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# Biosynthesis of Functionalized Silver Nanomaterials Utilizing Seeds of Black Rosehip (*Rosa pimpinellifolia* L.) and its Remarkable Antioxidative Capacity

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## ABSTRACT

**Background:** Silver nanoparticles (AgNPs) have gained significant attention due to their unique antimicrobial, catalytic and optical properties. This study focuses on the green synthesis of silver nanoparticles using Black rosehip (*Rosa pimpinellifolia L.*) seeds (Rp-AgNPs) and evaluates their biological activities.

**Methods:** Rp-AgNPs were synthesized using *R. pimpinellifolia* seed extract and characterized using multiple analytical techniques including UV-Vis spectroscopy, XRD, FTIR, TEM, SEM, EDAX, zeta potential analysis, DSC, SERS and XPS. The biological activities were assessed through DPPH free radical scavenging assay, cytotoxicity testing against H460 lung cancer cells and antimicrobial activity evaluation against various pathogens.

**Result:** The synthesized Rp-AgNPs exhibited spherical morphology with an average size of 51.27 nm and demonstrated stability with a zeta potential of -18.9. The nanoparticles showed enhanced antioxidant activity ($IC_{50}$ = 30.89 μg/mL) compared to the seed extract ($IC_{50}$ = 136.4 μg/mL). Antimicrobial testing demonstrated effectiveness against both Gram-positive and Gram-negative bacteria (MIC 50-100 μg/mL) and *Candida* strains (MIC 100-200 μg/mL).

**Key words:** Biological activity, Black rosehip (*Rosa pimpinellifolia* L.), Green synthesis, Silver nanoparticles.

## INTRODUCTION

Silver nanoparticles (AgNPs) are easy to produce by biological methods, that is low-cost and environmentally friendly (Garg *et al.*, 2020). AgNPs exhibit cellular interactions by binding to cell membrane proteins due to their small size (Kumar *et al.*, 2020). This size-dependent behavior affects both their cellular distribution and biological activity, with smaller AgNPs generally showing greater cytotoxicity (Avalos *et al.*, 2014). AgNPs have anticarcinogenic (Veeragoni *et al.*, 2022), anti-inflammatory (Haider *et al.*, 2020) and antimicrobial (Mousavi *et al.*, 2018) effects. In a complementary investigation, Rengarajan and colleagues achieved successful AgNP synthesis using Calotropis gigantea leaf extract. Their methodological approach incorporated multiple advanced characterization techniques, including UV-visible and FT-IR spectroscopy, electron microscopy (SEM and TEM), XRD and EDX analysis. The resulting nanoparticles showed varied morphologies (spherical to cubic) while retaining key bioactive compounds from the original extract, such as tannins, alkaloids, flavonoids and polyphenols. These results support the wide therapeutic potential of plant-derived AgNP composites in biological and pharmaceutical applications (Rengarajan *et al.*, 2024).

Medicinal and aromatic plants effect health and disease processes, especially due to presence of their phytochemical compounds and high antioxidant content that impart anticarcinogenic, antidiabetic (Tasci Çelikel *et al.*, 2024; Orkun Erkılıç and Bayraktar, 2025a; 2025b) and antimicrobial effects (Bayraktar and Tekce, 2018; Bayraktar *et al.*, 2023; Ozcan *et al.*, 2024). Black rose is the fruit of a shrubby plant belonging to the *Rosa* genus of the Rosaceae family that sheds its leaves in winter. It contains tannins, phenolic compounds such as quercetin, kaempferol and catechin (MármoL *et al.*, 2017); carotenoids (such as beta-carotene, lycopene, lutein and zeaxanthin) (Andersson *et al.*, 2011) as well as antioxidant (El Wafa *et al.*, 2025) anti-inflammatory (Rajabi-Moghadam *et al.*, 2025), antimicrobial and anticancer effects (Mallick *et al.*, 2024; Rajabi-Moghadam *et al.*, 2025).The *Rosa* genus, especially *Rosa pimpinellifolia*, is an interesting candidate for green synthesis with its rich phytochemical content (Strålsjö *et al.*, 2003). Recent studies have reported that it has the potential to treat non-small cell lung cancer (NSCLC) (MármoL *et al.*, 2017; Demir *et al.*, 2021; Kızıl, 2023). Therefore, this study aims to investigate the green synthesis of silver nanoparticles using Black rose seeds and evaluate their biological activities.

