL, 10%) and $FeCl_3$ (0.25 mL, 0.1%) were added to the reaction flask, vortexed then UV-Vis measurement was executed at 700 nm. The calibration curve of Trolox at various concentrations was drawn and the results of samples were calculated concerning to the TE [33].

Statistical analysis

GraphPad Prism was used for the statistical analysis of antioxidant assays. One-way ANOVA with Tukey's multiple comparison test was applied for the comparison of the means of each column with every other column. The results were determined by mean value ± SDs with $P < 0.05$.

Results and Discussion

UV-Vis spectroscopic analysis

The nanoparticles were synthesised using *E. pallida.* The colour change from yellow to dark brown revealed the reduction of Ag^+ into Ag^0 (Fig. 1) which accorded with the reported study [34]. UV-Vis analysis displayed the reduction of Ag^+ and confirmed the formation of Ep-AgNPs. A strong band (350-550 nm) in the visible region was observed which was the characteristic of silver nanoparticles. In addition, absorption in 410-500 nm in the UV-Vis spectrum confirmed the spherical nanoparticles [35]. The observation of maximum absorption at 468 nm also approved the formation of nanostructures (Fig. 1) [36].

Fourier-transform infrared spectroscopy

Plants contain secondary metabolites such as flavonoids, alkaloids, and terpenes with reducing ability. The functional group of responsible bioactive compounds for surface coating and effective stabilization of Ep-AgNPs were presented by FTIR analysis. Broadband at 3175 cm^{-1} in FTIR spectrum corresponds to the OH stretching, while 2032 cm^{-1} could be due to the CH stretching of alkyne. The characteristic carbonyl signal appeared at 1583 cm^{-1}. In addition, the signal at 1046 cm^{-1} could be assigned to the CN stretching of amine. The peak observed at 518 cm^{-1} represented the silver oxide which accorded to the reported study [37]. The slight change between the extract spectrum and nanoparticle spectrum proved the formation of nanoparticles. During the reaction, silver ions were reduced while some molecules in the extract were oxidized (Fig. 2).

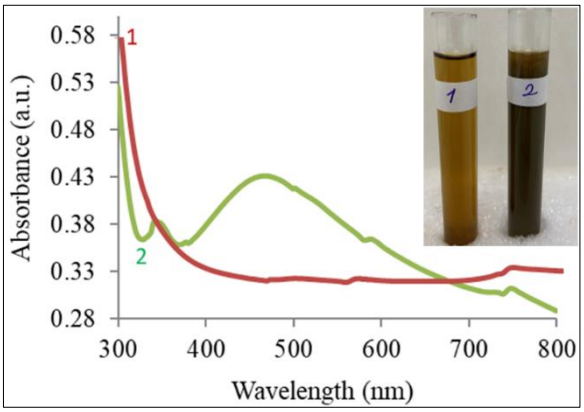

Fig. 1: Extract (1), Ep-AgNPs (2) and UV-vis spectrum of extract (1) and AgNPs (2).

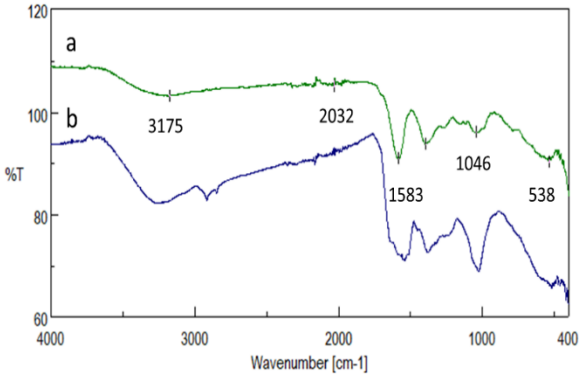

Fig. 2: FTIR spectrum of Ep-AgNPs from *E. pallida.* 1: 3175, 2: 2032, 3: 1583, 4: 1046, 5: 538

