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Phytochemical Profiling and Bioactivity of *Ziziphus nummularia* Extract and it's Optimization for Green Synthesis of Silver Nanoparticles

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Abstract: *Ziziphus nummularia*, a member of the Rhamnaceae family, is rich in bioactive compounds and possesses significant therapeutic potential. This study evaluated its phytochemical composition, antioxidant activity, antibacterial properties, and role in green nanoparticle synthesis. Methanolic leaf extract screening confirmed the presence of various phytochemicals. Antioxidant potential was assessed using DPPH and TAC assays, revealing strong ROS scavenging activity. The extract also showed effective antibacterial activity against both gram-positive and gram-negative bacteria. Furthermore, the leaf extract was successfully utilized for the green synthesis of silver nanoparticles, characterized by UV–visible spectroscopy, SEM, and XRD analysis. The findings highlight *Ziziphus nummularia* as a promising natural source for pharmaceutical and green nanotechnology applications.
Keywords: *Ziziphus nummularia* • Antioxidant activity • Antibacterial activity • Silver nanoparticles

Introduction

Plants contribute to healthcare through bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties (Evans et al 2009; Heinrich et al 2023). Medicinal plants are a rich source of antioxidants such as polyphenols carotenoids, and some vitamins (Pandey & Rizvi 2023). Plant-derived medications, rooted in folklore and Ayurvedic practices, are recognized as safe and effective (Napagoda et al 2022; Singh et al 2024). Despite the dominance of synthetic pharmaceuticals, medicinal plants continue to play a crucial role in drug development within the pharmaceutical industry (Butler and Capon 2026).

Plant-derived medications, rooted in folklore and Ayurvedic practices, are regarded as safe and effective (Singh et al 2024). The growing population and medication scarcity, along with the high costs and side effects of synthetic drugs, have increased interest in medicinal plants. Despite the focus on synthetic pharmaceuticals, medicinal plants continue to play a crucial role in drug development (Butler and Capon 2026). Extracts from *Ziziphus* are employed in traditional medicine for conditions like insomnia and skin disorders (Sakna et al 2023; Liu et al 2021).

Ziziphus nummularia, also known as Ramilak or Wild Jujube (Fig 1), is a thorny bush from the Rhamnaceae family with significant medicinal properties. Predominantly found in South and West Asia and several African countries, it thrives in arid climates.

Fig 1. *Ziziphus nummularia* (Rhamnaceae)

Ziziphus nummularia is traditionally used to treat ailments such as constipation, diarrhoea, helminthiasis, and skin disorders due to the presence of various phytochemicals

responsible for its biological activities (Kritika et al 1999). Various phytochemicals are required as reducing and capping agents in the green synthesis of nanoparticles. *Ziziphus nummularia* contains diverse phytochemicals that are useful for the synthesis of silver nanoparticles. The synthesized nanoparticles were characterized using UV–Vis spectroscopy, SEM, and XRD analysis (Kumar et al 2026; Bhandari et al 2026). This study demonstrated that *Ziziphus nummularia* leaf extract possesses phytochemicals, antioxidant, and antibacterial activities, and can be effectively used for green synthesis of silver nanoparticles for pharmaceutical and nanotechnology applications.

Materials and Methods

Sample collection : The biological replicates of the leaves of the *Ziziphus nummularia* plant used in the present study were collected in the month of January from the Chauras region of Srinagar, Garhwal., Uttarakhand, India.

Chemicals and reagents : All the chemicals and glassware were purchased from Sisco Research Laboratories Pvt. Ltd. (SRL), Chemical Manufacturers in Mumbai, India, Himedia Laboratories Pvt. Ltd. in Mumbai, India and Borosil respectively.

Preparation of methanolic extraction : The collected sample was first thoroughly washed and removes unwanted impurity. The leaves were dried at room temperature. The 50 grams of the obtained powder was homogenized in 300 millilitres of methanol and was kept in an orbital shaker for 48 hr at 50 rpm and 37˚C. The extract was prepared using the filtration process (Abcha et al 2021; Zhang et al 2022; Kumar et al 2025).

Quantitative phytochemical analysis (Table 1)

Alkaloids Determination:

Five grams of sample were mixed with 200 ml ethanol containing 10% acetic acid and kept for 4 h. After filtration, the extract was concentrated to one-fourth volume, followed by dropwise addition of NH_4OH to precipitate alkaloids. The precipitate was collected, washed, dried, weighed, and used to calculate alkaloid content percentage (Evans et al 2009; Kanagarajaet al 2025; Ozcan et al 2025). The percentage of alkaloid content within the sample was determined using the subsequent mathematical expression:

% Alkaloid = (Weight of alkaloid / Weight of sample) ×100

Table 1. Qualitative phytochemical analysis

SN	Phytochemical	Test	Procedure	Expected Outcome	References
1.	Alkaloids Test	Mayer's test	A few drops of Mayer's reagent were added to the filtrate	Formation of white or creamy precipitate	Auwal et al., 2014
2.	Flavonoids	Ammonia test	0.5 ml leaf extract + 4 ml ammonia solution + 1 ml conc. sulphuric acid	Strong yellow colour	Kabir et al., 2026
3.	Saponins	Foam test	1 ml leaf extract + 5 ml distilled water; shaken vigorously for 15 min	Formation of foam layer	Tiwari et al., 2011
4.	Steroids	Hesse's response	1 ml leaf extract + 10 ml chloroform + 1 ml conc. sulphuric acid added along the wall of test tube	Upper layer red; lower layer yellow with green fluorescence	Jena et al., 2025
5.	Fatty Acids	Spot test	0.5 ml leaf extract + 5 ml petroleum ether; placed on filter paper and allowed to evaporate	Transparent spot-on filter paper	Evans et al., 2009
6.	Tannins	Ferric chloride test	1 ml leaf extract + 10 ml distilled water + few drops of 1% aqueous ferric chloride solution	Intense green, purple, blue, or black coloration	Harborne et al., 1998
7.	Phenols	Ferric chloride test	1 ml leaf extract + distilled water + few drops of 10% ferric chloride	Dark green colour	Tiwari et al., 2011
8.	Xanthoproteins	H_2SO_4 test	1 ml leaf extract + few drops of conc. sulphuric acid + ammonia solution	Reddish-orange precipitate	Sofowora et al., 1993