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## MATERIALS AND METHODS

### Preparation of the plant extract

Plant specimens of *Rosa pimpinellifolia* L. were collected from Gümüşsu Village, Bayburt Province, Türkiye (N:40° 13′ 29.0064″; E: 40° 18′ 11.8620″, 1,817 m elevation) Rosehip species identification was confirmed by a botanist from the Department of Organic Agriculture, Faculty of Applied Sciences, Bayburt University.

### Green synthesis of silver nanoparticles (Rp AgNPs)

For the green synthesis of Rp-AgNPs, ground seeds were extracted in distilled water (100 g in 400 mL) at 45°C for 2 hours. The filtered extract was slowly added to silver nitrate solution (0.037 mM, 400 mL) and kept at 55°C for 2 hours until the color changed to brown, indicating nanoparticle formation. The synthesized Rp-AgNPs were purified by three centrifugation cycles (12,000 rpm, 20 minutes) and stored at 4°C (Gecer, 2021).

### Characterization of Rp AgNPs

Synthesized Rp-AgNPs were comprehensively characterized using several techniques. [1]UV-Vis spectrophotometry was performed with a Thermo Scientific Multiskan Go. Crystal structure was determined by XRD (Bruker D8 Discover, 2θ = 5°-80°). Average crystalline size (D) was calculated using the Debye–Scherrer formula:

$$D=\beta\cos\theta K\lambda \quad .... (1)$$

Where,
$\lambda$ = 0.154056 nm (CuKα).
$\beta$ = FWHM in radians.
$\theta$ = diffraction angle.
K = 0.9.

Structural characterization included FTIR (Perkin Elmer spectrum device, 4000-450 $cm^{-1}$) to examine bonds and functional groups (Büyüksırıt and Kuleaşan, 2014). Morphological analysis used TEM (FEI Talos-F200S, 200 kV) and SEM (FEI-Quanta FEG 450). TEM samples were prepared on carbon-coated copper grids (200 mesh) with solvent evaporation. Composition was analyzed by EDAX and EDX. Surface charge was determined using a Malvern Instruments Zetasizer Nano ZSP. Thermal properties were investigated by DSC (Perkin Elmer 8000, 5 mg samples, 10°C/min to 500°C) (Niraimathi *et al*., 2013). Surface plasmon resonance of AgNPs (typically ~400 nm for spheres), influenced by various factors, was examined for its role in SERS (Stamplecoskie *et al*., 2011). SERS analysis (WITech Alpha 300R) provided insights into biomedical molecule properties (Bindhu *et al*., 2015). XPS (SpecsGroup Flexmod-Germany) was used for atomic/molecular structure analysis, quantifying seed extract-AgNP binding and elemental composition of Rp-AgNPs (Ag and O).

### Antioxidant activity analysis

The antioxidant capacity of functionalized silver nanomaterials and *R. pimpinellifolia* seed aqueous extracts was evaluated using a DPPH. (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay with a 0.26 mM DPPH solution (SIGMA). Samples at various concentrations were incubated for 30 minutes at room temperature in the dark and absorbance was measured at 517 nm using UV-Vis spectroscopy (Fig 1). The DPPH scavenging effect was calculated using the equation:

$$\text{Scavenging (\%)} = \frac{(A - B)}{A} \times 100 \quad .... (2)$$

Where,
A = Absorbance of the blank.
B = Sample absorbance at 517 nm. (Erenler *et al*., 2017).

### Cytotoxicity assessment of Rp-AgNPs

Cytotoxicity was evaluated using the H460 non-small cell lung cancer cell line (ATCC, USA). Cells were cultured in RPMI medium (Gibco) with 10% FBS (Gibco) in 25 ml flasks and maintained at 37°C in 5% $CO_2$ for 24-48 hours, with PBS (Sigma) washing and trypsin-EDTA (Gibco) for detachment. Cell *via*bility was checked with trypan blue staining (Sigma) and cell counting was done using a Thoma slide after centrifugation (Gecer, 2021). Cell *via*bility was quantified using the WST-8 assay with a CVDK-8 kit (Ecotech Biotechnology®, Türkiye). The procedure included: 1. Seeding H460 cells in 96-well plates for 24 hours. 2. Treating with plant extract (200-12.5 µg/mL in PBS). 3. Incubating for another 24 hours. 4. Assessing *via*bility after 72 hours per kit instructions. 5. Adding 10 µl CVDK-8 reagent and incubating for 3 hours. 6. Measuring absorbance at 450 nm (MultiSkan Go, Thermo Scientific). Statistical significance was set at $p<0.05$.