X-ray diffraction

The crystalline shape of synthesised silver nanoparticles using *E. pallida* was determined by X-ray diffraction (Fig. 3). The spectrum (2θ) for 38.14°, 44.29°, 64.48° and 77.37° indexed to the (111), (200), (220), and (311) planes respectively exhibited the face-centered cubic structure (JCPDS No. 87-0720) [38]. The particle size was calculated by the Debye-Scherrer formula (1).

$$D = 0.9 \lambda / \beta \cos \theta \qquad (1)$$

Where D represented crystalline size, lambda (λ) designated the x-ray wavelength (nm), beta (β) defined the angular line as radian, and θ is the angle (degree). The particle size was found as 77.8 nm.

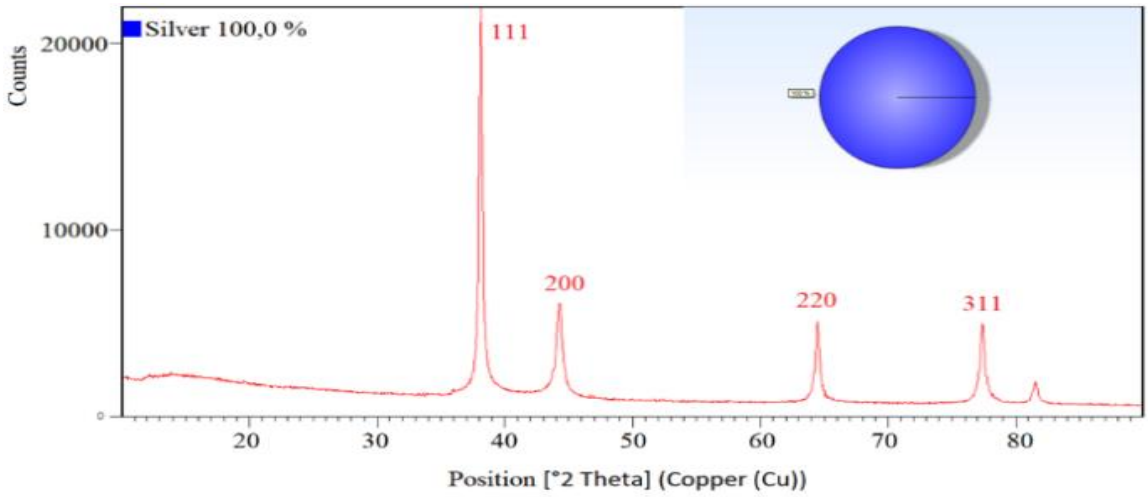

Fig. 3: XRD pattern of Ep-AgNPs.

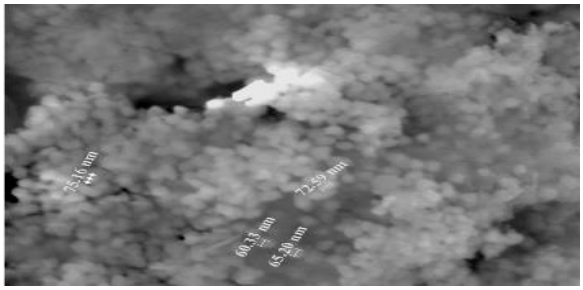

Fig. 4: SEM image of Ep-AgNPs.

Scanning Electron Microscope

SEM analysis revealed the size and morphological properties of Ep-AgNPs synthesised from *E. pallida* (Fig. 4). The spherical size of Ep-AgNPs was demonstrated in the range of 30-95 nm. The morphological form was significant for optical performance. But Ep-AgNPs included the entire region of visible light observed in the UV-Vis spectrum. The synthesised Ep-AgNPs have high density, homogeneously dispersed, and spherical size. Energy disperses analysis (EDX) presented the formation of nanoparticles (Fig. 5). EDS patterns of the Ep-AgNPs confirmed the existence of C, O, Mg, Al, and Ag. The peaks in EDX corresponded to the elements present in the Ep-AgNPs (Fig. 5). EDX analysis showed that the Ep-AgNPs was synthesised in high yield (100%).