9.	Quinones	Alcoholic KOH test	0.5 ml leaf extract + 5 ml alcoholic potassium hydroxide solution	Colour change from red to blue	Kumar et al, 2025
10.	Carboxylic Acids	Sodium carbonate test	1 ml leaf extract + few ml sodium carbonate solution	Occurrence of effervescence in the test tube	Harborne et al., 1998
11.	Phlobatannins	HCl test	2 ml leaf extract + 2–3 ml 10% hydrochloric acid	Red precipitate	Auwal et al., 2014
12.	Terpenoids	Salkowski test	Small volume of leaf extract + 2 ml chloroform + 3 ml concentrated sulphuric acid	Formation of a reddish-brown layer at the interface	Gul et al., 2017
13.	Oxalates	Acetic acid test	4 ml leaf extract + 2 ml acetic acid + 1 drop ferric chloride + 2 ml sulphuric acid	Brown ring at interface	Nanna et al., 2011
14.	Glycosides	Keller–Killani test	1 ml leaf extract + 1 ml glacial acetic acid + 2–3 drops ferric chloride + 2 ml concentrated sulphuric acid	Formation of reddish-brown colour at the junction of two layers	Peiris et al., 2023

Flavonoid Determination:

Ten grams of sample were extracted with 100 ml of 80% aqueous methanol for 24 h at room temperature. The extract was filtered, evaporated to dryness on a water bath, and weighed after obtaining a constant weight (Imtiaz et al 2025).

The percentage of flavonoids present in the sample was calculated using the following formula:

% Flavonoid = (Weight of flavonoid / Weight of sample) ×100

Terpenoids Determination:

For terpenoid estimation, 1 g leaf powder was soaked in ethyl alcohol for 24 h, filtered, and extracted with 10 ml petroleum ether. The ether extract represented the total terpenoid content (Bugul et al 2025).

The percentage of the total terpenoid content was expressed mathematically as follows:

% Terpenoid = {(Final weight of sample - Initial weight of sample) / Weight of sample} × 100

Antioxidant Assay

DPPH Radical Scavenging Activity Assay

A free, stable radical called DPPH is a free stable radical that can take an electron or a hydrogen ion to become stable. When antioxidants are present, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is converted to 2,2-diphenyl-picrylhydrazine, which produces a light-yellow solution (Blois 1958). Different concentrations of the methanolic leaf extract were taken, and the volume of each was made up to 1000µl using methanol. 2.5mL of 0.1mM methanolic solution of DPPH was added to the sample, and the mixtures were incubated at 27 °C for 20 min. Methanol was used as a blank. As a negative control, a test tube containing 5mL of DPPH solution and 1000µl of methanol was used. The mixture of commercial BHT, DPPH and methanol was used for a positive control and absorbance of the solution was measured at 517nm

Phosphomolybdenum (TAC) Assay

This assay is used to assess sample's total antioxidant capacity (TAC). Mo (IV) in the reaction mixture is reduced to Mo (V) and forms a complex that is green in colour (Prieto et al 1999). Different concentrations of the methanolic plant extract were taken, and the volume was made up to 1000µl using methanol. 3ml of the reagent solution was then added. As a blank, a reaction mixture was taken without a sample or standard. Test tubes were incubated in a water bath for 90 min at 95 °C (Untea et al 2018). At room temperature, the mixture was cooled, and the absorbance at 695 nm was measured in comparison to a control. The result was expressed in ascorbic acid equivalents per gram extract (AAEC) (Magalhães et al 2008).

Antibacterial Activity

To evaluate the antibacterial activity of *Ziziphus nummularia* extract, both Gram-positive and Gram-negative bacterial strains were tested. The Gram-positive strains

included *Staphylococcus aureus* (ATCC25293) and *Streptococcus pneumoniae* (ATCC49619), while *Escherichia coli* (Clinical strain) and *Klebsiella pneumoniae* (MTCC4030) represented the Gram-negative group. The agar well diffusion method was employed for the antibacterial activity. Nutrient agar and broth were prepared, sterilized, and poured into petri dishes, after which bacterial inoculum was uniformly spread on the agar surface.

Wells were created in the agar using a 100mm diameter cork borer, and each was filled with 100µl of the plant extract at varying concentrations (Zhang et al 2022). Streptomycin (25 µg) and Ampicillin (10 µg) were used as positive controls, while methanolic extract served as the negative control; plates were pre-diffused for 2 h and incubated at 37°C for 24 h to evaluate bacterial inhibition.

Silver nanoparticle optimization and synthesis

Silver nanoparticles were synthesized by following the method described by Ahmed et al., (2016). The plant was with silver nitrate solution taken in different concentration ratios. The thoroughly mixed solution was incubated under sunlight until the color of solution became dark brown. The dark brown colored solution was centrifuged 10,000 rpm for 10 minutes. The supernatant obtained was discarded and pellet was dissolved in ethanol and centrifuged. The solution so obtained was poured over a watch glass and air dried to evaporate ethanol. The dried powder was collected with the help of a brush and stored for characterization as nanoparticles.