### Antimicrobial activity analysis

### Disc diffusion assay

Rp-AgNPs' antimicrobial efficacy was assessed against a panel of pathogens (3 Gram-positive, 3 Gram-negative

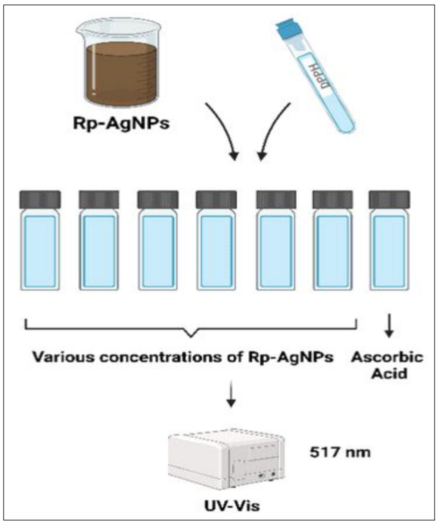



**Fig 1:** DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay.

bacteria, 4 yeast-like fungi) using disc diffusion and broth microdilution. Cultures revived on selective media were incubated, pure colonies transferred to liquid media and incubated and standardized suspensions were spread on fresh media. Sterile discs with Rp-AgNPs were placed on inoculated surfaces, incubated and inhibition zones measured (Boztaş *et al*., 2024).

**Broth microdilution method**

The minimum inhibitory concentration (MIC) of Rp-AgNPs against selected pathogens was determined using broth microdilution in 96-well plates. Wells were prepared with 95 µL liquid medium (Mueller Hinton broth for bacteria, Saboraud Dextrose broth for fungi) and 5 µL standardized inoculum (100 µL total). A two-fold serial dilution of Rp-AgNPs (100 µL added to first wells, 100 µL transferred down to the eighth well) was performed. Microplates were incubated at 37°C with shaking (40 rpm) for 24 hours. Then, 5 µL aliquots from each well were transferred to agar plates and incubated for another 24 hours at 37°C. The MIC was the highest concentration showing no microbial growth (Keskin *et al*., 2023).

**Statistical analysis**

All experiments were performed in triplicate and the results were analyzed using GraphPad Prism software (version 8.0.1, United States). The DPPH free radical scavenging activity was evaluated through one-way ANOVA followed by Tukey's multiple comparisons test. Data are presented as mean±standard de*via*tion, with statistical significance set at $p<0.05$. Cytotoxicity data, also obtained from triplicate experiments, were analyzed using Student's t-test and the results are expressed as mean±standard de*via*tion, with statistical significance similarly defined at $p<0.05$.

## RESULTS AND DISCUSSION

The pulverized Black rosehip seeds (100 g) were ground using a coffee grinder and heated with distilled water (400 mL) at 45°C for 2 hours.. The mixture was filtered through Whatman filter paper and a silver nitrate solution (0.04 mM, 400 mL) was gradually added to the extract. The reaction mixture was further heated at 60°C for 2 hours, during which the color changed from orange to dark brown. After the reaction, Rp-AgNPs were collected by repeated centrifugation at 12,000 rpm for 20 minutes and washed thoroughly with distilled water. Finally, the Rp-AgNPs were dried *via* lyophilization (Fig 2).

The successful synthesis of silver nanoparticles was confirmed by UV–Vis spectroscopy, with a maximum absorption peak at 476 nm, consistent with the typical range of 400–480 nm reported in the literatüre (Fig 3),(Sreeram *et al*., 2008).

The XRD analysis of Rp-AgNPs (Fig 4) revealed prominent peaks at 38°, 44°, 64° and 77°, corresponding to the fcc planes (111, 200, 220 and 311) of AgNPs (Rahimi-Nasrabadi *et al*., 2014). Additional peaks likely indicate metabolites coating the nanoparticles. The calculated crystallite size was 51.93 nm (Rahimi-Nasrabadi *et al*., 2014).