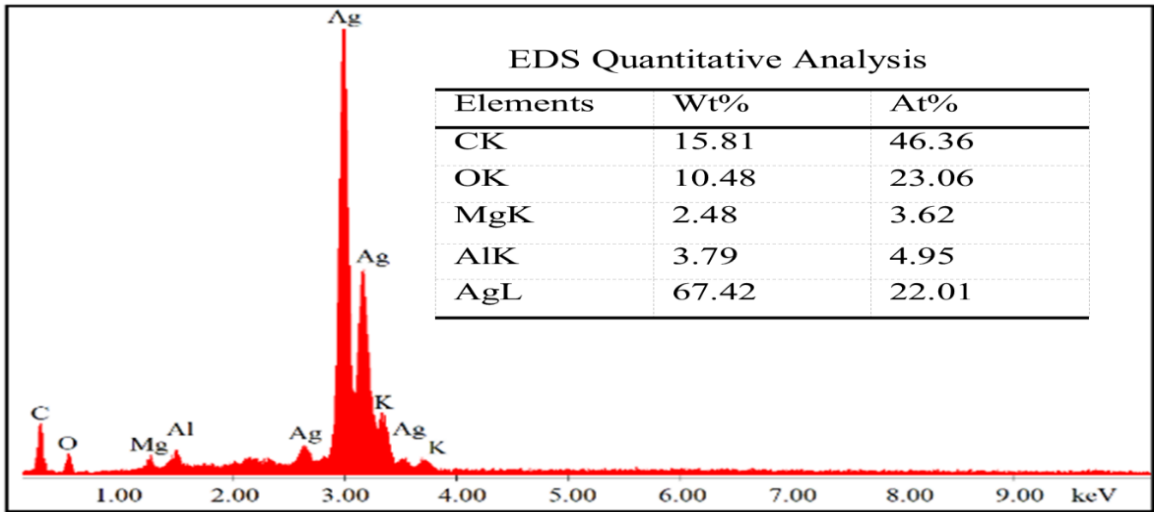

Fig. 5: EDX spectrum and quantitative analysis of Ep-AgNPs.

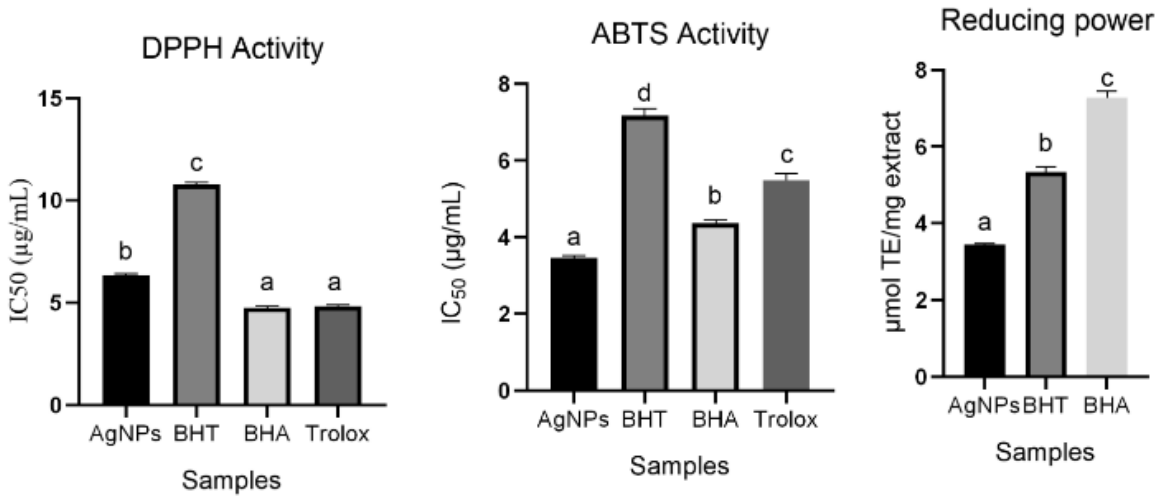

Fig. 6: Antioxidant activity of Ep-AgNPs and standards. The different letters for each assay indicate the significantly different of activity ($P < 0.05$).

