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Antioxidant and Antimicrobial Properties of Different Silver Nanoparticles Produced by Green Synthesis

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Abstract

Nanoparticles are materials that can be used in a wide range from medicine to industry. In recent years, especially the fruits, flowers, leaves, and roots of plants have come to the fore in nanoparticle synthesis because they are environmentally friendly and economical. *Rosa damascena* is a plant that is used both in foods such as jams, desserts, and beverages, and in many cosmetic products such as perfumes, creams, and lotions due to its pleasant smell and taste. In addition to its pleasant aroma, valuable bioactive components are among the main uses of these flowers. *Berberis crataegina* fruit is a wild shrub fruit that can be consumed by humans but is unknown to many. This study aims to examine the antibacterial and antioxidant properties of silver nanoparticles produced from *Rosa damascena* flowers and *Berberis crataegina* fruits, both of which are rich in anthocyanins. For this, first of all, the produced silver nanoparticles were evaluated using SEM and SEM EDX. In addition, the size and properties of the nanoparticle were defined by performing XRD and FTIR analyses. Furthermore, these nanoparticles were subjected to antioxidant and antibacterial analyses. As a result, two different silver nanoparticles with high antioxidant properties were synthesized from both. However, nanoparticles synthesized from *R. damascena* flowers showed more antimicrobial activity than nanoparticles synthesized from *Berberis crataegina* fruits.

Key Words: Antimicrobial activity, Antioxidant capacity, *Berberis crataegina, R. damascena*

1. Introduction

Nanotechnology has significantly impacted the therapeutic, diagnostic, biomedical, industrial, environmental protection, and scientific research fields throughout recent decades (Farokhzad & Langer, 2009; Guerra et al., 2018). Nanoparticles (NP) are particles that make up nanomaterials and have diameters between 1 and 100 nm (Algotiml et al., 2022). For a wide range of biological applications, NP provides a very appealing platform (Jain et al., 2021).

There are many different kinds of metal NP, such as iron, platinum, gold, thallium, silver, titanium, cerium, and others (Piñón-Segundo et al., 2013). Silver nanoparticles (AgNPs) are one of the most important and intriguing nanomaterials among the several metallic NPs used in biological applications (Karmous et al., 2020; Yesilot & Aydin, 2019).

Numerous methods, including physical, chemical, and biological ones, can be used to produce nanoparticles (Chen et al., 2008). Various AgNP kinds, forms, sizes, and crystal materials have been created using various physicochemical techniques as a result of research and applications. These physicochemical techniques, however, have major drawbacks, including being costly, costing a lot of time and energy, and also producing extremely substantial chemical by-products (Bagheri & Banihashemi, 2015; Ueno et al., 2015). The biological synthesis of the nanoparticle, or "green synthesis," of AgNPs, on the other hand, is a natural, affordable, and eco-friendly procedure (Ali et al., 2015; Maddinedi et al., 2017). Furthermore, green-produced nanoparticles have great yields, solubility, and stability. AgNPs may create well-defined sizes and shapes under ideal circumstances for traditional study using biological techniques, which are straightforward, quick, non-toxic, dependable, and environmentally friendly (Rai et al., 2016; Yesilot & Aydin, 2019).

Recently, green biosynthesis techniques have become quite popular for creating nanoparticles employing a variety of biological systems, including yeast, fungus, bacteria, and plant extracts (Khan et al., 2017; Kowshik et al., 2002; Shahverdi et al., 2007). The most well-known of them is the manufacture of controlled-physicochemical AgNPs based on plant extracts (Dipankar & Murugan, 2012; Keshari et al., 2020).

The potential for natural antioxidants and antibacterial agents found in plants makes them very interesting (Stanković et al., 2016). Customers and companies desire to switch from synthetic antioxidants to natural ones due to their potential harm (Branen, 1975; Pokorný, 1991).