The above-explained procedure was performed at different concentrations of plant extract and $AgNO_3$ at different time intervals of reaction. To observe the formation of nanoparticles, the absorbance of the reaction mix was measured in a range of 400-500 nm wavelengths in a spectrophotometer (Systronics), after that nanoparticle were characterized by the XRD and SEM (Kumar et al 2026, Bhandari et al.2026, Kumar et al., 2025).

Results and discussion

Qualitative screening of phytochemicals

The phytochemical screening of crude methanolic extract of leaves of *Ziziphus nummularia* showed the presence of important secondary metabolites like alkaloids, flavonoids, saponins, tannins, and many more, as shown in Table 2.

The presence of phytochemicals such as Flavonoid, Saponin, Steroid, Tannin**,** Fatty acid**,** Phenol, Xanthoprotein, Quinone**,** carboxylic acid**,** Phlobatanin**,** Terpenoid, Oxalate, and Glycoside is shown in Fig 2.

Table 2. Qualitative analysis of phytochemicals present in *Ziziphus nummularia* leaves extract

PHYTOCHEMICALS	LEAVES
Alkaloids	**Present**
Flavonoids	**Present**
Saponins	**Present**
Steroids	**Absent**
Tannins	**Present**
Fatty acid	**Present**
Phenol	**Present**
Xanthoprotein	**Present**
Quinone	**Absent**
Carboxylic acid	**Present**
Phlobatannin	**Present**
Terpenoid	**Present**
Oxalate	**Present**
Glycoside	**Present**

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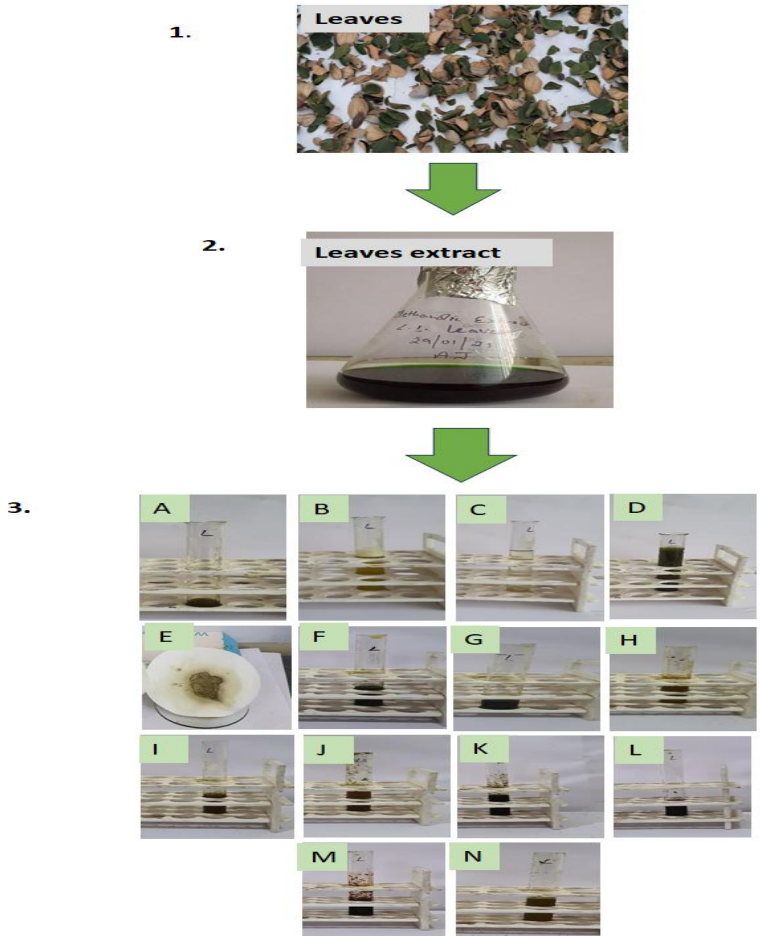

Fig 2. Image 1- Leaves of *Z. nummularia*
Image 2- shows preparation of *Z. nummularia* leaf extract
Image 3- shows preliminary phytochemical screening of the extract.
(A) Flavonoid (**B)** Saponin **(C)** Steroid **(D)** Tannin **(E)** Fatty acid **(F)** Phenol
(G) Xanthoprotein (**H)** Quinone **(I)** carboxylic acid (**J)** Phlobatannins **(K)** Terpenoid
(L) Oxalate **(M)** Glycoside **(N)** Protein.

Quantitative analysis of phytochemicals

Estimation of total Alkaloid content:

The alkaloid content in the *Z. nummularia* leaves extract was determined to be 0.11%. as shown in table 3. These compounds are naturally occurring phytochemicals that contain nitrogen. Therefore, the assessment of alkaloids in *Z. nummularia* is essential for understanding the range of its pharmaceutical activities (Sonia and Singh 2019; Joseph and Thangavel 2023)**.**

Estimation of total flavonoid content

The current study revealed that the total flavonoid content present in *Z. nummularia* leaves extract is 1.79% as shown in table 3.

Table 3. Quantification of phytochemicals

Sample part	Phytochemical	Percentage availability
Leaves	Alkaloid	0.11
Leaves	Flavonoid	1.79
Leaves	Terpenoid	0.13

Estimation of total terpenoid content

Total terpenoid content accumulated in leaf extract of *Z. nummularia* is 31% as shown in table 3, which is very high in comparison of other secondary metabolites present in the plant extract. This shows that terpenoids are dominant secondary metabolite present in *Z.*

nummularia and is mainly responsible for its pharmaceutical properties. (Tholl 2015).

