

Green production of silver nanoparticles from *Eryngium planum* and evaluation of its antimicrobial effect on *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT:

Background and Objective: The rapid development of nanotechnology over the past decade has opened new and unique horizons in science. Among various nanomaterials, silver nanoparticles have attracted significant attention due to their strong antimicrobial properties and are considered one of the most important types of nanoparticles. Various methods are available for the green synthesis of silver nanoparticles. The use of plant extracts for silver nanoparticle synthesis is a low-cost, economical, and environmentally friendly approach. The aim of the present study was to investigate the green synthesis of silver nanoparticles using *Chuchagh* plant leaves and to evaluate the antibacterial effects of the synthesized nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. **Methods:** Initially, total phenolic content, total flavonoid content, DPPH antioxidant assay, GC–MS analysis for terpenoids, and LC–MS analysis for alkaloids were employed to identify and characterize the bioactive compounds present in the *Chuchagh* plant. After confirming the plant's potential for nanoparticle formation, UV–visible spectroscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM) were used to confirm the synthesis and characterize the silver nanoparticles. In the final stage, the antibacterial activity of the synthesized silver nanoparticles against *Escherichia coli* and *Staphylococcus aureus* was evaluated. **Results:** Due to the presence of phenolic, alkaloid, and terpenoid compounds, the *Chuchagh* plant extract successfully facilitated the formation of silver nanoparticles. The synthesized silver nanoparticles exhibited significant antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. **Conclusion:** The results demonstrated that the leaf extract of the *Chuchagh* plant possesses a high potential for the synthesis of high-purity silver nanoparticles. A careful analysis of the results revealed that increasing the concentration of silver nitrate leads to an enhanced synthesis of silver nanoparticles.

Keywords: Silver nanoparticles; Green synthesis; *Chuchagh*; Antibacterial activity

INTRODUCTION

Silver is one of the most commercially utilized nanomaterials, with annual production of 500 tons of silver nanoparticles, and this is expected to increase in the coming years. Its significant and primary role has been identified in biomolecular sensing, analysis, biosensors, and medical diagnostics, and it exhibits strong antimicrobial effects, along with antifungal and anti-inflammatory activities (1). Silver nanoparticles have been used for various antimicrobial, anticancer, and anticoagulant purposes, as well as for wound-healing treatments (2). Several techniques exist for the synthesis of silver nanoparticles, such as ion sputtering, chemical reduction, sol-gel, and others (1). Plant-based synthesis of nanoparticles is faster, safer, lighter, and can also be performed at low temperatures (3). Studies have shown that silver nanoparticles not only exist on

the cell surface but also penetrate inside the cell. The presence of particles within the cell supports the hypothesis that silver kills and destroys microbes by forming bonds with phosphorus and sulfur present in cellular compounds such as DNA (4). Chamakura et al. found that silver nanoparticles interact with the bacterial cell wall and its cytoplasmic membrane, leading to the formation of pores in the cell wall. Silver ions form insoluble compounds with sulfhydryl groups in the cell wall, ultimately leading to bacterial death (5).

Given the high potential of plants as a source of nanoparticles, this study aimed to employ a green biological method for the synthesis of silver nanoparticles as an alternative to conventional methods (6). In this context, extracts from the plant *Chuchagh*, native to northern regions of the country, were used to convert silver ions into nanoparticles biologically. This

plant is used to treat iron deficiency in patients with thalassemia. Its medicinal and therapeutic properties, popular among people in northern Iran, include use as a stimulant, tonic, diuretic, expectorant, anti-inflammatory, and disinfectant for the treatment of anemia, inflammation, gastrointestinal infections, appetite stimulation, and kidney stone treatment (7). This plant also aids in treating whooping cough and serves as a remedy for rheumatic diseases. *Chuchagh* contains beneficial compounds that help prevent cancer and cardiovascular diseases and reduce bone weakening (8).

Rigi (9) investigated the green synthesis of silver nanoparticles using American mulberry fruit extract. He concluded that plants have a high capacity to reduce metals via their metabolic pathways, a process known as nanoparticle biosynthesis. Shahvali Bar (10) examined the effects of silver nanoparticles on the growth and physiological and biochemical indices of lemon balm and concluded that nanomaterials play an essential role in increasing agricultural productivity through the use of biological nanoparticles. Nanoparticles have been used in plants as pesticides, protective agents, and nutrients. Resai (11) studied the effect of salinity on the biosynthesis of silver nanoparticles by leaf extracts of purple basil, savory, and thyme (from the Lamiaceae family). In the present study, the green synthesis of silver nanoparticles using leaf extracts of *Ocimum basilicum* var. *purpurascens*, *Hyssopus officinalis*, and *Satureja hortensis* was investigated under control (0) and 50, 100, and 150 micromolar sodium chloride treatments.

Based on previous research on nanoparticles, the aim and rationale for our study on the production of silver nanoparticles from the *Chuchagh* plant stem from Iran's diverse climate. Consequently, it is the native habitat of many medicinal plants, many of which contain valuable properties and metabolites. Therefore, there is a critical need to identify the secondary metabolites produced by these plants and to study and evaluate their antibacterial properties. In fact, to establish a presence in the global market and in the scientific and medical fields, it is essential to pursue this knowledge.

