

**MICROWAVE-ASSISTED GREEN SYNTHESIS OF SILVER NANOPARTICLES USING
ETHANOLIC LEAF EXTRACT OF CORDIA SEBESTENA L.: CHARACTERISATION
AND IN VITRO ANTIBACTERIAL EVALUATION**Sneka C.¹, P. Perumal², K. Amuthavalli^{1*}¹Department of Pharmaceutical Chemistry, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N. Palayam, Gobi (Tk), Erode (Dt) – 638 506, Tamil Nadu, India.²Professor and Principal, JKK Munirajah Institute of Health Sciences College of Pharmacy, T.N. Palayam, Gobi (Tk), Erode (Dt) – 638 506, Tamil Nadu, India.***Corresponding Author: K. Amuthavalli**

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ABSTRACT**Background:** The rise of antimicrobial resistance has renewed interest in silver nanoparticles (AgNPs) as broad-spectrum antibacterial agents, and plant-mediated synthesis offers a route that avoids the toxic reducing agents and energy costs of conventional chemical methods. *Cordia sebestena* L. (Boraginaceae), an ornamental tree used in folk medicine for respiratory complaints, fevers and wounds, is rich in phenolic and flavonoid constituents capable of reducing silver ions, but its use as a bioreductant under microwave irradiation has received little attention.**Methods:** Dried leaves were defatted with petroleum ether and macerated in ethanol for 48 h. The extract was subjected to qualitative phytochemical screening and used to reduce aqueous silver nitrate, with incubation for 24 h in the dark followed by microwave irradiation at 240 W for 15 min. The resulting AgNPs were characterised by UV–visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), and their colloidal stability was monitored for 60 days. Antibacterial activity of the extract and the AgNPs was evaluated by disc diffusion at 250, 500 and 1000 µg/mL against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*, with amoxicillin as the reference standard. **Results:** The extract contained alkaloids, terpenoids, flavonoids, coumarins, steroids, phenolics and tannins, and saponins. AgNP formation was indicated by a yellow-to-brown colour change and a surface plasmon resonance band near 430 nm. FTIR spectra showed bands attributable to C–H, C=C, aromatic, C–N and C–O vibrations, consistent with phytochemical capping. SEM showed polydisperse, spherical, non-agglomerated particles with a mean size of 13 ± 0.63 nm. The colloid showed no sedimentation or change in absorbance over 60 days. Zones of inhibition increased with concentration for both preparations, and at every concentration the AgNPs produced larger zones than the crude extract. At 1000 µg/mL the AgNPs gave zones of 24, 22, 21 and 20 mm against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae* respectively, comparable to the standard. **Conclusion:** Ethanolic leaf extract of *C. sebestena* is an effective reducing and capping agent for the rapid, microwave-assisted synthesis of small, stable, spherical AgNPs with concentration-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria, and greater activity than the extract alone.**KEYWORDS:** *Cordia sebestena*; Silver nanoparticles; Green synthesis; Microwave-assisted synthesis; Antibacterial activity; Surface plasmon resonance.**1. INTRODUCTION**

Nanotechnology concerns the design and use of materials with at least one dimension in the range of 1 to 100 nm, a scale at which physical, chemical and biological

behaviour departs markedly from that of the corresponding bulk material.^[1] The very high surface-area-to-volume ratio of nanoparticles, together with quantum confinement effects, gives rise to altered

optical, catalytic and electronic properties that have been exploited across medicine, agriculture, electronics and environmental remediation.^[2,3] In medicine specifically, nanoparticles are now used in diagnostics, imaging, targeted drug delivery and antimicrobial therapy.^[4]

Among metallic nanoparticles, silver nanoparticles (AgNPs) have received the most sustained attention, owing principally to their broad-spectrum antimicrobial activity and their distinctive optical behaviour.^[5,6] Silver has been used as an antimicrobial agent for centuries, and at the nanoscale its activity is substantially enhanced because the large surface area favours the release of silver ions and increases contact with microbial cells.^[7,8] AgNPs have consequently been incorporated into wound dressings, medical device coatings, textiles, food packaging and water purification systems.^[9] Their antibacterial action is thought to be multimodal, involving adhesion to and disruption of the bacterial cell membrane, generation of reactive oxygen species, and interaction of released silver ions with thiol groups of proteins and with phosphorus-containing molecules including DNA.^[10,11,12] Because this mechanism acts on several targets at once, the development of resistance is considered less likely than with conventional single-target antibiotics, which is of particular importance given the global increase in multidrug-resistant pathogens.^[13,14]