Antioxidant Assay

DPPH Radical Scavenging Activity Assay

To assess the activities of the samples, a comparison was made against a control solution consisting of 0.5 mL of DPPH solution and 4.5 mL of methanol. The activity was determined using the following formula:

DPPH Radical scavenging activity (% RSA) = [(Ac–As)/Ac] × 100

Where Ac is the absorbance value of the control, and As is the absorbance value of the added test sample solution.

A lower IC50 number denotes a higher level of antioxidant activity in the test sample, which is measured by the IC50 value. It is calculated as the amount of antioxidant required to reduce the initial DPPH concentration by 50%. By using the intercept and slope values obtained by linear regression analysis of DPPH reduction vs leaves extract concentration (Fig 3), the IC 50 value of BHT (butylated hydroxytoluene) and Z. nummularia was obtained to be 56.978 µg/ml and 107.2 µg/ml, respectively. The previous investigations have demonstrated similar findings by showing noteworthy antioxidant activity by extracts of different parts of *Ziziphus nummularia* in relation to DPPH (Mesmar et al 2022; Imran et al 2024).

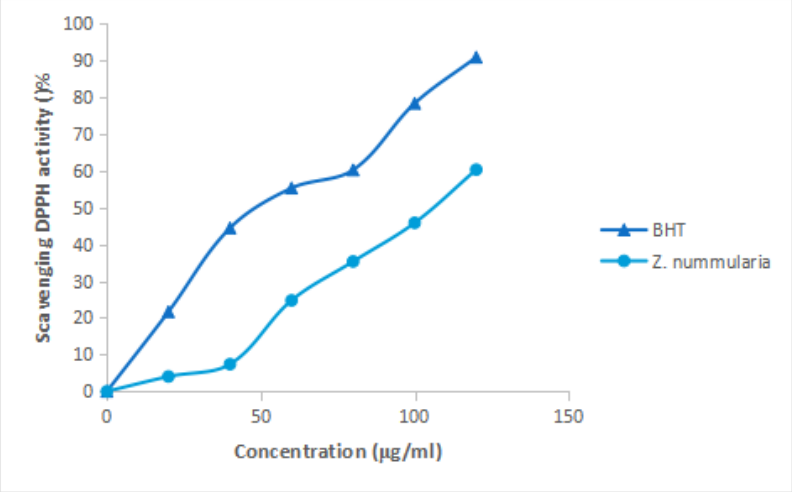

Fig 3. DPPH Activity of BHT (Standard) and *Ziziphus nummularia* leaf extract at different concentrations (%Radical Scavenging Activity vs Concentration in µg/ml)

Phosphomolybdenum (TAC) Assay

The results showed increasing total antioxidant capacity with an increase in concentration (20 -140 µg/ml) of the methanolic leaves extract of *Ziziphus nummularia*.

The highest TAC activity of 14.7 % was observed at 140 µg/ml concentration of extract, and the lowest activity of 13.94 % was found at 20 µg/ml concentration of extract, as shown in Fig 4.

Antibacterial activity

The extract exhibited moderate inhibitory effects against *K. pneumoniae* and *E. coli*, with zones of inhibition ranging from 11.0 to 13.0 mm. Notably, *K. pneumoniae* showed a consistent response across all concentrations, with the highest inhibition (13.0 mm) observed at both 200 and 1000 µg/ml. *E. coli* exhibited a similar pattern, with peak activity (13.0 mm) at 600 µg/ml.

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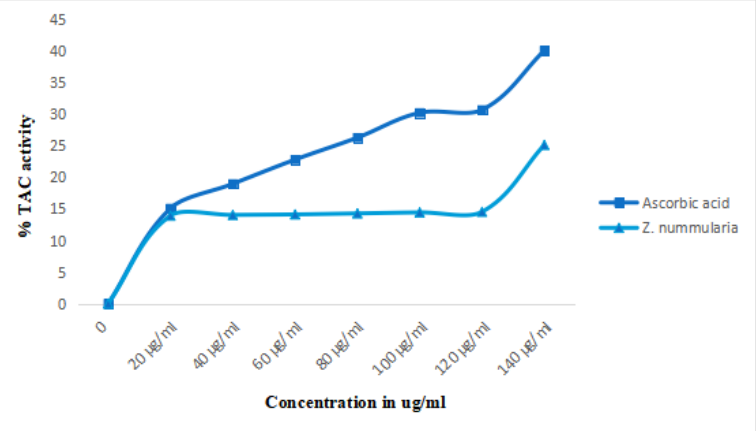


Fig 4: Total antioxidant capacity in % of different concentrations (in µg/ml) of methanolic *Z. nummularia* leaves extracts.

In contrast, *S. pneumoniae* showed no inhibition at lower concentrations, but a measurable zone of 11.0 mm was observed at 1000 µg/ml, indicating limited sensitivity at higher doses. *S. aureus* was completely resistant to the methanol extract at all concentrations tested (Table 4).

Table 4. Antibacterial activity of *Z. nummularia* leaf extract against *K. pneumonia*, *E. coli*, *S. pneumonia*, and *S. aureus*.