MATERIALS AND METHODS:

A species of the *Chuchagh* plant was collected from the northern regions of the country (Gilan Province), and in the Sharif University laboratory, the species of both plants was examined and finally confirmed. For the experiment, 5 grams of powdered *Khalevash* and *Chuchagh* leaves were weighed and placed separately in beakers. Then, 100 mL of distilled water was added to each and heated on a hotplate at 110°C for 20 minutes. During this time, a magnetic stirrer was placed in the beaker to ensure mixing. After extraction, the aqueous extract was filtered through a fine sieve, and the remaining fine particles were further filtered using Whatman No. 42 filter paper. The absorption of the extract was measured using a spectrophotometer in the wavelength range of 300 to 700 nm. Distilled water was

used as the blank in this measurement. (In this study, all experiments were conducted separately for *Chuchagh* and *Khalevash* plants.)

Preparation of Silver Nitrate Solutions

To prepare a 60 mM silver nitrate solution, 2.54 g of silver nitrate was weighed and dissolved in deionized water in a 250 mL volumetric flask to reach the final volume. In this study, silver nitrate from Merck, Germany, was used.

To prepare a 30 mM silver nitrate solution, 125 mL of the 60 mM silver nitrate solution from the previous step was transferred to a 250 mL volumetric flask and diluted to volume with deionized water.

To prepare a 15 mM silver nitrate solution, 125 mL of the 30 mM solution was transferred to a 250 mL volumetric flask and diluted to volume with deionized water.

All flasks containing silver nitrate were wrapped in foil to prevent a reaction with silver. Then, 5 mL of the filtered *Chuchagh* plant extract (*Eryngium planum*) was placed in a 50 mL Falcon tube, and 20 mL of 15 mM silver nitrate solution was added. Separately, 30 mM and 60 mM silver nitrate solutions were each added to two Falcon tubes containing 5 mL of *Chuchagh* plant extract. All three Falcon tubes were incubated for 24 hours at 27°C.

Absorption Spectrum of the Two *Chuchagh* Plants:

A portion of each solution was centrifuged at 4000 rpm for 15 minutes to allow the precipitate to settle to the bottom of the Falcon tube. The absorption was then measured using a spectrophotometer in the wavelength range of 300 to 700 nm, with the diluted plant extract used as the blank. Additionally, distilled water was used as a blank to observe changes in the extract's absorption profile. In this study, a HACH DR500 spectrophotometer was used.

Materials and Methods of Secondary Metabolites:

The ethanolic extract of the *Chuchagh* plant was obtained by maceration. In this method, *Chuchagh* plant powder was mixed with 72% ethanol at a ratio of 1:12 in an Erlenmeyer flask fitted with an aluminum-foil-covered lid at room temperature. The sample was placed on a shaker at 132 rpm for 48 hours. After that, the mixture was passed through filter paper (Whatman No. 4) to obtain the ethanolic extract. Then, the extract was concentrated using a rotary evaporator under vacuum at 62 °C, and the solvent was removed. For drying the extracts, the solvent was removed using a vacuum oven at 45 °C and 15 mmHg. The extracts were stored until use in sterile, sealed glass containers in the dark and in a refrigerator at 4 °C. Initially, the dried *Chuchagh* (each separately) and the solvent were combined in a flask, which was placed on a heater. After some time, the water in the flask began to boil, and, together with the evaporated essential oil, it entered the condenser, where

it was converted into liquid and then entered the burette. The system was kept in this condition for twelve hours to ensure that all the essential oils were extracted, after which the heater was turned off. Inside the burette, water and essential oil were collected. Since essential oils are insoluble in water, the essential oil and water did not mix, forming two phases. During the first hour of essential oil extraction, the essential oil floated on the water; however, after several hours, when the essential oil was extracted entirely, the Chuchagh essential oil became denser than water and settled to the bottom of the burette. After 11 hours, the stopcock of the burette was opened, and the essential oil was collected in a separate container.

A portion of the obtained ethanolic extract, after concentration, was prepared as follows for the identification of its compounds by GC/MS. The extract was placed in a freezer at -12°C for 48 hours to separate its waxes and fats. Then, in the same freezer, the ethanolic extract phase was separated using filter paper. The ethanolic extract phase was transferred to an Erlenmeyer flask fitted with a lid, and hexane was added in an equal volume. The mixture was placed on a shaker at 186 rpm for about 1 hour to become fully homogeneous. Then it was poured into a separatory vessel and left undisturbed for 15 minutes to allow phase separation. The hexane phase was then prepared for injection into the GC/MS for separation. After injection of the hexane-prepared extracts and the essential oil, the GC/MS instrument produced chromatograms of the essential oil and the extract. By examining the chromatograms and mass spectra obtained from a gas chromatograph equipped with a mass spectrometer, the compounds present in the extract and the essential oil of the Chuchagh plant were identified.