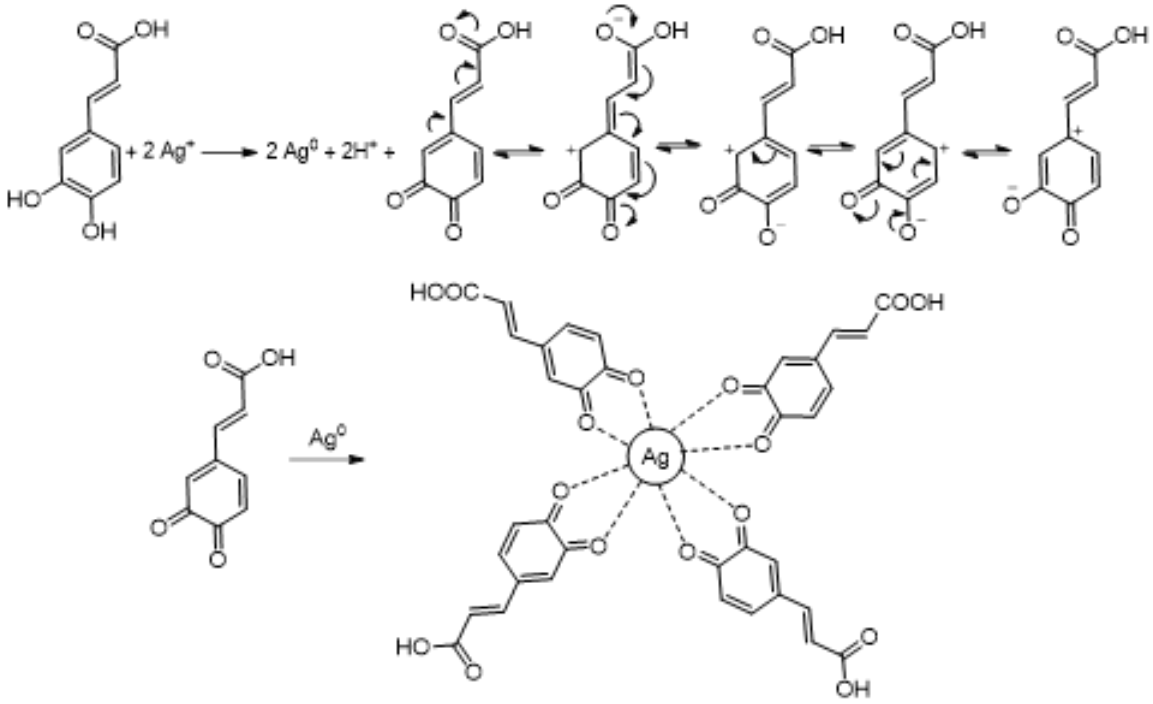

Fig. 7: Plausible reaction mechanism for synthesis of Ep-AgNPs. Caffeic acid was used as a reducing agent. After reduction, molecule becomes stable by resonance structures.

Antioxidant Activity

Antioxidant activity of Ep-AgNPs was carried out using DPPH$^{\bullet}$ scavenging, ABTS$^{\bullet+}$ scavenging, and reducing power assays. Ep-AgNPs exhibited excellent DPPH$^{\bullet}$ activity with a value of 6.34 (IC_{50}, µg/ml) compared to the standard BHT (10.78, IC_{50}, µg/ml). The same trend was observed for the ABTS$^{\bullet+}$ activity in which Ep-AgNPs showed the outstanding activity (3.46, IC_{50}, µg/ml) with the comparison of BHT (7.17, IC_{50}, µg/ml), BHA (4.36, IC_{50}, µg/ml) and Trolox (5.49, IC_{50}, µg/ml). In reducing power activity, Ep-AgNPs have a tremendous effect with a value of 3.45 (µmol/mg sample) in comparison to BHT 5.34 (µmol/mg sample) and