Plant	**Concentration µg/ml**	**Bacterial strain zone of inhibition (mm)**			
		K. pneumoniae	***E. coli***	***S. pneumoniae***	***S. aureus***
Methanolic extract of Z. *nummularia*	200	13.0 ± 0.0	11.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
	400	11.0 ± 0.0	12.0 ± 0.5	00.0 ± 0.0	00.0 ± 0.0
	600	11.0 ± 0.5	13.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
	800	12.0 ± 0.5	12.0 ± 0.5	00.0 ± 0.0	00.0 ± 0.0
	1000	13.0 ± 1.0	11.0 ± 0.0	11.0 ± 0.5	00.0 ± 0.0
Control	Methanol	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
	Ampicillin	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
	Streptomycin	23.0 ± 0.0	26.0 ± 0.0	22.0 ± 0.0	28.0 ± 0.0

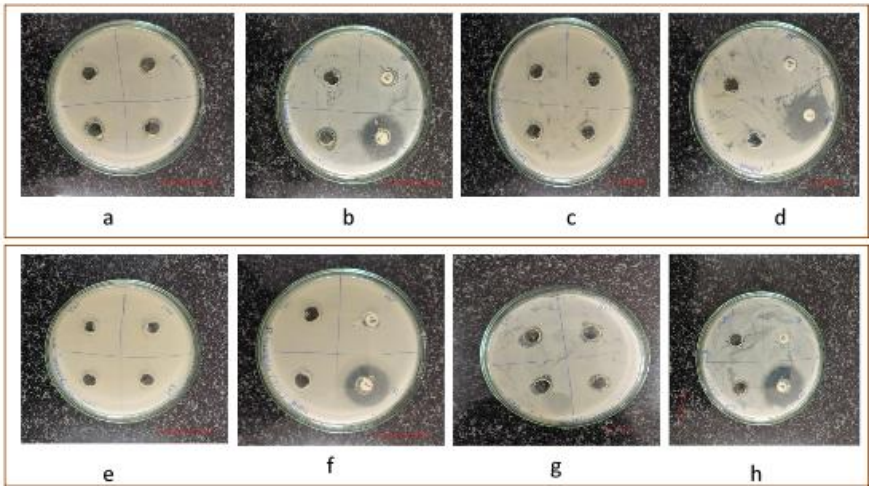


Figure 5. Antibacterial activity of Z *nummularia against K pneumonia, K pneumonia control, S aureus, S aureus, S pneumonia, and S pneumonia* (a- *K pneumonia, b- K pneumonia control, c- S aureus d- S aureus control* e- *S pneumonia, f- S pneumonia control, g- E. coli, h- E coli control).*

Negative controls using methanol and ampicillin showed no inhibition**,** confirming that the observed activity was due to the plant extract. Streptomycin, used as a positive control, exhibited strong antibacterial activity against all strains, with inhibition zones ranging from 22.0 to 28.0 mm, as shown in Fig 5**.** These findings suggest that the methanol extract of *Z. nummularia* possesses moderate antibacterial properties, particularly against Gram-negative bacteria, and could be a potential source of bioactive compounds for antimicrobial development. It was reported that the methanolic extract of Ziziphus showed good antibacterial activity against both Gram-negative and Gram-positive bacteria. Antibacterial activity against *E. coli* suggests that the plant extracts may come good against uropathogens (Semwal et al 2017). Moreover, due to heavy and uncontrolled use of antibiotics many of the uropathogens have started showing resistance against commonly available antibiotics in such scenarios traditional medicinal practice involving plants like *Z. nummularia* may serve potential therapeutic agents (Agrawal et al 2023; Semwal et al 2021). On the back of our observations this particular plant may be explored further with in different formulations and different parts of it as well.

Table 5. IC50 values for antioxidants and antibacterial activities

Activity type	**Target**	**IC_{50} (µg/ml)**
Antioxidant (DPPH)	*Z. nummularia* extract	**107.2**
Antioxidant (DPPH)	BHT	**56.98**

Table 5 shows the IC50 values of the extracts for antioxidant and antibacterial activities. The IC_{50} is widely used to quantify the potency of an extract in radical-scavenging or microbial inhibition assays: it represents the concentration required to achieve 50% inhibition of a defined activity (e.g. free-radical scavenging or bacterial growth) (Srinivasan and Lloyd 2024). In your study, the methanolic extract of *Ziziphus nummularia* showed an IC_{50} of 107.2 µg/ml in the DPPH assay, indicating moderate antioxidant potency compared to the standard Butylated hydroxytoluene (BHT), which had IC_{50} = 56.98 µg/ml. Together, these results show that the extract does possess antioxidant effects (Itam et al 2021).

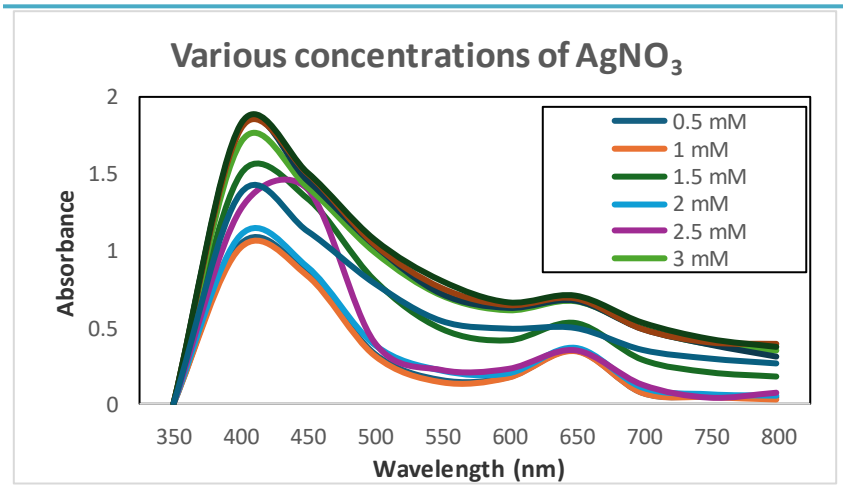
Optimization of silver nanoparticle synthesis using *Z. nummularia's* leaf extract

The change in the color of the solution of silver nitrate and leaves extract from dark green to colloidal brown served as visual confirmation of the formation of silver nanoparticles. This color change of the solution is caused by the excitation of surface plasmon vibrations.