The DPPH method was selected to evaluate antioxidant capacity under in vitro conditions. Chuchagh plant extracts were subjected to antioxidant assays. Free radical scavenging activity of DPPH was assessed using the described method. For the assay, 0.70 mL of DPPH methanolic solution (100 μM) was mixed with the sample extract or standard (ascorbic acid) at different concentrations (0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mg/mL) in the same volume. The mixtures were shaken vigorously and incubated in the dark at room temperature for 20 minutes. The absorbance of DPPH with methanol and methanol without DPPH was measured using a UV/Vis DU spectrophotometer. For flavonoids, the assay was performed using the aluminum chloride colorimetric method.

Method for Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

To determine MIC and MBC, after 24 hours of incubation of the bacteria in diluted broth medium, the optical density was evaluated by visual observation and spectrophotometry. The lowest concentration at which

no turbidity was observed was determined as the minimum inhibitory concentration (MIC) of growth. To determine the minimum bactericidal concentration (MBC), from the MIC tube, one higher dilution and one lower dilution were selected, and 10 μL of each was transferred using a sampler onto blood agar medium and surface-cultured. The plates were incubated for 24 hours at 37°C in an incubator. Afterward, the lowest dilution at which no bacterial growth was observed was determined as the minimum bactericidal concentration (MBC).

Broth Microdilution Method for Determination of Growth Inhibition Values:

To perform the test using the microdilution method, first 30 μL of nutrient broth culture medium was dispensed into the first well (A1). Then, 70 μL of bacteriocin was added to the first well of a sterile microplate. Subsequently, 50 μL from the first well was transferred vertically to the second well using serial dilutions, and 50 μL from the second well was transferred to the third well; finally, 50 μL from the last well was discarded. In well A2, 40 μL of culture medium and 60 μL of bacteriocin were added. Then, 50 μL from the first well was transferred vertically to the second well using serial dilutions, and 50 μL from the second well was transferred to the third well; finally, 50 μL from the last well was discarded. In well A3, 50 μL of culture medium and 50 μL of bacteriocin were added. Then, 50 μL from the first well was transferred vertically to the second well using serial dilutions, and 50 μL from the second well was transferred to the third well; finally, 50 μL from the last well was discarded. Afterward, 50 μL of a bacterial suspension equivalent to one McFarland standard was added to all wells, and the microplate was incubated at 37°C for 21 hours in a shaking incubator.

Findings:

Biosynthesis of Silver Nanoparticles Using Chuchagh Extract:

After adding silver nitrate to the extract, the extract turned brown, indicating the initial reduction of silver ions and the formation of nanoparticles in the *Chuchagh* leaf extract.

Observation of Color Change and Formation of Black Precipitate:

Shortly after adding silver nitrate solutions to the Chuchagh plant extract, the solutions darkened; after 24 hours, the darkening intensified, and a black nanoparticle precipitate was clearly observed at the bottom of the Falcon tubes. As shown in Figures (1) and (2), after 24 hours, the solution containing 60 mM silver nitrate produced the most tremendous amount of precipitate and became the darkest, followed by the solution containing 30 mM silver nitrate, which produced a greater amount of precipitate. The solution containing 15 mM silver nitrate showed the least color darkening and the lowest amount of black precipitate.

Portions of these solutions were stored in a refrigerator for subsequent antibacterial testing.



Figure 1: Mixture of *Chuchagh* plant extract with silver nitrate (15, 30, 60 mM) 24 hours after reaction.

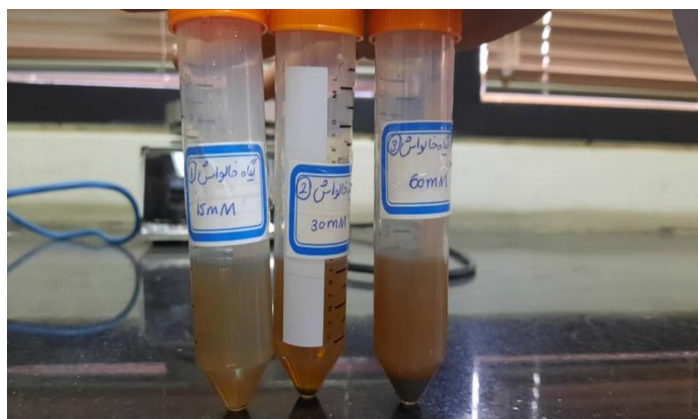


Figure 2: Mixture of *Khalvash* plant extract with silver nitrate (15, 30, 60 mM) 24 hours after reaction.

Investigation of Solution Absorbance: Ultraviolet–Visible Spectrophotometric Studies in the *Chuchagh* Plant:

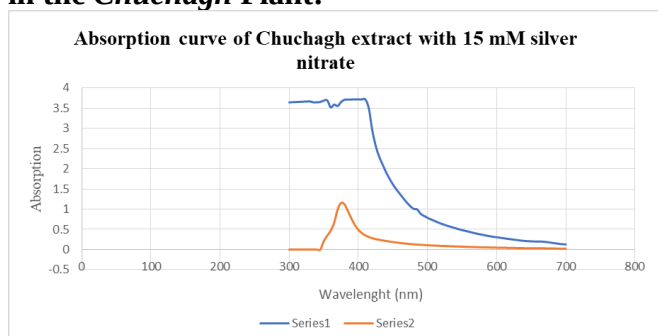


Figure 3: Absorption curve of *Chuchagh* extract with 15 mM silver nitrate.