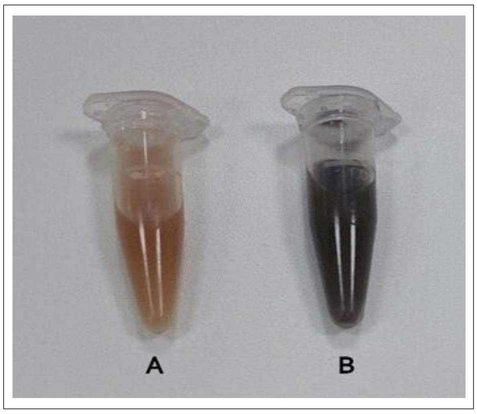



**Fig 2:** Aqueous solutions of *Rosa pimpinellifolia* L. seeds (A) and Rp-AgNPs (B).

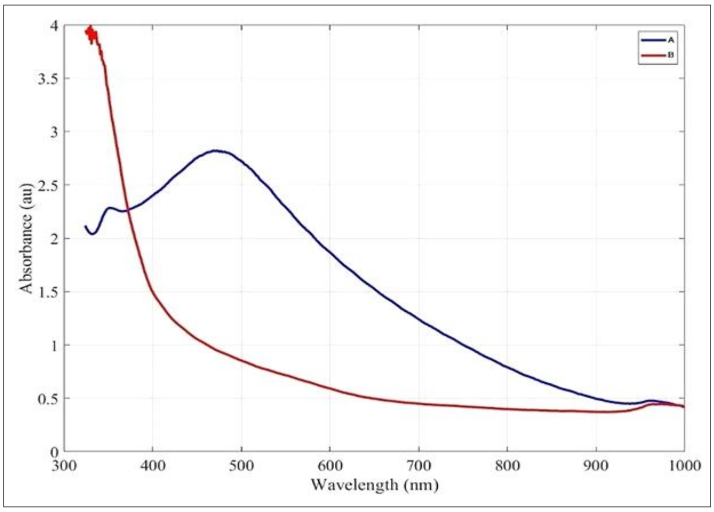



**Fig 3:** UV-Visible absorption spectra of Rp-AgNPs (A) and Aqueous solutions of *Rosa pimpinellifolia* L. seeds (B).

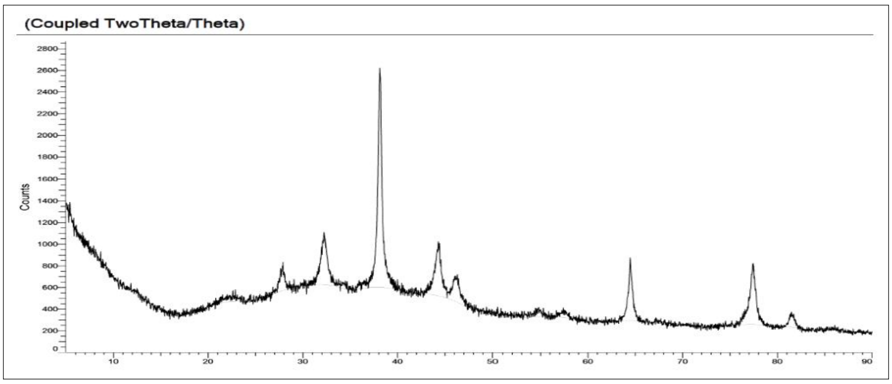



**Fig 4:** XRD spectrum of Rp-AgNPs.

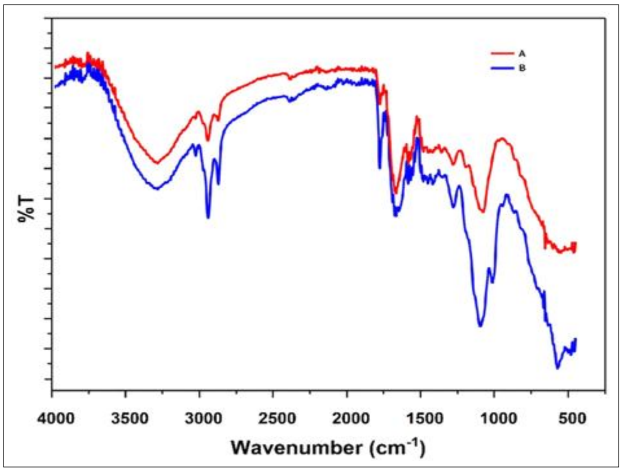
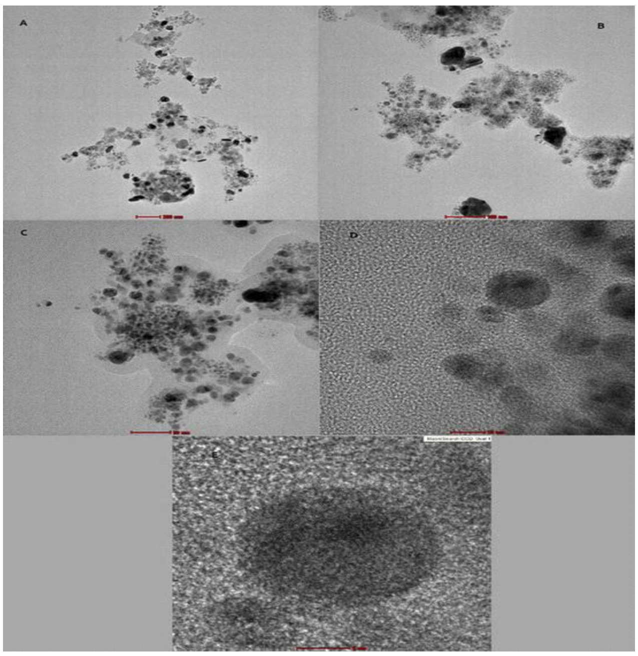



**Fig 5:** FTIR spectra of Rp-AgNPs (A) and Aqueous solutions of *Rosa pimpinellifolia* L. seeds (B).




A) 200 nm, B) 100 nm, C) 50 nm, D) 10 nm and E) 5 nm (magnified view).