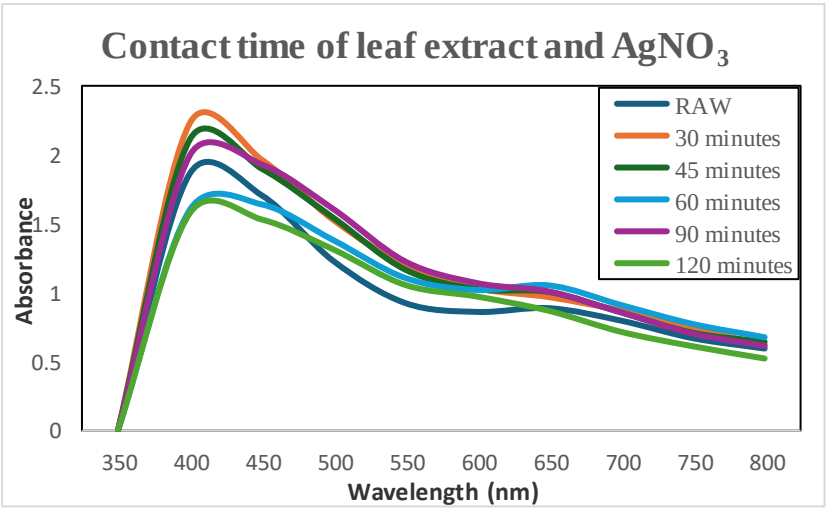
The study revealed that to synthesize AgNPs from the methanolic extract of *Ziziphus nummularia* leaves, 10 mL of leaf extract must be dissolved in 100 mL of 4.5 mM $AgNO_3$ for 30 min (Table 6). The different operational parameters required for the optimum synthesis of green AgNPs are shown in Fig 6 and the concentration and conditions that gave the best surface plasmon resonance (SPR) peaks are shown in (Table 6).

Table 6: Parameters optimized for the synthesis of Silver Nanoparticles using *Ziziphus nummularia* leaf extract

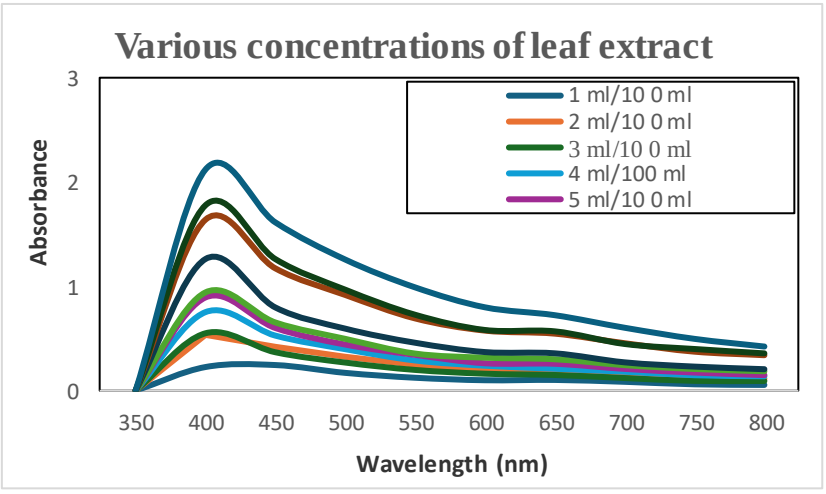
PARAMETERS	**Optimization**
Methanolic leaf extract	10 mL leaf extract per 100 mL solvent
Silver nitrate concentration	4.5 mM
Contact time	30 minutes



(A)



(B)



C)

Fig 6: Optimization of AgNP synthesis using different leaf extract concentrations, (A) $AgNO_3$ concentrations, (B) contact times, (C) of *Ziziphus nummularia* leaf extract.

Characterization of silver nanoparticles synthesized using *Ziziphus nummularia*

UV-Visible Spectroscopy:

UV-Visible spectroscopic measurements were taken to determine the surface plasmon resonance (SPR) peaks of the green-

synthesized silver nanoparticles. The SPR peaks were observed at 405 nm. This result signifies the formation of AgNPs (Figure 7).

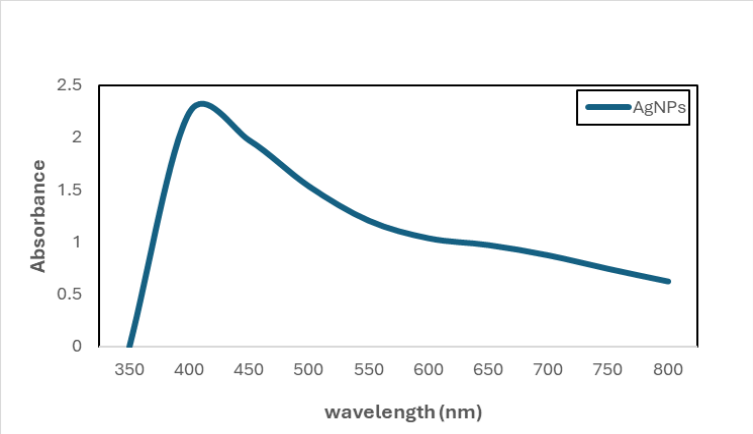


Figure 7: UV- Visible spectroscopy of AgNPs synthesized using *Ziziphus nummularia* leaf extract.

Scanning Electron Microscopy (SEM)

SEM analysis, as clear from Fig 8, revealed the agglomerated morphology of AgNPs synthesized using *Z. nummularia* leaf extract. The nanoparticles are seen to have a predominantly hexagonal shape. For the leaf-synthesized AgNPs, the size of the nanoparticle was found to be around 5.19 nm. The XRD analysis determined distinctive peaks at (2θ) 21.342, 32.252, 37.045, 40.310, 76.107 in correspondence to (24.5), (98.3), (42.5), (100.0), (0.8) intensity, respectively, as depicted in Fig 9, authenticated by reference code 96-150-9195 of the JCPDS database.