In the past, there has been a great deal of interest in the plant extracts that were used as medicines by ancient civilizations (Grabley & Thiericke, 1999; Uzun et al., 2004), and antimicrobial compounds derived from plants are still a valuable resource in the battle against infectious diseases today. Various studies have shown a significant increase in the incidence of bacterial resistance to many antibiotics (Finch, 1998; Kunin, 1993). Therefore, it has become necessary to investigate the chemical compounds found in plants (Nascimento et al., 2000; Phillipson, 1991; Xu & Lee, 2001).

Rosa damascena is a member of the Rosaceae family and is widely distributed in Turkey, Bulgaria, Spain, France, Syria, India, Morocco, Tunisia, Saudi Arabia, and China (Chevallier, 1996; Commission, 2015; Liu et al., 2020; Takahashi et al., 2019). *R. damascena* is mostly used as decoration, fragrance, food additive, and medicinal treatment such as constipation, antistress activity, stomach ulcer, and cardiovascular diseases (Akram et al., 2020; Naveena & Thamaraiselvi, 2020). *R. damascena* flower essential oil is valuable, and the main producers of rose essential oil in the world are Bulgaria, Turkey, and Morocco (Mahboubi, 2015; Tosun et al., 2002). Additionally, Rosa *damascena* exhibits antibacterial, antioxidant, antitussive, anti-HIV, antidepressant, hypoglycaemic, anti-inflammatory, and analgesic properties (Akram et al., 2020; Labban & Thallaj, 2020; Naveena & Thamaraiselvi, 2020). Various useful compounds, including vitamins, flavonoids, carotenes, carbohydrates, organic acids, trace elements, phenolic and aromatic compounds are found in roses, according to research in the literature. Most of these compounds have been reported to have valuable properties such as antimutagenic, antioxidant, antimicrobial, anticancer, and anti-inflammatory, retarding or inhibiting oxidation processes (Bitis et al., 2017; Crespo et al., 1999; Jabłońska-Ryś et al., 2009; Kähkönen et al., 1999; Kaisoon et al., 2011; Krishnaiah et al., 2011; Kumar et al., 2009; Lamien-Meda et al., 2008; Mlcek & Rop, 2011; Nishihara & Nakatsuka, 2011; Yang & Shin, 2017).

Berberis crataegina is from the Berberidaceae family and has small oval leaves and yellow flowers. The fruit ripens in late summer and takes on a dark purple-to-black color (Baytop, 1963; Işikli & Yilmaz, 2014). In Turkey, it is called 'karamuk' or 'kadın tuzluğu' by the people (Baytop, 1994; Işikli & Yilmaz, 2014). *B. crataegina* fruit, which grows wild in Asia and Europe, has strong antioxidant properties. It is also rich in phenolic compounds such as chlorogenic acid, gallic acid, vanillic acid, p-coumaric acid, 4-hydroxybenzoic acid, syringic acid, trans-ferulic acid, caffeic acid, and sinapic acid (Eroğlu et al., 2020).

Particularly in the last decade, interest in phyto-nanotechnology has increased tremendously (Mehata, 2015). Plants can be easily processed as they are non-toxic compared to other biological sources and are therefore suitable sources for the synthesis of AgNPs. Many plants have been used to synthesize nanomaterials (Gardea-Torresdey et al., 2003). In this work, AgNP was produced using green biosynthesis using *B. crataegina* fruit and *R. damascena* flowers. The current study has aimed to characterize the physicochemical properties of synthesized *R. damascena* AgNPs (RAgN) and *B. crataegina* AgNPs (BAgN) and evaluate their antioxidant, and antimicrobial activities.

2. Material and Methods

2.1. Nanoparticle synthesis

40 grams of dried *R. damascena* samples were combined with 600 ml of distilled water. It was mixed on a magnetic stirrer at 60°C at 800 rpm for 4 hours. 600 ml of a 1 mM $AgNO_3$ solution was combined with 300 ml of extract that had been filtered via filter paper. A brown discoloration similar to mud color was observed after 24 hours of incubation at room temperature. The range of 300 to 600 nm was utilized to measure absorbance. It was then centrifuged at 5000 rpm for 20 minutes. The precipitate was separated, and it was then dried for six hours at 80°C in an oven.