**Fig 6:** TEM image of Rp-AgNPs nanoparticles. Image scale bar.

FTIR analysis was performed to identify functional groups in the *R. pimpinellifolia* seed extract involved in the synthesis and stabilization of silver nanoparticles. The spectra of the extract and Rp-AgNPs (Fig 5) revealed peaks at 3304.33 $cm^{-1}$ and 2926.6 $cm^{-1}$ (O-H stretching), 2855.12 $cm^{-1}$ and 2111.63 $cm^{-1}$ (N-H and C≡C stretching), 1743.53 $cm^{-1}$ (C=O stretching), 1637.05 $cm^{-1}$ (C=C stretching), 1548.06 $cm^{-1}$ (N-O stretching), 1450.16 $cm^{-1}$ (C-H bending), 1317.96 $cm^{-1}$ (C-F stretching and O-H bending), 1242.21 $cm^{-1}$ (C-O and C-N stretching), 1063.63 $cm^{-1}$ (S=O and C-O stretching), 969.28 $cm^{-1}$ and 896.13 $cm^{-1}$ (C=C bending) and 586.43 $cm^{-1}$ (C-I stretching).

The morphology and size distribution of nanoparticles produced through biological synthesis were examined using TEM, a robust analytical method. The TEM image reveals individual silver particles and illustrates the polydispersity of the nanoparticles. Notably, the silver nanoparticles exhibit an almost spherical shape (Fig 6).

The surface morphology and dimensions of silver nanoparticles produced under optimal conditions were assessed through SEM, as illustrated (Fig 7).

The surface morphology was also identified to possess a spherical nature, with an average size measuring 51.27 nm (Fig 8).

In the EDAX analysis, a distinct peak of silver metal was observed at 3 kV, confirming the presence of silver in the complex. Additionally, the peaks corresponding to other elements in the EDAX spectrum are illustrated (Fig 9).

Zeta potential measures the electrical potential at the solid-liquid interface, influencing nanoparticle stability. Rp-AgNPs showed a zeta potential of -18.9, indicating stability and particle repulsion (Fig 10), (Gecer, 2021).

Differential scanning calorimetry (DSC) was used to analyze the thermal stability of Rp-AgNPs. The DSC curve showed endothermic and exothermic processes, with an exothermic peak at 92°C indicating desorption of organic components from the nanoparticle surface (Fig 11).