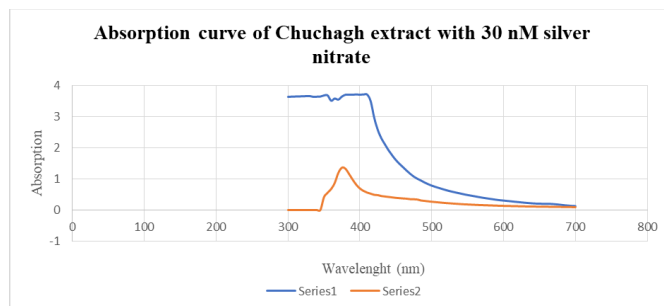


Figure 4: Absorption curve of *Chuchagh* extract with 30 mM silver nitrate.

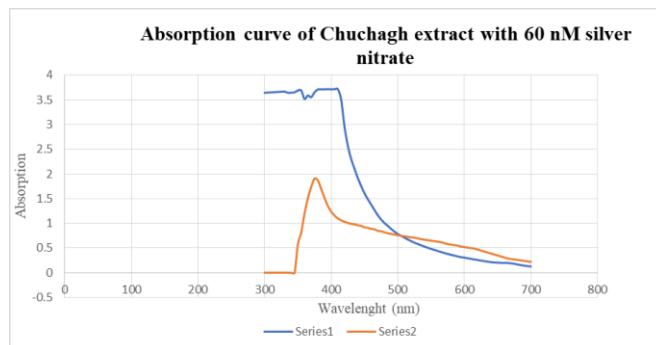


Figure 5: Absorption curve of *Chuchagh* extract with 60 mM silver nitrate.

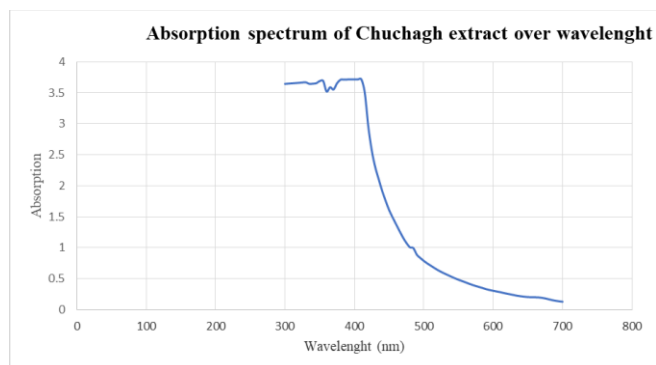


Figure 6: Absorption spectrum of *Chuchagh* extract over wavelength.

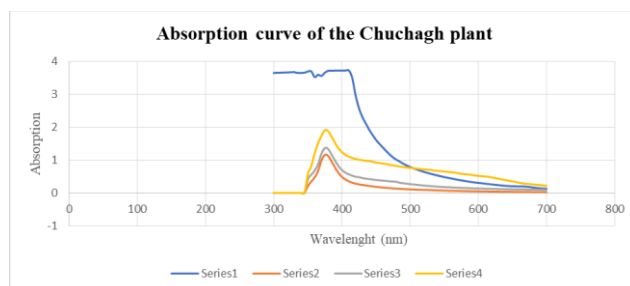


Figure 6: Absorption Curve of the *Chuchagh* Plant

To better observe the peak, the extract solution, diluted 1:5 with distilled water, was used as a blank, and the spectrum was scanned again over 300–700 nm. The maximum absorbance was observed at approximately 370 nm, corresponding to the absorption of silver nanoparticles. An increase in silver nitrate concentration led to greater nanoparticle-related absorbance.

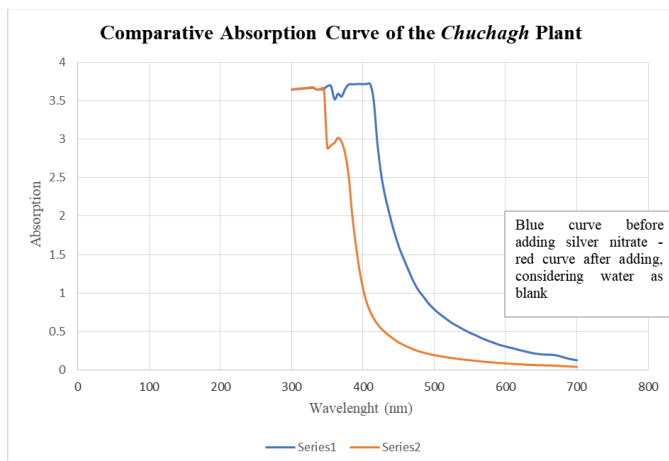


Figure 7: Comparative absorption curve of Chuchagh extract.

Figures 4–6 show the absorption spectra of the Chuchagh plant (*Eryngium planum*). The blue spectrum represents the extract before silver nitrate addition, and the red spectrum represents the extract after silver nitrate addition, with distilled water as the blank. The red peak observed around 370 nm corresponds to the formation of silver nanoparticles. As shown in Figures 4-1, 4-2, and 4-3, the absorption of plant extracts treated with 15, 30, and 60 mM silver nitrate was compared with the absorption of the Chuchagh extract before silver nitrate addition. After adding silver nitrate, the absorption peak shifted to approximately 370 nm, indicating the formation of silver nanoparticles.