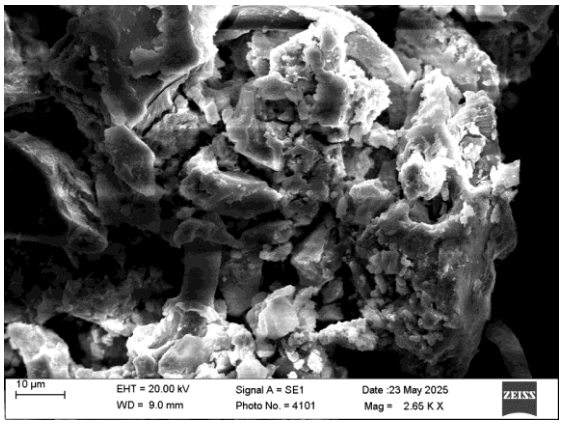


Figure 8. Scanning Electron Microscopy of AgNPs synthesized using *Z. nummularia* leaf extract.

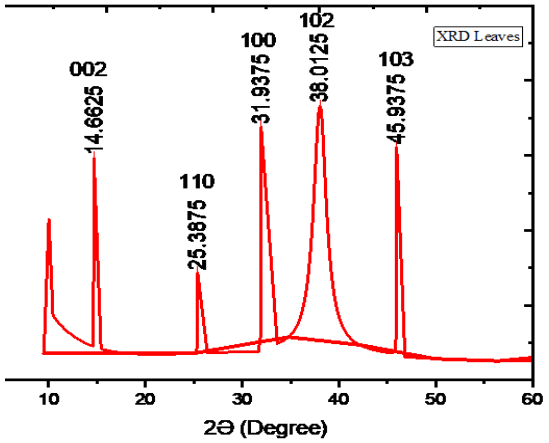


Figure 9.XRD analysis of AgNPs synthesized using *Z. nummularia* leaf extract.

Conclusion

Ziziphus nummularia has been traditionally reported to be effective in treating body aches, nausea, indigestion, dermatitis, and wound healing, indicating its broad medicinal potential. The present study further validates these traditional uses, as the results demonstrated the presence of phytochemicals in *Ziziphus nummularia*, its antioxidant potential, and notable antibacterial activity against several pathogenic strains, as shown by significant zones of inhibition, along with considerable enzymatic antioxidant activity. Furthermore, the conditions for synthesizing silver nanoparticles from *Ziziphus nummularia*

extract were successfully optimized, which opens up a plethora of opportunities for enhancing its biological applications. The present study confirms that *Ziziphus nummularia* possesses promising medicinal properties and may serve as a valuable source for the development of plant-based therapeutic formulations with green nanoparticles synthesized using it.

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References

Abcha I, Ben Haj Said, L, Salmieri S, Criado P, Neffati M & Lacroix M (2021). Optimization of extraction parameters, characterization and assessment of bioactive properties of Ziziphus lotus fruit pulp for nutraceutical potential. *European Food Research and Technology*, 247(9), 2193-2209.

Agrawal S, Rana P S & Saklani P (2023). Exploration of Leaves of Picrorhiza kurroa for their Medicinal Potential by Phytochemical Analysis and Antibacterial Activity. *Environment and Ecology*, 41(4B), 2693-2700.

Ahmed S, Ahmad M, Swami B L & Ikram S (2016). A review on plants extracts mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of advanced research*, 7(1), 17-28.

Auwal, M S, Sanda K A Grema H A &Mairiga I A (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum*, *5*(2), 95-100.

Bagul M V S, Shimpi M R B, Ansari M A & Bodele M S J (2025). Textbook of Pharmacognosy and Phytochemistry-I. Chyren Publication.

Bhandari N, Kumar S, Rawat M, Rana P S, Karki K & Saklani P (2026). Biogenic silver nanoparticles' optimization parameters and their application for wastewater treatment. *Plant Science Today*. In Press

Blois M S (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*(4617), 1199–1200.

Butler M S & Capon R J (2026). Natural product inspired antibiotics approved for human use–1943 to 2025. *Natural Product Reports.*

Evans W C (2009). Trease and Evans' pharmacognosy. Elsevier Health Sciences.

Gul R, Jan S U, Faridullah S, Sherani S & Jahan N (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. *The Scientific World Journal.*, 1–7.

Harborne J B (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis (3rd ed.). Chapman & Hall, London.

Heinrich M, Appendino G, EfferthT, Fürst R, Izzo A A, Kayser O, Pezzuto J M & Viljoen A (2023). Best practice in research: Consensus statement on ethnopharmacological field studies. *Journal of Ethnopharmacology, 302*, 115856.

Imran S, Yamin B I B I., Qayyum A, Sobia N I S A, Shahzad R, Alharbi S A & Ansari M J (2024). Biological and phytochemical analysis of fractionated extracts of Ziziphus nummularia against pathogenic bacteria, oxidative stress and cytotoxicity. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 52(3), 13663-13663.

Imtiaz F, Ahmed D, Mohammed O A, Younas U & Iqbal M (2025). Optimized recovery of phenolic and flavonoid compounds from medicinal plant extracts for enhanced antioxidant activity: A mixture design approach. *Results in Chemistry*, 13, 101960.

Itam A, Wati M S, Agustin V, Sabri N, Jumanah R A & Efdi M (2021). Comparative study of phytochemical., antioxidant, and cytotoxic activities and phenolic content of Syzygium aqueum (Burm. F. Alston f.) extracts growing in West Sumatera Indonesia. T*he Scientific World Journal.*, (1), 5537597.