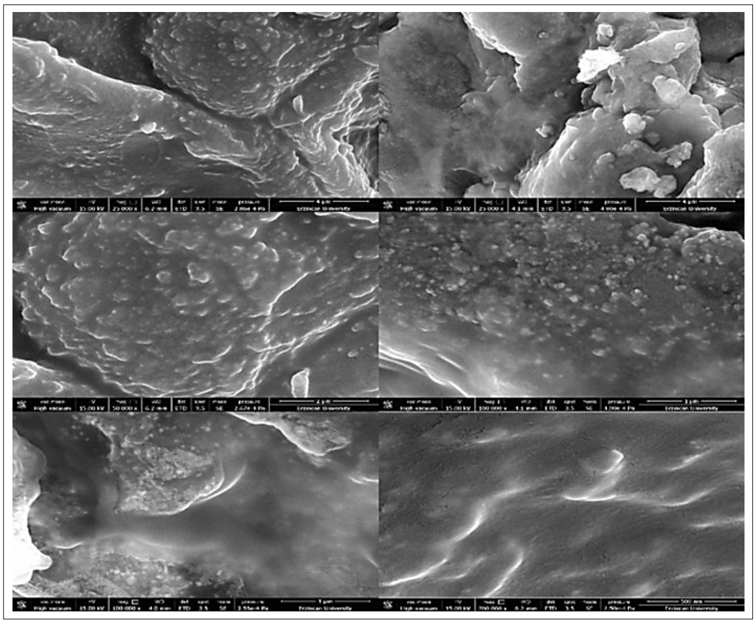


**Fig 7:** SEM images of Rp-AgNPs magnified at 25,000×, 50,000×, 100,000× and 200,000×.

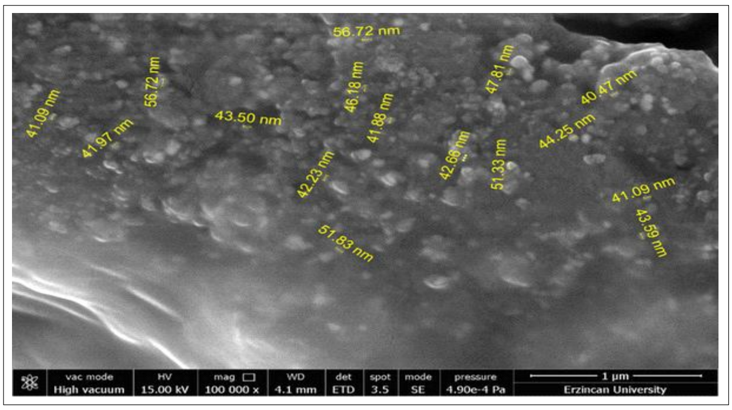



**Fig 8:** SEM image of nanoparticles from 16 points.

Surface-enhanced Raman scattering (SERS) is a highly sensitive technique for detecting and characterizing metal nanoparticles (Bindhu *et al*., 2015). The Raman spectra of silver nanoparticles (AgNPs) showed vibrational modes at 238 $cm^{-1}$ (Ag-O stretching) (Linic *et al*., 2015). 613 $cm^{-1}$ (C-S-C) and 1301 $cm^{-1}$ (anti-symmetric C=O stretching) (Joshi *et al*., 2018) (Fig 12).

X-ray Photoelectron Spectroscopy (XPS) was used to analyze the surface composition and chemical state of Rp-AgNPs. The XPS spectrum (Fig 13) revealed Ag 3d