Results of Secondary Metabolite Analysis:

Table 1: Total Flavonoid Content in Chuchagh Plants

	Total flavonoid content of Chuchagh (µg QE/g dry weight)
1	1484.556489
2	1487.586744
3	1473.087116
Average	1481.74345
Standard Deviation	7.648182884

As shown in [Table 1](#), the total flavonoid content of Chuchagh was measured in three replicates, and the

mean value was calculated. The results indicate that the highest flavonoid extract was obtained from sample 2.

Table 2: Total Phenolic Content in Chuchagh Plants

	Total phenol content of Chuchagh (mg GAE/g dry weight)
1	161.5609942
2	158.6935625
3	154.2221909
Average	158.1589159
Standard Deviation	3.698498813

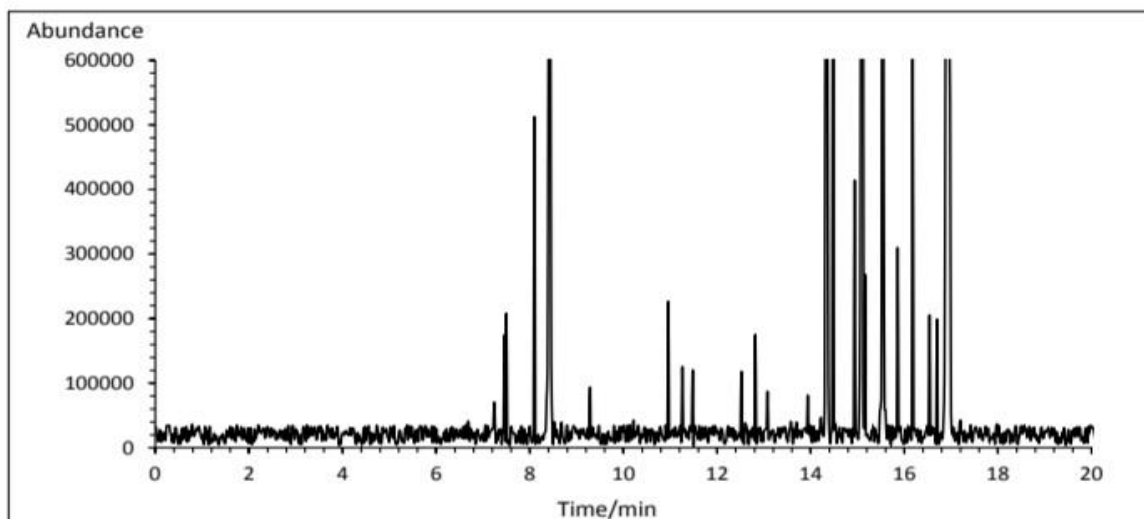
As shown in [Table 2](#), the total phenol concentration was measured three times at the Chuchagh plant, and the average of these measurements was calculated. Based on the results, the highest phenolic content in Chuchagh corresponds to sample 1.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is one of the most widely used methods for evaluating the antioxidant activity of plant extracts and, more generally, natural antioxidants. Butylated hydroxyanisole (BHA) and ascorbic acid were used as standards in this study.

Table 3: DPPH Radical Scavenging Activity of Chuchagh Extracts

Chuchagh Concentration (µg/mL)					
	10	50	100	250	500
1	3.160 282	13.98 268	35.56 7	51.24 197	66.66 508
2	1.495 963	16.14 673	37.18 514	51.28 531	66.04 148
3	2.951 323	13.21 355	37.03 403	50.13 498	66.92 03
Vita min C	20.72 224	35.30 079	—	79.48 535	89.08 423
TBH Q	22.43 831	36.88 955	66.75 374	83.46 854	91.85 637

As shown in [Figure 8](#), the percentage of free radical inhibition increased with increasing concentration of Chuchagh extract. Therefore, Chuchagh demonstrates strong antioxidant potential.



Peak IDs	RT (min)	Concentration (%)
1. α -Thujene	1. 6.68	1. 0.308957
2. β -Pinene	2. 7.24	2. 0.52613
3. Sabinene	3. 7.46	3. 1.309966
4. Myrcene	4. 7.50	4. 1.559379
5. α -Terpinene	5. 8.10	5. 3.839681
6. δ -3-Carene	6. 8.42	6. 7.631857
7. 1,8-Cineole	7. 8.52	7. 0.300062
8. Limonene	8. 8.68	8. 0.295228
9. <i>trans</i> -Linalool oxide	9. 9.29	9. 0.700671
10. Terpinen-4-ol	10. 10.22	10. 0.319497
11. Linalool acetate	11. 10.96	11. 1.698029
12. 2-Decenal	12. 11.25	12. 0.941614
13. Perillaldehyde	13. 11.48	13. 0.903762
14. Cubebene	14. 12.52	14. 0.885943
15. α -Copaene	15. 12.60	15. 0.288031
16. β -Bourbonene	16. 12.82	16. 1.312214
17. Cyperene	17. 13.08	17. 0.65332
18. α -Cedrene	18. 13.58	18. 0.30482
19. α -Gurjunene	19. 13.70	19. 0.295632
20. β -Caryophyllene	20. 13.94	20. 0.60812