B. crataegina fruit was collected from Kayseri at the end of summer. To 50 grams of dried *B. crataegina* fruit, 750 ml of distilled water was added. It was stirred for 4 hours at 650 rpm at 60°C on a magnetic stirrer. 400 ml of extract filtered through filter paper and 800 ml of 1 mM $AgNO_3$ solution were mixed. The solution was left at room temperature for 24 hours. Absorbance was measured between 300 - 600 nm. The solution was centrifuged at 5000 rpm for 20 minutes. The precipitate was separated, and it was then dried for six hours at 80°C in an oven.

2.2. Characterization of nanoparticles

SEM and EDX analyses were carried out with a JEOL JSM 6510 Scanning Electron Microscope to determine the elemental composition and shape of the nanoparticles. XRD analyses were carried out with the RIGAKU ULTIMA IV X-Ray Diffraction Spectrometer. FTIR analyses were performed with a Perkin Elmer Spectrum 100 FT-IR Spectrophotometer.

2.3. Antioxidant capacity assays

Three distinct techniques were used to conduct *in vitro* antioxidant capacity testing (DPPH, CUPRAC, and ABTS). Antioxidant capacity analyses were made by modifying the DPPH method of Makhlouf-Gafsi et al. (Makhlouf-Gafsi et al., 2018). For this, methanol was used to produce a 100µM DPPH radical solution. The samples generated at various concentrations were mixed with 3.9 ml of DPPH radical. The radical scavenging rate was then computed by recording the absorbance at 517 nm.

The samples generated at various concentrations were mixed with $7.5x10^{-3}$M methanolic neocuprin solutions, 0.01 M $CuCl_2$ solution, and 1M CH_3COONH_4 (pH: 6.5) buffer. After half an hour of incubation, copper ion reduction capacity was

determined by absorbance measurement at 450 nm. Antioxidant analysis according to the CUPRAC method was performed according to the method of Apak et al. (Apak et al., 2007).

By combining 2.45 mM potassium persulfate solution with 2 mM ABTS solution, an ABTS radical was created. With the help of 0.1 M phosphate buffer (pH 7.4), the absorbance of the radical solution was stabilized to a range of 0.750 to 0.800 nm. To the samples prepared at various concentrations, 2 ml of ABTS radical was added. At 734 nm, absorbances were measured following a 30-minute incubation period (Bursal et al., 2013; Re et al., 1999).

2.4. Antimicrobial activity test

Antimicrobial activity analyses by Disk Diffusion technique (National Committee for Clinical Laboratory Standards., 1997) were performed on *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. Thus, the effect of nanoparticles on gram-negative bacteria, gram-positive bacteria, and fungi was investigated.

2.5. Statistical Analysis

Trials were conducted in triplicate. Statistical analysis was examined by one-way ANOVA. As a result, Both RAgN and BAgN showed significant antioxidant activity and antimicrobial activity. In both analyses, the significance values were $p<0.05$.

3. Results and Discussion

Pictures of the *Rosa damascena* flowers and *B. crataegina* fruits are given in Figure 1.

R. damascena flowers *B. crataegina* fruits

Fig. 1. *Rosa damascena* flower and *Berberis crataegina* fruit

UV-VIS spectrum of silver nanoparticles synthesized from *R. damascena* flower and *B. crataegina* fruit between 300-600 nm is given in Figure 2.

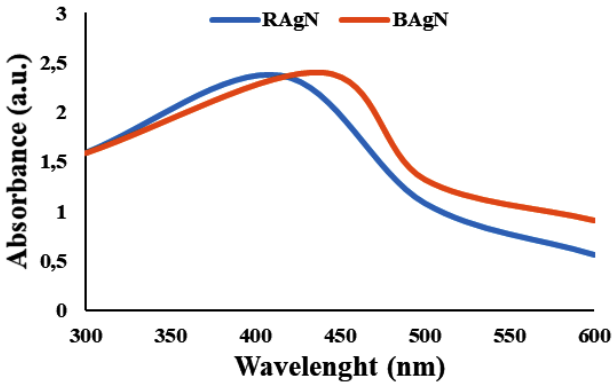


Fig. 2. UV-VIS spectrum of RAgN and BAgN

According to the UV-VIS results, characteristic surface plasmon resonance (SPR) peaks of silver nanoparticles, corresponding to silver nanoparticle biosynthesis, were observed at 415 and 440 nm (Arshad et al., 2022).