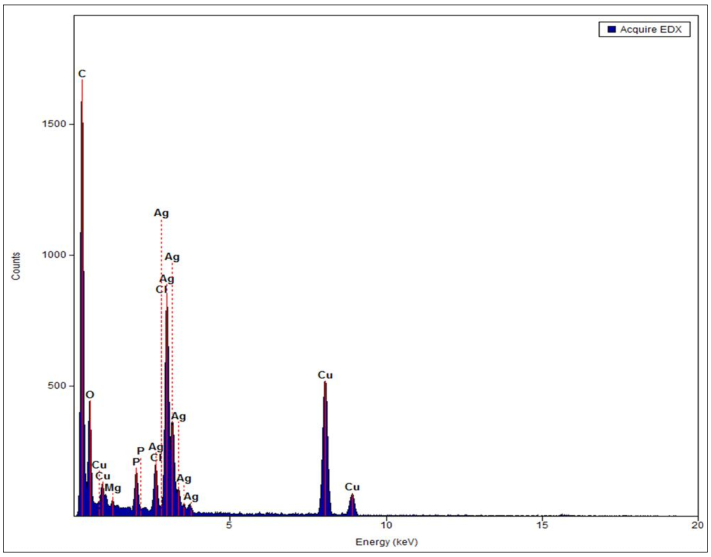



**Fig 9:** EDAX spectrum of silver nanoparticles synthesized utilizing black rosehip seeds.

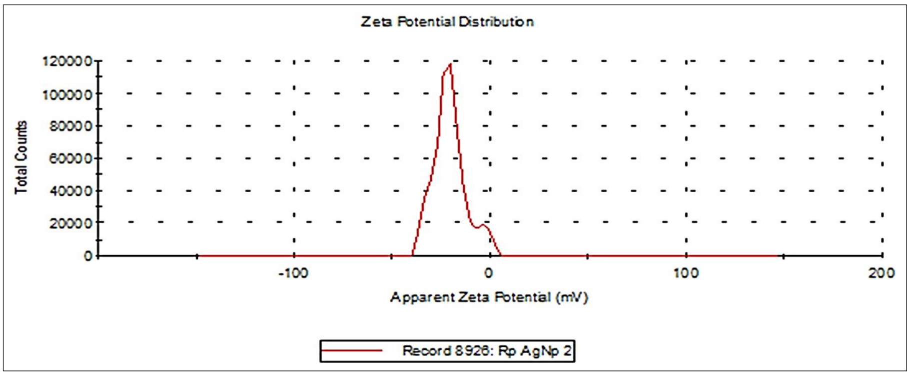



**Fig 10:** Zeta potential distribution of Rp-AgNPs.

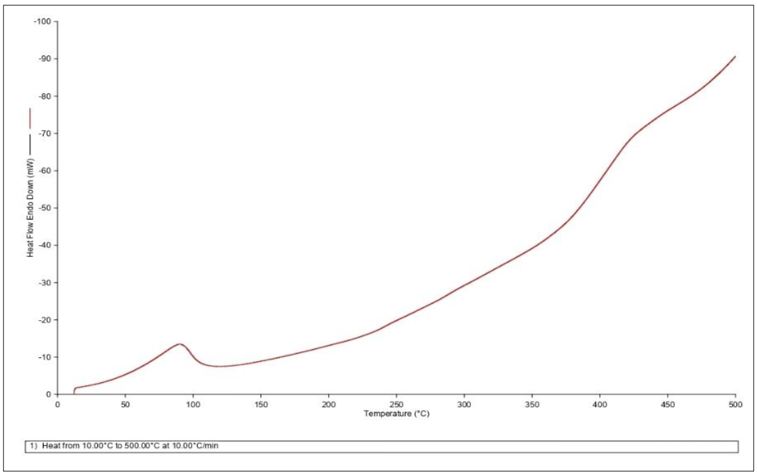



**Fig 11:** Differential scanning calorimetry (DSC) of Rp-AgNPs.

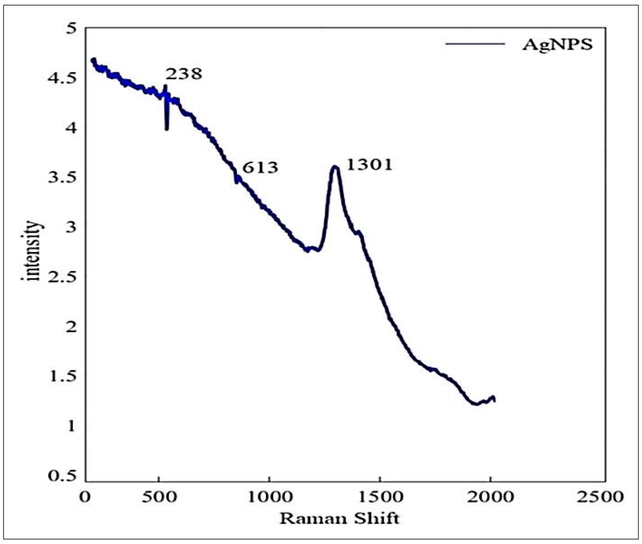



**Fig 12:** RAMAN scattering spectra of the Rp-AgNPs.

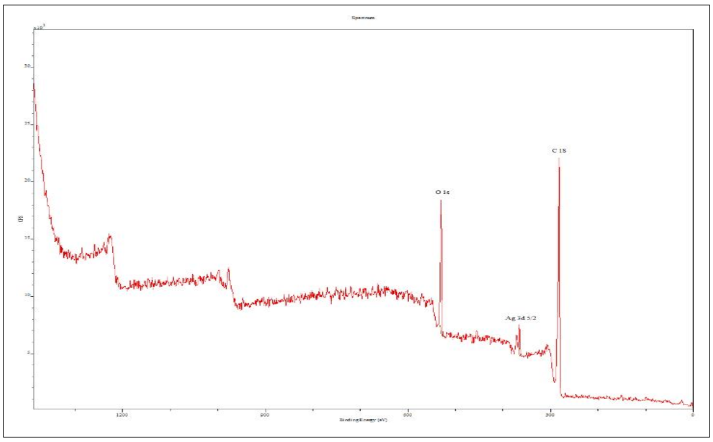



**Fig 13:** XPS spectra of Ag 3d 5/2, O 1s and C 1s peak of the Ag NPs functionalized with seed aqueous extract.

5/2 peaks at binding energies of 365.1 and 369.0 eV, corresponding to oxidized silver (Ag-O) (Maiti *et al*., 2016).

DPPH free radical scavenging activity showed that ascorbic acid (IC50: 11.53 µg/mL) had the highest activity, followed by Rp-AgNPs ($IC_{50}$: 30.89 µg/mL) and *R. pimpinellifolia* seed extract ($IC_{50}$: 136.4 µg/mL). This is the first study on silver nanoparticles functionalized with *R. pimpinellifolia* seeds.

The antimicrobial activity of Rp-AgNPs synthesized using *Rosa pimpinellifolia* seed extract was evaluated against various pathogenic microorganisms. The disk diffusion method and broth microdilution technique were employed to assess *in vitro* activity, with results summarized in Table 1.