21. γ -Elemene	21. 14.22	21. 0.349684
22. β -Gurjunene	22. 14.35	22. 9.220904
23. α -Guaiene	23. 14.47	23. 6.92445
24. α -Humulene	24. 14.93	24. 3.104768
25. α -Curcumene	25. 15.10	25. 18.16749
26. γ -Muurolene	26. 15.16	26. 2.01154
27. β -Selinene	27. 15.34	27. 0.236926
28. α -Selinene	28. 15.40	28. 0.15521
29. α -Muurolene	29. 15.54	29. 9.993259
30. γ -Cadinene	30. 15.72	30. 0.044825
31. β -Bisabolene	31. 15.86	31. 2.322722
32. δ -Cadinene	32. 16.18	32. 4.659859
33. α -Cadinene	33. 16.53	33. 1.538551
34. Elemol	34. 16.70	34. 1.491263
35. Ledol	35. 16.92	35. 14.50115
36. Caryophyllene oxide	36. 17.21	36. 0.319705
37. Guaiol	37. 17.99	37. 0.274778

Figure 8: GC-MS Results of Terpenoids in the Chuchagh Plant

The chemical composition of Chuchagh essential oil was analyzed by GC-MS. The primary and characteristic compounds in the essential oil include

curcumin, gamma-3-caren, beta-gurjunene, alpha-guaiene, gamma-cadinene, alpha-terpinene, and others.

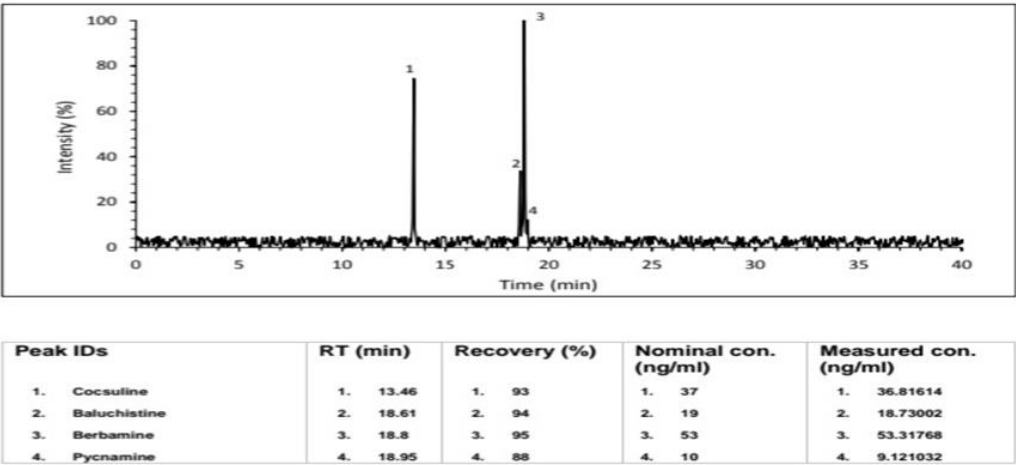


Figure 9: LC-MS Results of Alkaloids in the Chuchagh Plant

According to the LC-MS analysis of alkaloids, the Chuchagh plant contains four alkaloid compounds, listed in order of highest concentration: Berbamine, Cocculine, Baluchistine, and Phycnamine. The GC-MS and LC-MS results indicate that the plant contains both terpenoids and alkaloids.

Results of Electron Microscopy Analysis of Silver Nanoparticles in *Chuchagh*:

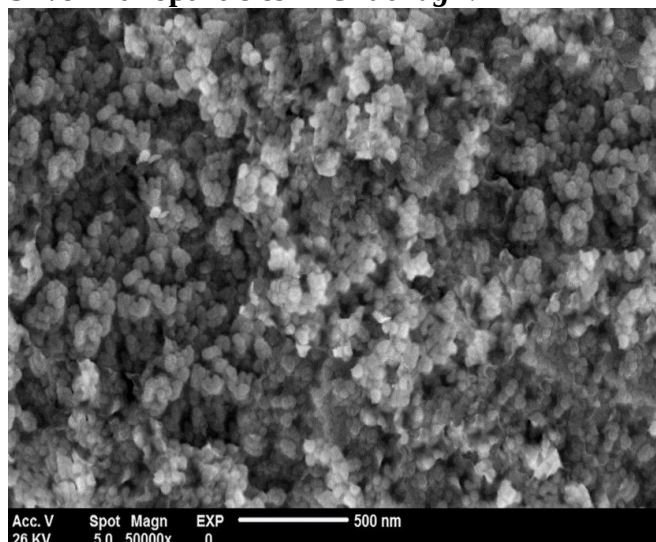


Figure 10: TEM Image of Silver Nanoparticles Synthesized from *Chuchagh*

To examine particle characteristics, including shape, size, and distribution, transmission electron microscopy (TEM) was used. The images correspond to the green synthesis of silver nanoparticles in the leaf extract of the *Chuchagh* plant, treated with 60 mM silver nitrate. The images were captured at 40,000 $\times$ magnification and 80 kV voltage. TEM images of nanoparticles synthesized from the *Chuchagh* plant indicate that the particles ranged from approximately 15 to 20 nm and that the majority were spherical ([Figure 10](#)).