BHA 7.29 (µmol/mg sample). Consequently, silver nanoparticles synthesised from *E. pallida* have significantly higher activity than that of the standards. Hence, Ep-AgNPs are promising for usage in food as well as pharmaceutical industries (Fig. 6). *Persea americana* fruit peel aqueous extract was used for the synthesis of gold and silver-gold alloy nanoparticles that displayed good antioxidant activity [39]. Silver nanoparticles were synthesized using *Annona muricata* leaf extract and they exhibited considerable antioxidant, anti-diabetic, antimicrobial, and cytotoxic activities [3]. In addition, silver, gold, and silver-gold alloy nanoparticles were synthesized from *Opuntia ficus-indica* which displayed antimicrobial and antioxidant activity [40].

E. pallida included the caffeic as a major product. Hence reaction mechanism for synthesis of silver nanoparticles was showed using caffeic acid (Fig. 7).

Conclusion

Eco-friendly, cheap, easy synthesis of Ep-AgNPs was achieved in high yield. The structure of nanoparticles was determined fully by spectroscopic analyses. Due to the significance of *Echinacea* species, especially *E. pallida* in medicine, the synthesised AgNPs from *E. pallida* could be a promising material for food, drugs, and medicinal materials.

Declaration of conflicting of interests

No conflict of interest was declared.

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References

1. R. K. Bachheti, A. Fikadu, A. Bachheti and A. Husen, Biogenic fabrication of nanomaterials from flower-based chemical compounds, characterization and their various applications: A review, *Saudi. J Biol. Sci.,* **27**, 2551 (2020). doi:https://doi.org/10.1016/j.sjbs.2020.05.012.
2. T. A. J. de Souza, L. R. R. Souza and L. P. Franchi, Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity, *Ecotox. Environ. Safe.,* **171**, 691 (2019). doi:https://doi.org/10.1016/j.ecoenv.2018.12.095.
3. J. Badmus, S. Oyemomi, O. Adedosu, T. Yekeen, M. Azeez, E. Adebayo, A. Lateef, U. Badeggi, S. Botha and A. Hussein, Photo-assisted bio-fabrication of silver nanoparticles using Annona muricata leaf extract: exploring the antioxidant, anti-diabetic, antimicrobial, and cytotoxic activities, *Heliyon,* **6**, e05413 (2020). doi:https://doi.org/10.1016/j.heliyon.2020.e05413.
4. I. Oladipo, A. Lateef, M. Azeez, T. Asafa, T. Yekeen, S. Ogunsona, H. Irshad and S. Abbas, in *IOP Conference Series: Mat. Sci. Eng.* (IOP Publishing, 2020), vol. 805, pp. 012035.
5. M. Azeez, F. Durodola, A. Lateef, T. Yekeen, A. Adubi, I. C. Oladipo, E. Adebayo, J. Badmus and A. Abawulem, in *IOP Conference Series: Mat. Sci. Eng*. (IOP Publishing, 2020), vol. 805, pp. 012043.
6. E. N. Gecer, R. Erenler, C. Temiz, N. Genc and I. Yildiz, Green synthesis of silver nanoparticles from *Echinacea purpurea* (L.) Moench with antioxidant profile, *Particul. Sci. Technol.,* **40**, 50 (2022). doi:https://doi.org/10.1080/02726351.2021.1904309.
7. T. S. Alomar, N. AlMasoud, M. A. Awad, M. F. El-Tohamy and D. A. Soliman, An eco-friendly plant-mediated synthesis of silver nanoparticles: Characterization, pharmaceutical and biomedical applications, *Mater. Chem. Phys.,* **249**, 123007 (2020). doi:https://doi.org/10.1016/j.matchemphys.2020.123007.
8. K. Geetha, M. Umadevi, G. Sathe and R. Erenler, Spectroscopic investigations on the orientation of 1, 4-dibromonaphthalene on silver nanoparticles, *Spectrochim. Acta A Mol. Biomol. Spectrosc.,* **116**, 236 (2013). doi:https://doi.org/10.1016/j.saa.2013.07.039.
9. M. Bordoloi, R. K. Sahoo, K. J. Tamuli, S. Saikia and P. P. Dutta, Plant Extracts Promoted Preparation of Silver and Gold Nanoparticles: A Systematic Review, *Nano,* **15**, 2030001 (2020)
10. L. Castillo-Henríquez, K. Alfaro-Aguilar, J. Ugalde-Álvarez, L. Vega-Fernández, G. Montes de Oca-Vásquez and J. R. Vega-Baudrit, Green Synthesis of Gold and Silver Nanoparticles from Plant Extracts and Their Possible Applications as Antimicrobial Agents in the Agricultural Area, *Nanomaterials,* **10**,