SEM images of silver nanoparticles synthesized from *R. damascena* are given in Figure 3. Although RAgN nanoparticles are extremely small, *R. damascena* gave very efficient results in the synthesis of silver nanoparticles. *R. damascena* gave a reaction product similar to the sludge and slime appearance with $AgNO_3$ solution.

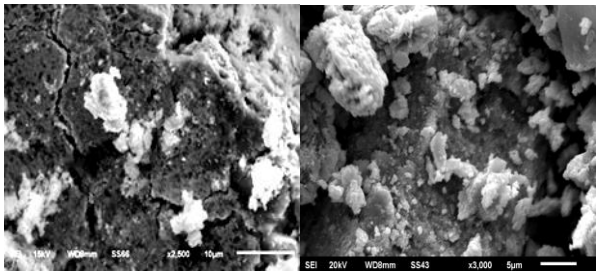
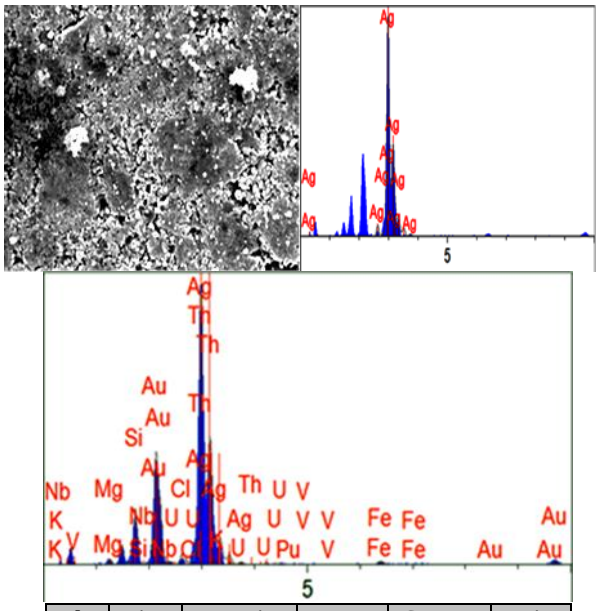


Fig. 3. SEM images of RAgN

SEM-EDX results and graph of RAgN synthesized from *R. damascena* are given in Figure 4.



Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units
Mg	Ka	27.58	3.169	0.329	wt.%
Al	Ka	93.12	4.115	0.993	wt.%
Si	Ka	277.56	5.461	2.665	wt.%
V	Ka	3.96	2.935	0.076	wt.%
Fe	Ka	27.88	2.984	0.780	wt.%
Nb	La	84.43	4.287	2.478	wt.%
Ag	La	1,355.48	10.633	55.722	wt.%
Au	La	29.83	2.523	24.456	wt.%
Th	Ma	279.22	6.438	12.501	wt.%
			Total	100.000	wt.%

Fig. 4. SEM-EDX analysis results of RAgN

FTIR analysis results of RAgN are given in Figure 5.