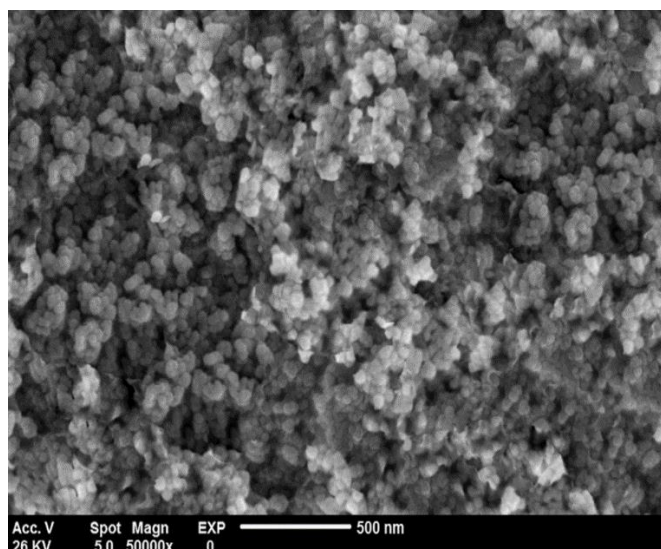


Figure 11: SEM Analysis of *Chuchagh* Nanoparticles

Scanning electron microscopy is one of the most effective analytical methods and has numerous applications across various fields. Images of silver nanoparticles synthesized from the *Chuchagh* plant were obtained at a magnification of 50,000 $\times$. Based on the results of apparent color change, absorption spectroscopy, TEM, and SEM imaging, as well as the

analysis of secondary metabolites—including total phenols, total flavonoids, DPPH assay, GC-MS for terpenoids, and LC-MS for alkaloids—the successful green biosynthesis and formation of silver nanoparticles were confirmed. Therefore, the obtained extract can be used for evaluating antibacterial activity ([Figure 11](#)).

Microbial Results:

The microbial results include the well diffusion test, disk diffusion test, and microdilution test, which were examined in this study. The findings obtained are summarized in [Table 2](#).

Table 2: Well Diffusion Assay Results Against *Staphylococcus aureus* and *Escherichia coli* for 15, 30, and 60 mM Solutions

Row	Well Diffusion Test Against <i>Staphylococcus aureus</i>		
	Laboratory Code	Sample Name	Diameter (mm)
1	1400/P/33-1	<i>Chuchagh</i> 30 mM	12
3	1400/P/33-3	<i>Chuchagh</i> 60 mM	12
3	1400/P/33-4	<i>Chuchagh</i> 15 mM	11
4	Blank-Control	R	R

Row	Well Diffusion Test Against <i>Escherichia coli</i>		
	Laboratory Code	Sample Name	Diameter (mm)
1	1400/P/33-1	<i>Chuchagh</i> 30 mM	6
2	1400/P/33-3	<i>Chuchagh</i> 60 mM	8
3	1400/P/33-4	<i>Chuchagh</i> 15 mM	6
4	Blank-Control	R	R

In the well diffusion assay, measurement of the inhibition zones showed that the 30 mM and 60 mM *Chuchagh* plant solutions produced the largest zones, with a 12 mm diameter, against *Staphylococcus aureus* (*S. aureus*), demonstrating the most excellent antibacterial effect. Therefore, it can be concluded that the silver nanoparticles derived from the *Chuchagh* plant can play an effective role in inhibiting *S. aureus*. Measurement of the inhibition zone in this assay also indicated that the largest zone, with a diameter of 8 mm, was observed for the 60 mM *Chuchagh* plant solution ([Table 2](#)).

Table 3. Disc Diffusion Assay Results Against *S. aureus* and *E. coli* for 15, 30, and 60 mM Chuchagh Solutions

Row	Disk Diffusion Test Against <i>Staphylococcus aureus</i>		
	Laboratory Code	Sample Name	Diameter (mm)
1	1400/P/33-1	Chuchagh 30 mM	12
3	1400/P/33-3	Chuchagh 60 mM	11
4	1400/P/33-4	Chuchagh 15 mM	11
7	Blank-Control	R	R

Row	Disk Diffusion Test Against <i>Escherichia coli</i>		
	Laboratory Code	Sample Name	Diameter (mm)
1	1400/P/33-1	Chuchagh 30 mM	6
3	1400/P/33-3	Chuchagh 60 mM	8
4	1400/P/33-4	Chuchagh 15 mM	8
7	Blank-Control	R	R

In the disk diffusion test against *Staphylococcus aureus*, the largest inhibition zone was observed with the 30 mM Chuchagh plant solution. In the disk diffusion test against *Escherichia coli*, the largest inhibition zones were observed with the 15 mM and 60 mM Chuchagh plant solutions (Table 3).