1763 (2020). doi:https://doi.org/10.3390/nano10091763.
11. A. Lateef, O. Darwesh and I. Matter, in *Microbial Nanobiotechnology*. (Springer, 2021), pp. 1.
12. A. Lateef, S. A. Ojo and J. A. Elegbede, The emerging roles of arthropods and their metabolites in the green synthesis of metallic nanoparticles, *Nanotechnol. Rev.*, **5**, 601 (2016). doi:http://dx.doi.org/10.1515/ntrev-2016-0049.
13. H. Aksit, S. M. Çelik, Ö. Sen, R. Erenler, I. Demirtas, I. Telci and M. Elmastas, Complete isolation and characterization of polar portion of *Mentha dumetorum* water extract, *Rec. Nat. Prod.*, **8**, 277 (2014).
14. R. Erenler, S. Yilmaz, H. Aksit, O. Sen, N. Genc, M. Elmastas and I. Demirtas, Antioxidant activities of chemical constituents isolated from *Echinops orientalis* Trauv, *Rec. Nat. Prod.*, **8**, 32 (2014).
15. N. Türkmen, A. Öz, A. Sönmez, T. Erol, D. Gülümser, B. Yurdakul, Ö. Kayır, M. Elmastas and R. Erenler, Chemical Composition of Essential Oil from *Rosmarinus Officinalis* L. Leaves, *J. New Results Sci.*, **6**, 27 (2014).
16. M. Elmastaş, İ. Telci, H. Akşit and R. Erenler, Comparison of total phenolic contents and antioxidant capacities in mint genotypes used as spices/Baharat olarak kullanılan nane genotiplerinin toplam fenolik içerikleri ve antioksidan kapasitelerinin karşılaştırılması, *Turk. J. Biochem.*, **40**, 456 (2015). doi:https://doi.org/10.1515/tjb-2015-0034.
17. A. Sahin Yaglioglu, B. Akdulum, R. Erenler, I. Demirtas, I. Telci and S. Tekin, Antiproliferative activity of pentadeca-(8E, 13Z) dien-11-yn-2-one and (E)-1,8-pentadecadiene from *Echinacea pallida* (Nutt.) Nutt. roots, *Med. Chem. Res.*, **22**, 2946 (2013). doi:https://doi.org/10.1007/s00044-012-0297-2.
18. A. R. Tufekci, S. Kucuk, F. Gul and İ. Demirtas, Determination of Phytochemical Contents of Some Medicinal Aromatic Plants (Echinacea pallida, Melissa officinalis, Hypericum perforatum and Sideritis syriaca) Belonging to Antalya Region, *Eur. J. Bio. Chem. Sci.*, **1**, 29 (2018).
19. R. Erenler, I. Telci, M. Ulutas, I. Demirtas, F. Gul, M. Elmastas and O. Kayir, Chemical Constituents, Quantitative Analysis and Antioxidant Activities of *Echinacea purpurea* (L.) Moench and *Echinacea pallida* (Nutt.) Nutt, *J. Food Biochem.*, **39**, 622 (2015). doi:https://doi.org/10.1111/jfbc.12168.