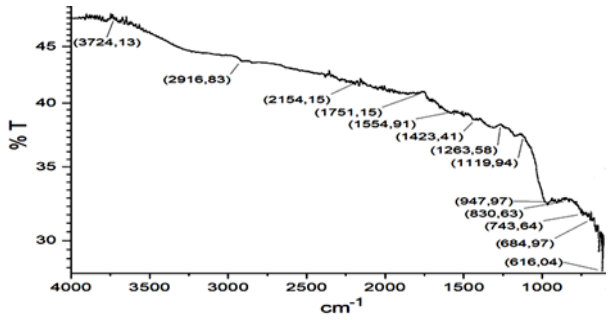


Fig. 5. FTIR analysis results of RAgN

3724.13, 2916.83, 2348,88, 2154.15, 1751.15, 1554.91, 1423.41,1263.58, 1119.94, 947.97, 830.63, 743.64, 684.97, 616.04 cm^{-1} intermolecular bonds gave vibrational peaks. The vibration peak of 3724.13 cm^{-1} in the FTIR analysis result of RAgN indicates the tension of the O-H bond in alcohols and phenols (Hosseini et al., 2020; Şahin et al., 2022). Alkanes' saturated C-H bond is what causes the 2916.83 cm^{-1} signal to appear further, the 2348.88, 2154.15 cm^{-1} vibration peaks are due to the C≡N bonds in the peptide bonds (Şahin et al., 2022). A vibration peak of 1751.15 cm^{-1} indicates the presence of a C=O bond, and a vibration peak of 1554.91 cm^{-1} indicates a C=C bond (Hosseini et al., 2020). 1263.58, 684.97, and 616.04 cm^{-1} peaks indicate the C-O bonding (Periasamy et al., 2022). The FTIR results show the presence of different molecular groups that stabilize the silver nanoparticle (Adebayo-Tayo et al., 2022).

The XRD plot of the RAgN nanoparticle is given in Figure 6.

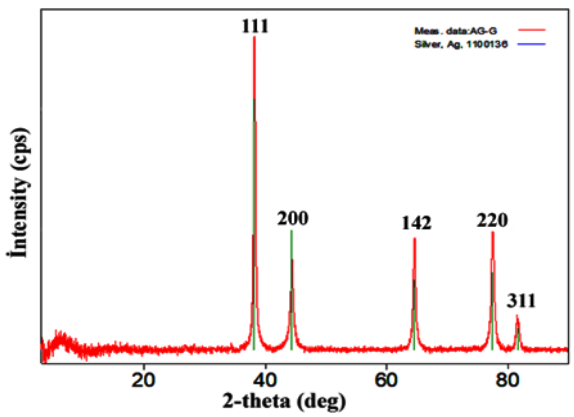


Fig. 6. The XRD graph of RAgN

2θ angles for RAgN were found as 38.141, 44.341, 64.498, 77.436, 81.54. According to the Debye–Scherrer equation: (D=kλ/βcosθ), the crystal size of RAgN was calculated as approximately 20.59 nm, while k: 0.9, λ:0.154 (Giri et al., 2022). A face-centered cubic structure was obtained from the XRD model of RAgN (Giri et al., 2022). The RAgN characteristic peaks (111), (200), (220), and (311) conform to Four Bragg's standard data (JCPDS No. 89-3722) (Baker et al., 2005; Giri et al., 2022; Shameli et al., 2010).

SEM images of (BAgN) silver nanoparticles synthesized from *B. crataegina* fruit are given in Figure 7.

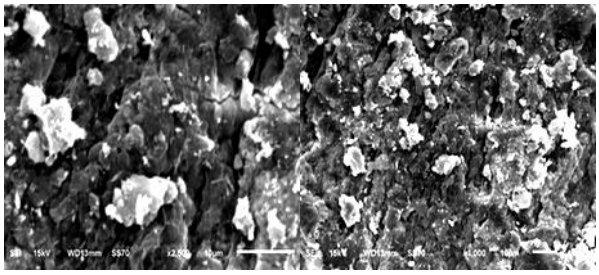
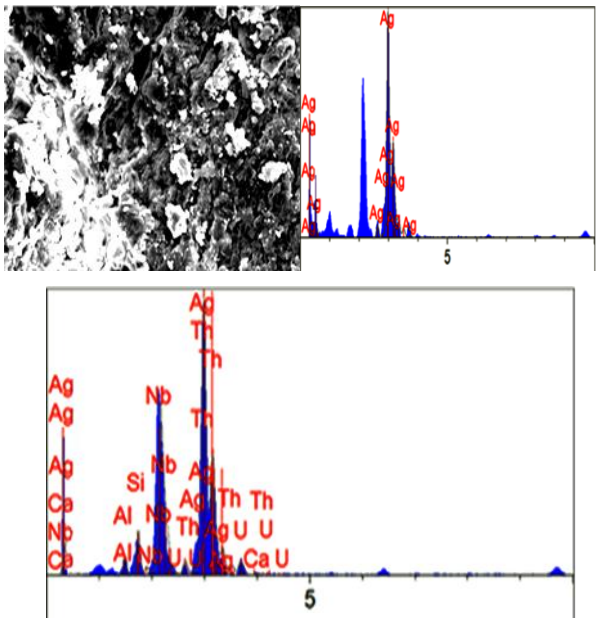