The increasing issue of antibiotic resistance (Nikolich and Filippov, 2020) has driven interest in alternative antimicrobial strategies, such as nanoparticle-based approaches (Murugaiyan *et al*., 2022). *R. pimpinellifolia* has a high polyphenol content (121.38 mg GAE/100 g fresh weight) and shows tolerance to various stresses (Fattahi *et al*., 2012). Rp-AgNPs at 100 µg/mL showed inhibition zones of 10-14 mm for bacteria and 7-8 mm for *Candida* strains, with MIC values of 50-100 µg/mL for bacteria and 100-200 µg/mL for *Candida*. Rp-AgNPs demonstrated *in vitro* antimicrobial activity against all tested pathogens, consistent with previous findings (Fattahi *et al*., 2012).

Hormones are biomolecules that are involved in many physiological processes that affect the growth and development of cancer cells (Bayraktar and Bayraktar, 2019; Bayraktar, 2020). Plant-synthesized AgNPs have a promising potential in anticancer treatments because they can affect the development of some types of cancer by affecting the endocrine system. Juarez-Moreno *et al*. (2017) demonstrated effective cytotoxicity across multiple cancer cell lines without DNA damage, suggesting potential applications in combination therapy. Studies with various plant-derived AgNPs have reported significant anticancer activity, with $IC_{50}$ values ranging from 28.125 µg/mL for lung cancer to 62.5 µg/mL for breast cancer cells (Vijayan *et al*., 2018; Valsalam *et al*., 2019). Green-synthesized AgNPs

**Table 1:** *In vitro* antimicrobial activities of silver nanoparticles (Rp-AgNPs) prepared using *Rosa pimpinellifolia* seed extract.

| Microorganisms | IZD | MIC | AMP | FLU | |
|---|---|---|---|---|---|
| Gram (+) | *Bacillus cereus* ATCC 14579 | 14 | 50 | 29 | - |
| | *Enterococcus faecalis* ATCC 49452 | 12 | 100 | 26 | - |
| | *Streptococcus mutans* ATCC 35668 | 11 | 100 | 28 | |
| | *Staphylococcus aureus* NCTC 10788 | 13 | 50 | 27 | - |
| Gram (-) | *Acinetobacter baumannii* ATCC 19606 | 11 | 100 | 14 | - |
| | *Escherichia coli* NCTC 9001 | 10 | 100 | 13 | - |
| | *Pseudomonas aeruginosa* NCTC 12924 | 12 | 100 | 11 | - |
| | *Salmonella typhimurium* RSSK95091 | 11 | 100 | 12 | - |
| Yeast like fungi | *Candida albicans* SB1 | 7 | 200 | - | 25 |
| | *Candida glabrata* SB5 | 8 | 200 | - | 26 |
| | *Candida krusei* SB8 | 8 | 200 | - | 21 |
| | *Candida albicans* ATCC 10231 | 7 | 200 | - | 21 |

*IZD: Inhibition zone diameter; MIC: Minimum inhibition concentration; AMP: Ampicillin; FLU: Fluconazole.

are particularly promising due to their ability to generate ROS and penetrate cells (Jabeen *et al*., 2021). Furthermore, silver nanoparticles synthesized using black rosehip seed water extract maintained their antibacterial activity for over 8 weeks when stored at 4°C.

## CONCLUSION

In our current study, Rp-AgNPs showed strong antimicrobial activity against bacterial and fungal pathogens and remained stable for over 8 weeks at 4°C. It is thought that Rp-AgNPs will be beneficial and contribute to the treatment of wound infections using environmentally friendly methods thanks to their potential in combating antibiotic resistance. Future research should address these gaps to better explore the biomedical potential of these flavonoid-enriched AgNPs, which also show promise for food and pharmaceutical applications.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**Preprint statement**

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**Disclaimers**

The authors are solely responsible for the content and its accuracy, which does not necessarily reflect their institutions' views. They accept no liability for any losses from its use.

**Informed consent**

This study was approved by the Ethics Committee of Bayburt University (Decision No: 37/04, Date: 04/30/2024) in accordance with the Declaration of Helsinki principles. All methods were carried out in accordance with relevant guidelines and regulations and all experimental protocols were approved by the Ethics Committee of Bayburt University. The study utilized the NCI-H460 lung cancer cell line obtained from American Type Culture Collection (ATCC | USA). As this research was conducted using commercially available cell lines and did not involve direct human participants, human material, or human data collection, it did not require clinical trial registration, participant consent, or consent to publish.

**Conflict of interest**

The authors declare no conflicts of interest and the study was not influenced by funding or sponsorship.

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