Table 4: Microdilution Assay Results Against *Staphylococcus aureus* and *Escherichia coli* for 15, 30, and 60 mM Chuchagh Solutions

Row	MIC test by the microdilution method in exposure to <i>Staphylococcus aureus</i>		
	Laboratory Code	Sample Name	In microliters per milliliter μ l/ml
1	1400/P/33-1	Chuchagh 30 mM	5.12
2	1400/P/33-3	Chuchagh 60 mM	5.12
3	1400/P/33-4	Chuchagh 15 mM	5
	Blank-Control	R	R

Row	MIC Test by the Microdilution Method Against <i>Escherichia coli</i>		
	Laboratory Code	Sample Name	In microliters per milliliter μ l/ml
1	1400/P/33-1	Chuchagh 30 mM	12.5

2	1400/P/33-4	Chuchagh 60 mM	0.78
3	1400/P/33-3	Chuchagh 15 mM	3.12
	Blank-Control	R	R

The MIC (Minimum Inhibitory Concentration) test on *Staphylococcus aureus* showed that, for the Chuchagh plant, the lowest inhibitory concentration in the 15 mM Chuchagh solution was 5 μ g/mL. The MIC test on *Escherichia coli* showed that, for the Chuchagh plant, the lowest inhibitory concentration in the 60 mM Chuchagh solution was 0.78 μ g/mL. Therefore, it can be concluded that different concentrations of biosynthesized Chuchagh solutions exhibit antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* (Table 4).

DISCUSSION:

In the present study, the addition of Chuchagh seed extract to silver nitrate solution resulted in a color change from transparent to dark yellow, indicating the formation of silver nanoparticles. This color change is attributed to the excitation of surface plasmon vibrations by the nanoparticles. It has also been shown that UV-Vis absorption spectroscopy can be used to study the shape and size of metallic nanoparticles. In all synthesis methods, a key piece of evidence for the formation of silver nanoparticles at the nanoscale is the presence of a broad absorption band in the 380–450 nm region. Silver nanoparticles exhibit unique optical properties, and this peak arises from the collective excitation of free electrons in the small particles. According to Mie theory, the interaction of light with the electrons in metal nanoparticles produces an absorption band whose shape and intensity depend on particle size and uniformity.

In the present study, three concentrations of silver nitrate (15, 30, and 60 mM) were added to the Chuchagh extracts. As the silver nitrate concentration increased to 60 mM, more precipitate formed at the bottom of the vial, and the solution turned dark brown. Consequently, the highest silver nitrate concentration produced the highest AgNP concentration, consistent with previously reported results. Huang et al. (75) demonstrated that increased absorption with higher extract doses indicates greater silver nanoparticle production, attributed to the presence of more biomacromolecules capable of reducing silver ions at higher concentrations.

Transmission electron microscopy (TEM) images revealed individual silver nanoparticles and some aggregated particles, with the majority exhibiting spherical shapes. In this method, Chuchagh extract was used as a stabilizing agent to prevent nanoparticle agglomeration. The separation observed between silver nanoparticles in the TEM images is attributed to the protective effects of the Chuchagh plant extract. TEM images of Chuchagh-treated plant tissue showed nanoparticles in the nanometer range, with uniform

shape and size of approximately 20 nm. The particles in the images predominantly exhibited spherical morphology. Data published by Bali et al. (12) indicate that the ability to accumulate metal ions varies significantly among plant species. However, overall, across all species, the amount of accumulation is generally related to the initial metal concentration and exposure time.

The formation of these nanoparticles was confirmed by scanning electron microscopy (SEM). The results showed that, under identical conditions, nanoparticles synthesized using mint were more abundant and more stable than those produced using eucalyptus. This indicates that mint has a greater capacity to produce silver nanoparticles (12).

The results showed that the primary and characteristic terpenoid compounds in *Chuchagh* essential oil include curcumin, gamma-3-carene, beta-gurjunene, alpha-guaiene, gamma-cadinene, alpha-terpinene, and others. Regarding the DPPH assay, it can be interpreted that as the concentration of *Chuchagh* samples increased, their ability to scavenge free radicals also increased. Therefore, as concentration increased, the antioxidant capacity of the *Chuchagh* plant increased. The antioxidant capacity of grape extracts (skin, seed, and pulp) was also generally evaluated. Concentrations were assessed using the most common antioxidant standard. In the DPPH assay, the results clearly indicated that free radical production is significantly dependent on the concentration and DPPH inhibitory activity (13).

CONCLUSION:

The results showed that the leaf extract of the *Chuchagh* plant has a high potential for producing silver nanoparticles with high purity. Careful examination of the results indicates that increasing the concentration of silver nitrate increases the synthesis of silver nanoparticles. Additionally, longer reaction times led to greater silver nanoparticle synthesis. The findings indicate that silver ions were reduced to silver atoms using the aldehydes present in the *Chuchagh* leaf extract, and that, ultimately, silver nanoparticles were prepared and synthesized using this extract. These observations demonstrate that the metabolites in the *Chuchagh* leaf extract, including terpenoids and aldehydes, can reduce silver ions and convert them to elemental silver, thereby facilitating the production of silver nanoparticles.

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