Fig. 7. SEM results of BAgN

SEM-EDX results of silver nanoparticles synthesized from *B. crataegina* fruit are given in Figure 8.



	Line	Intensity (c/s)	Error 2-sig	Conc	Units
Al	Ka	55.92	4.642	1.757	wt.%
Si	Ka	148.45	5.209	3.815	wt.%
Ca	Ka	49.27	4.035	1.916	wt.%
Nb	La	594.39	7.550	25.350	wt.%
Ag	La	1,030.29	9.421	61.453	wt.%
Th	Ma	62.37	4.927	5.709	wt.%
			Total	100.00	wt.%

Fig. 8. SEM-EDX results of BAgN

FTIR analysis results of silver nanoparticles synthesized from *B. crataegina* fruit are given in Figure 9.

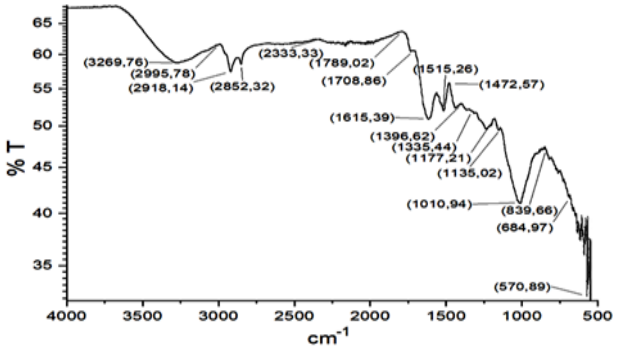


Fig. 9. FTIR results of BAgN

The molecular bonds of BAgN gave vibration peaks in the range of 3269-570 cm^{-1}. There are 3269.76, 2995.78, 2852.32, 2918.14, 2333.33, 1789.02, 1708.86, 1615.39, 1515.26, 1472.57, 1396.62, 1335.44, 1177.21, 1335.44, 1177.21, 1135.02, 1010.94, 839.66, 684.97, and 570.89 cm^{-1} many vibration peaks. 3269.76, cm^{-1} vibration peak shows the tension of the O-H bond (Hosseini et al., 2020). The peaks of 2995.78, 2852.32, and 2918.14 cm^{-1} show the C-H bond, while the vibrational peak of 2333.33 cm^{-1} shows the C≡N bond (Şahin et al., 2022). In addition, vibration peaks of 1789.02, and 1708.86 cm^{-1} may indicate the C=O bond, and vibration peaks of 1515.26, and 1472.57 cm^{-1} may indicate the C=C bond (Hosseini et al., 2020). 1396.62, 1335.44, 1177.21, 1335.44, 1177.21, 1135.02, 1010.94 cm^{-1} peaks indicate the C-O bond (Periasamy et al., 2022). Numerous functional groups that stabilize BAgN are shown by FTIR findings (Adebayo-Tayo et al., 2022). XRD analysis results of BAgN are

given in Figure 10.