20. B. Bayir, H. Gündüz, T. Usta, E. Şahin, Z. Özdemir, Ö. Kayır, Ö. Sen, H. Akşit, M. Elmastaş and R. Erenler, Chemical Composition of Essential Oil from *Marrubium Vulgare* L. Leaves, *J. New Results Sci.*, **6**, 44 (2014).
21. R. Erenler, O. Sen, H. Aksit, I. Demirtas, A. S. Yaglioglu, M. Elmastas and İ. Telci, Isolation and identification of chemical constituents from *Origanum majorana* and investigation of antiproliferative and antioxidant activities, *J. Sci. Food Agr.*, **96**, 822 (2016). doi:https://doi.org/10.1002/jsfa.7155.
22. R. Erenler, T. Adak, T. Karan, M. Elmastas, I. Yildiz, H. Aksit, G. Topcu and M. A. Sanda, Chemical Constituents isolated from *Origanum solymicum* with Antioxidant activities, *The Eurasia Proceed. Sci. Tech. Eng. Math.*, **1**, 139 (2017)
23. E. Dede, N. Genc, M. Elmastas, H. Aksit and R. Erenler, Chemical Constituents Isolated from *Rhododendron ungernii* with Antioxidant Profile, *Nat. Prod. J.*, **9**, 238 (2019). doi:https://doi.org/10.2174/2210315508666181024114812.
24. R. Erenler, G. Nusret, M. Elmastaş and Ö. Eminağaoğlu, Evaluation of antioxidant capacity with total phenolic content of *Galanthus krasnovii* (Amaryllidaceae), *Turk J Biodiv*, **2**, 13 (2019)
25. R. Erenler, B. Meral, O. Sen, M. Elmastas, A. Aydin, O. Eminagaoglu and G. Topcu, Bioassay-guided isolation, identification of compounds from *Origanum rotundifolium* and investigation of their antiproliferative and antioxidant activities, *Pharm. Biol.*, **55**, 1646 (2017). doi:https://doi.org/10.1080/13880209.2017.1310906.
26. A. Guzel, H. Aksit, M. Elmastas and R. Erenler, Bioassay-guided isolation and identification of antioxidant flavonoids from *Cyclotrichium origanifolium* (Labill.) Manden. and Scheng., *Pharmacogn. Mag.*, **13**, 316 (2017). doi:https://doi.org/10.4103/0973-1296.204556.
27. M. Elmastas, S. M. Celik, N. Genc, H. Aksit, R. Erenler and İ. Gulcin, Antioxidant activity of an anatolian herbal tea—*Origanum minutiflorum*: isolation and characterization of its secondary metabolites, *Int. J. Food Prop.*, **21**, 374 (2018). doi:https://doi.org/10.1080/10942912.2017.1416399.