Jena N, Vimala S B, Patra A, Sharma B P, Hossain E & Kumar S (2025). Methods for ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for pharmacological evaluation of medicinal plants. *Journal of Biodiversity and Conservation*, 9(2), 87-107.

Joseph M K & Thangavel I (2023). GC-MS/HPTLC Analysis, Antioxidant, Anti-inflammatory and Antimicrobial Efficacy of Garcinia cambogia. *Free Radicals & Antioxidants*, 13(1).

Kabir D.& Lawan I (2026). Qualitative Phytochemical Screening Methods of Bioactive Compounds from Medicinal Plants. *J. Mater. Environ. Sci.*, 17 (2), 251, 266.

Kanagaraja A, Sakthivelu M & Raman P (2025). Methods for Isolation and Characterization of Plant Alkaloids. In Natural Products: Phytochemistry, Botany, Metabolism of Alkaloids, Phenolics and Terpenes (pp. 1-29). Berlin, Heidelberg: Springer Berlin Heidelberg.

Kirtikar K R & Basu B D (1999). Indian medicinal plants 2nd ed. Dehradun: International Book Distributors, 1012.

Kumar S, Sharma Y, Khandelwal V, Rawat K & Patil A (2025). Applications of nanotechnology in fertilizers: a review study. *Sustainable Chemistry for the Environment*, *10*, 100247.

Kumar S, Bhandari N, Rawat M & Saklani P (2025). Phytochemical Screening and In vitro antibacterial activity of Petroleum Ether extract of Ageratum conyzoides. *Journal of mountain research*, *20* (1), 449-456.

Kumar S, Bhandari N, Rawat M & Saklani P (2026). Application of Cu nanofertilizer in sustainable agriculture. *Biochem. Cell. Arch*. (26),1 29-39.

Liu S J, Lv Y P, Tang Z S, Zhang Y, Xu H B, Zhang D B, ... & Wei S M (2021). Ziziphus jujuba Mill., a plant used as medicinal food: a review of its phytochemistry, pharmacology, quality control and future research. *Phytochemistry Review*s, 20(3), 507-541.

Magalhães L M, Segundo M A, Reis S & Lima J L (2008). Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica chimica acta*, 613(1), 1-19.

Mesmar J, Abdallah R, Badran A, Maresca M, Shaito A & Baydoun E (2022). Ziziphus nummularia: a comprehensive review of its phytochemical constituents and pharmacological properties. *Molecules*, 27(13), 4240.

Napagoda M & Wijesundara D (2022). Medicinal plants as sources of novel therapeutics: The history, present, and future. Chem. Nat. Prod. Phytochem. *Pharm. Med. Plants*, 3, 4-18.

ÖZCAN İ İ (2025). Medicinal plant phytochemicals: sustainable extraction, optimization and industrial applications. Innovative Studies in Agriculture, *Forestry and Aquaculture* 2025, 53.

Pandey K B & Rizvi S I (2023). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity, 2023*, 1–9.

Peiris S, Fernando D T K, Senadeera S P N N & Ranaweera C B (2023). Phytochemical screening for medicinal plants: guide for extraction method.

Prieto P, Pineda M & Aguilar M.(1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum complex. *Analytical Biochemistry, 269*(2), 337–341.

Rizwan K, Majeed I, Bilal M, Rasheed T, Shakeel A & Iqbal S (2022). Phytochemistry and diverse pharmacology of genus mimosa: a review. *Biomolecules*, 12(1), 83.

Sakna S T, Maghraby Y R, Abdelfattah M S & Farag M A (2023). Phytochemical diversity and pharmacological effects of triterpenes from genus Ziziphus: a comprehensive review. *Phytochemistry Reviews*, 22(6), 1611-1636.

Semwal A C, Saklani P & Mathuria Y P (2017). Urinary tract infection among children and adolescents of Garhwal Region of Uttarakhand, India. *African Journal of Microbiology Research*, 11(36), 1392-1398.

Semwal AC, Rana PS, Saklani P. (2021) Sensitivity and Resistivity Pattern of some commonly used Antibiotics against Urinary Tract Infection (UTI) among elderly individuals from Garhwal Region of Uttarakhand, India. India. J. Mountain Res. 16(3):155-62.

Singh S, Bhandari N, Debbarma B, Patil A, Kumar S & Arya M (2024). Comprehensive study of Alpine lichen species in Uttarakhand: Characterization, phytochemical analysis and ethnobotanical Relevance. *Afr. J. Biological Sci*, *6*(5), 10023-10054.

Sofowora A (1993). Screening plants for bioactive agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, 134-156.

Sonia S & Singh S (2019). Phytoconstituents of Ziziphus nummularia (Burm. f.) Wight & Arn. leaves extracts using GC-MS spectroscopy. *Res. Rev. J. Life Sci*, 9, 109-118.

Srinivasan B & Lloyd M D (2024). Dose–response curves and the determination of IC50 and EC50 values. *Journal of Medicinal Chemistry*, 67(20), 17931-17934.

Tiwari P, Kumar B, Kaur M, Kaur G, & Kaur H (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98-106.

Tholl D (2015). Biosynthesis and biological functions of terpenoids in plants. Biotechnology of isoprenoids, 63-106.

Untea A, Lupu A, Saracila M & Panaite T (2018). Comparison of ABTS, DPPH, phosphomolybdenum assays for estimating antioxidant activity and phenolic compounds in five different plant extracts.

Zhang F & Cheng W (2022). The mechanism of bacterial resistance and potential bacteriostatic strategies. *Antibiotics*, 11(9), 1215.