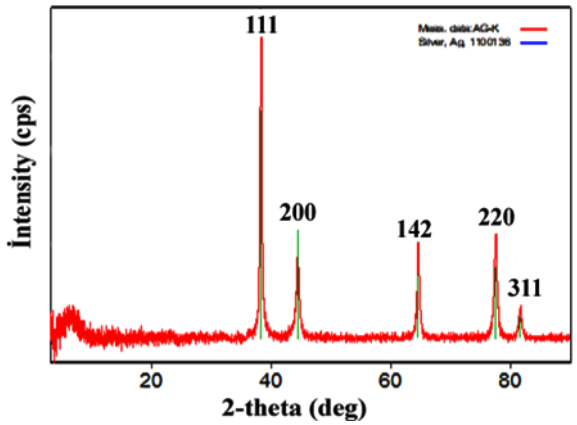


Fig. 10. The XRD graph of BAgN

2θ angles of BAgN; 38.181, 44.357, 64.503, 77.417, 81.683. The crystal size of BAgN was determined to be roughly D=18.04 nm using the Debye-Scherrer equation. In accordance with standard values, the XRD data show that the BAgN (111), (200), (220), and (311) peaks are face-centered cubic (Giri et al., 2022).

Antioxidant analysis results of RAgN and BAgN are given in Table 1.

Table 1. Antioxidant analysis results of RAgN and BAgN

Methods	RAgN (µg/ml)	BAgN (µg/ml)	Trolox (µg/ml)
DPPH(IC_{50})	502,9	618,45	22,29
CUPRAC($A_{0,5}$)	68,96	70,42	18,75
ABTS(IC_{50})	270,21	562,59	15,20

According to the antioxidant analysis results of RAgN, BAgN, and Trolox as standard antioxidants, the concentrations that inhibit 50% of free radicals in both DPPH and ABTS methods (IC_{50}) and the concentrations corresponding to 0.5 absorbance in the CUPRAC method ($A_{0.5}$) are given in Table 1. IC_{50} values were calculated from the graph created using different concentrations and % radical inhibition values of the samples. In the CUPRAC technique, the increase in absorbance is directly proportional to the amount of antioxidant capacity. In this method, a graph was created using absorbance values corresponding to different concentration values. From this graph, the concentration corresponding to 0.5 absorbance ($A_{0.5}$) was calculated. According to these results, RAgN showed better antioxidant properties than BAgN.

The antimicrobial analysis results of RAgN and BAgN are given in Figure 11.

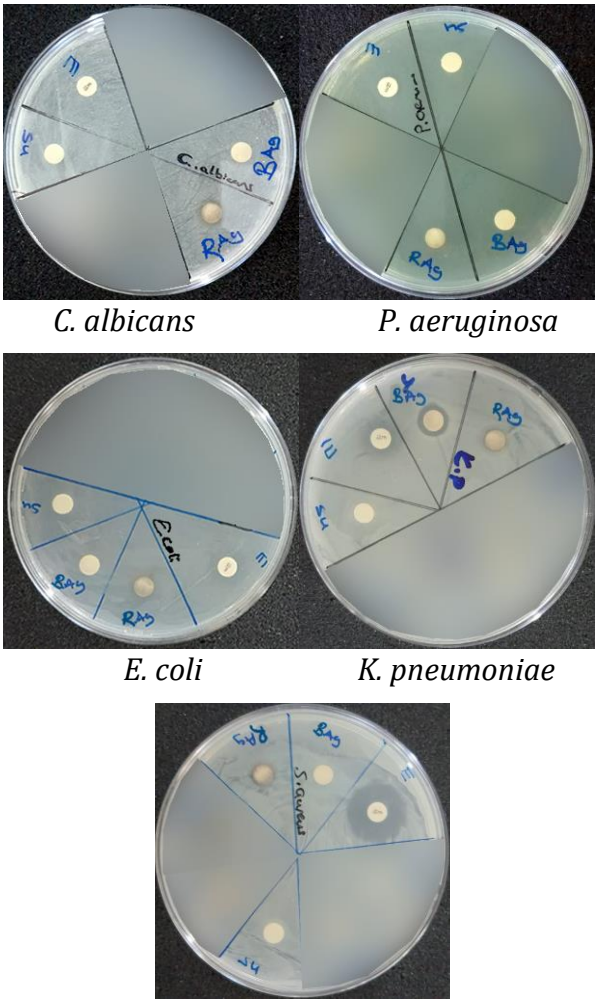


Fig. 11. The antimicrobial analysis results of RAgN and BAgN

Table 2. The antimicrobial analysis results of RAgN and BAgN (zone diameters (mm))