28. R. Erenler and B. Dag, Biosynthesis of silver nanoparticles using *Origanum majorana* L. and evaluation of their antioxidant activity, *Inorg. Nano-Met. Chem.*, **52**, 485 (2021). doi:https://doi.org/10.1080/24701556.2021.1952263.
29. N. Genc, I. Yildiz, R. Chaoui, R. Erenler, C. Temiz and M. Elmastas, Biosynthesis, characterization and antioxidant activity of oleuropein-mediated silver nanoparticles, *Inorg. Nano-Met. Chem.*, **51**, 411 (2021). doi:https://doi.org/10.1080/24701556.2020.1792495.
30. T. Karan, R. Erenler and B. M. Bozer, Synthesis and characterization of silver nanoparticles using curcumin: cytotoxic, apoptotic, and necrotic effects on various cell lines, *Z. Naturforsch. C*, (2022). doi:https://doi.org/10.1515/znc-2021-0298.
31. M. Elmastas, L. Ozturk, I. Gokce, R. Erenler and H. Y. Aboul-Enein, Determination of antioxidant activity of marshmallow flower (*Althaea officinalis* L.), *Anal. Lett.*, **37**, 1859 (2004). doi:https://doi.org/10.1081/AL-120039431.
32. I. Demirtas, R. Erenler, M. Elmastas and A. Goktasoglu, Studies on the antioxidant potential of flavones of *Allium vineale* isolated from its water-soluble fraction, *Food Chem.*, **136**, 34 (2013). doi:https://doi.org/10.1016/j.foodchem.2012.07.086.
33. P. Koysu, N. Genc, M. Elmastas, H. Aksit and R. Erenler, Isolation, identification of secondary metabolites from *Salvia absconditiflora* and evaluation of their antioxidative properties, *Nat. Prod. Res.*, **33**, 3592 (2019). doi:https://doi.org/10.1080/14786419.2018.1488700.
34. E. Burlacu, C. Tanase, N.-A. Coman and L. Berta, A review of bark-extract-mediated green synthesis of metallic nanoparticles and their applications, *Molecules*, **24**, 4354 (2019). doi:https://doi.org/10.3390/molecules24234354.
35. F. Ameen, S. AlYahya, M. Govarthanan, N. ALjahdali, N. Al-Enazi, K. Alsamhary, W. Alshehri, S. Alwakeel and S. Alharbi, Soil bacteria Cupriavidus sp. mediates the extracellular synthesis of antibacterial silver nanoparticles, *J. Mol. Struct.*, **1202**, 127233 (2020). doi:https://doi.org/10.1016/j.molstruc.2019.127233.
36. A. Sahin Yaglioglu, R. Erenler, E. N. Gecer and N. Genc, Biosynthesis of Silver Nanoparticles Using Astragalus flavesces Leaf: Identification, Antioxidant Activity, and Catalytic Degradation of Methylene Blue, *J. Inorg. Organomet. Polym. Mater.*, (2022). doi:https://doi.org/10.1007/s10904-022-02362-5.
37. M. Iftikhar, M. Zahoor, S. Naz, N. Nazir, G. E.-S. Batiha, R. Ullah, A. Bari, M. Hanif and H. M. Mahmood, Green Synthesis of Silver Nanoparticles Using *Grewia optiva* Leaf Aqueous Extract and Isolated Compounds as Reducing Agent and Their Biological Activities, *J. Nanomater.*, **2020**, (2020). doi:https://doi.org/10.1155/2020/8949674.
38. S. B. Aziz, G. Hussein, M. Brza, S. J Mohammed, R. T Abdulwahid, S. Raza Saeed and A. Hassanzadeh, Fabrication of Interconnected Plasmonic Spherical Silver Nanoparticles with Enhanced Localized Surface Plasmon Resonance (LSPR) Peaks Using Quince Leaf Extract Solution, *Nanomaterials*, **9**, 1 (2019). doi: https://doi.org/10.3390/nano9111557.
39. A. E. Adebayo, A. M. Oke, A. Lateef, A. A. Oyatokun, O. D. Abisoye, I. P. Adiji, D. O. Fagbenro, T. V. Amusan, J. A. Badmus, T. B. Asafa, L. S. Beukes, E. B. Gueguim-Kana and S. H. Abbas, Biosynthesis of silver, gold and silver–gold alloy nanoparticles using Persea americana fruit peel aqueous extract for their biomedical properties, *Nanotechnol. Environ. Eng.*, **4**, 13 (2019). doi:https://doi.org/10.1007/s41204-019-0060-8.
40. E. A. Adebayo, J. B. Ibikunle, A. M. Oke, A. Lateef, M. A. Azeez, A. O. Oluwatoyin, A. V. AyanfeOluwa, O. T. Blessing, O. O. Comfort and O. O. Adekunle, Antimicrobial and antioxidant activity of silver, gold and silver-gold alloy nanoparticles phytosynthesized using extract of *Opuntia ficus-indica*, *Rev. Adv. Mater. Sci.*, **58**, 313 (2019). doi:https://doi.org/10.1515/rams-2019-0039.