Microorganisms	Erythromycin (15µg)	BAgN (40mg/ml)	RAgN (20mg/ml)
P. aeruginosa	8.0	-	8.5
K. pneumoniae	10.0	7.5	8.0
E. coli	14.0	-	8.0
S. aureus	19.5	-	10.0
C. albicans	14.0	-	8.5

The antioxidant and antimicrobial properties of silver nanoparticles synthesized with two anthocyanin-rich samples gave different results. Silver nanoparticles synthesized from *R. damascena* flowers and *B. crataegina* fruit both exhibited antioxidant properties. RAgN nanoparticles exhibited lower antioxidant properties than Trolox and higher than BAgN. According to these results, the components or phenolic compounds of *R. damascena* that participate in the silver nanoparticle structure may have more antioxidant properties than the silver nanoparticle-forming components of *B. crataegina* fruit. Because bioactive components and phenolic compounds are important in terms of antioxidant properties (Balasundram et al., 2006). *R. damascena* flower extract has been reported to have strong radical scavenging and antioxidant capacity according to DPPH radical reduction and phosphomolybdenum methods (Özkan et al., 2004). In terms of antimicrobial properties, RAgN showed more antimicrobial effects than BAgN (Figure 11 and Table 2). Due to the high antioxidant and antimicrobial properties of RAgN, it has been revealed that *R. damascena* flower extract has been reported to inhibit *Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Enterococcus feacalis, Escherichia coli, Klebsiella pneumoniae, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, P. fluorescens, Salmonella enteritidis, S. typhimurium, Staphylococcus aureus* and *Yersinia enterocolitic* (Özkan et al., 2004). *R. damascena* flowers are an extremely suitable plant for producing nanoparticles with antioxidant and antimicrobial features. In the literature, the ability of silver nanoparticles to fight bacteria synthesized from *Rosa damascena* flowers was investigated and its inhibition effect on *S. aureus, K. pneumonia,* and *E. coli* bacteria was determined (Peron et al., 2021). Along with the outcomes that corroborate these outcomes, the inhibitory effect of silver nanoparticles synthesized from *R. damascena* flowers on *P. aeruginosa* and *C. albicans* was also revealed in this study. While BAgN showed antioxidant properties, it showed an inhibition effect only on *K. pneumoniae* bacteria. Chitosan-based film synthesized from *B. crataegina* fruit previously showed antioxidant and antimicrobial properties (Kaya et al., 2018). In experiments, BAgN showed antioxidant properties, but its antimicrobial effect was low. The antioxidant and antimicrobial properties of *B. crataegina* fruit have been previously examined and it has been reported that it has a strong antioxidant property in addition to its antimicrobial properties (Ercan, 2024). This might be the result of *B. crataegina* fruit's antioxidant components contributing more to the creation of nanoparticles.

Using different samples as reducing agents in silver nanoparticle synthesis can significantly affect the antioxidant and antimicrobial activity. Silver nanoparticles are agents that can be used in the treatment of various wounds and burns (Bold et al., 2022; Gherasim et al., 2020). Because of their antioxidant qualities, silver nanoparticles made from *R. damascena* flowers and *B. crataegina* fruit may be considered potential ingredients in these creams. RAgN can be used safely in antimicrobial products that come into touch with the skin because of its exceptional antibacterial properties.

4. Conclusion

Herbal samples used for silver nanoparticle synthesis by green synthesis affect the activity of the nanoparticle. Silver nanoparticles synthesized from both *R. damascena* and *B. crataegina* fruits are materials that can be safely used in health and cosmetics due to their antioxidant and antimicrobial properties. Especially pink *R. damascena* can stand out because it is a strong antimicrobial in silver nanoparticle synthesis. Silver nanoparticles of this flower and fruit, which are widely found in nature and have a pleasant taste, can be an

important material for products that can be used in different areas such as food, health, and cosmetics.

Disclosure statement

The authors state that they have no conflicts of interest.

Author Contribution

LE, Nanoparticle synthesis, antioxidant analyses and evaluations

ÜTE, Antimicrobial analysis and evaluations

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