AgNPs can be produced by physical, chemical and biological methods.^[15] Physical methods such as evaporation–condensation and laser ablation require substantial energy input and specialised equipment, while chemical reduction methods typically depend on reducing agents such as sodium borohydride and hydrazine and on synthetic stabilisers that are toxic and leave hazardous residues on the particle surface.^[15,16] These residues are a particular concern where the particles are intended for biomedical use. Biological, or green, synthesis addresses these limitations by using microorganisms, algae or plant extracts as the source of both reducing and capping agents.^[17,18] Plant-mediated synthesis is generally preferred over microbial routes because it avoids the need to maintain cell cultures, can be scaled readily, and is typically faster.^[19,20] Plant extracts contain secondary metabolites, notably polyphenols, flavonoids, terpenoids and alkaloids, that reduce Ag^+ to Ag^0 and simultaneously adsorb to the nascent particle surface, stabilising it against aggregation.^[21,22] Since the early demonstrations of plant-mediated synthesis using *Azadirachta indica* and *Aloe vera*,^[23,24] a very wide range of species has been investigated.^[25,26]

The rate of plant-mediated reduction at room temperature can be slow, often requiring hours to days for completion, and slow nucleation tends to produce broad size distributions. Microwave irradiation has emerged as a useful means of accelerating the process.^[27] Microwave heating is volumetric and rapid, producing uniform nucleation throughout the reaction mixture, which in turn

favours the formation of smaller particles with narrower size distributions in markedly shorter reaction times.^[28,29] Microwave-assisted green synthesis of AgNPs has been reported using plant peel and leaf extracts as well as biomolecules such as glutathione, with good control over particle size and morphology,^[30,31,32] and life-cycle assessment has indicated favourable environmental performance relative to conventional heating.^[33]

Cordia sebestena L. (Boraginaceae), commonly known as the geiger tree or scarlet cordia, is an evergreen tree native to the Caribbean and northern South America that is now widely cultivated throughout the tropics, including India, as an ornamental.^[34] In traditional medicine, preparations of the leaves are used for bronchitis, coughs, fevers and influenza, the sap is applied to wounds, and poultices of the leaves are used for headaches and sprains.^[35,36] Phytochemical studies have reported the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, tannins and sterols in various parts of the plant,^[35,37] together with a range of volatile constituents in the leaf, flower and fruit,^[38] and sebestenoids with enzyme-inhibitory activity have been isolated from the plant.^[39] The leaf extracts have been shown to possess appreciable phenolic content and antioxidant activity,^[40] and antibacterial activity has been reported for the crude plant.^[41] This phytochemical profile, and in particular the abundance of polyphenols, makes the leaf a plausible source of reducing and capping agents for AgNP synthesis, and synthesis of AgNPs using *C. sebestena* has been reported previously.^[42] However, the use of the ethanolic leaf extract under microwave-assisted conditions, together with an assessment of long-term colloidal stability and a direct comparison of the antibacterial activity of the AgNPs with that of the extract from which they were prepared, has not, to our knowledge, been described.

The present study therefore aimed to synthesise AgNPs by a microwave-assisted green route using ethanolic leaf extract of *C. sebestena*, to characterise the particles by UV–visible spectroscopy, FTIR and SEM, to evaluate their stability over 60 days, and to compare the *in vitro* antibacterial activity of the AgNPs with that of the crude extract against representative Gram-positive and Gram-negative bacteria.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Silver nitrate (AgNO_3) and all other chemicals and solvents were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nutrient agar and nutrient broth were used for microbiological work. Double-distilled water was used throughout, and Whatman No. 1 filter paper was used for all filtration steps.

2.2. Plant profile

C. sebestena is a slow-growing evergreen tree reaching 7.5 to 9 m in height with a spread of 6 to 7.5 m, a

symmetrical rounded to vase-shaped crown and coarse-textured foliage. It bears conspicuous orange-red tubular flowers and tolerates drought, salt spray and a broad range of well-drained soils, but not frost, which accounts for its wide planting as a street and coastal ornamental

throughout the tropics. It is known in Tamil as *accinaruvili*, in Hindi as *lal lasora*, and in English variously as geiger tree, scarlet cordia and sebesten plum. The taxonomic classification is given in Table 1.

Table 1: Taxonomic classification of *Cordia sebestena* L.

Rank	Taxon
Kingdom	Plantae
Subkingdom	Viridiplantae
Division	Tracheophyta
Class	Magnoliopsida
Superorder	Asteranae
Order	Boraginales
Family	Boraginaceae
Genus	<i>Cordia</i> L.
Species	<i>Cordia sebestena</i> L.

2.3. Plant material and extraction

Fresh leaves of *C. sebestena* were collected, washed thoroughly with running tap water followed by distilled water, shade-dried and ground to a fine powder. [Insert collection site, date, name of the botanist who authenticated the plant, and herbarium voucher specimen number.] The powdered leaf (50 g) was first defatted with petroleum ether by maceration to remove lipids, waxes and chlorophyll. The defatted marc was then

macerated with ethanol at room temperature for 48 h. The extract was filtered through sterile muslin cloth, concentrated, and stored for phytochemical screening and nanoparticle synthesis. Defatting before extraction was adopted to limit interference from non-polar constituents and to enrich the extract in the polar polyphenols and flavonoids principally responsible for silver ion reduction (Fig. 1).^[17,21]

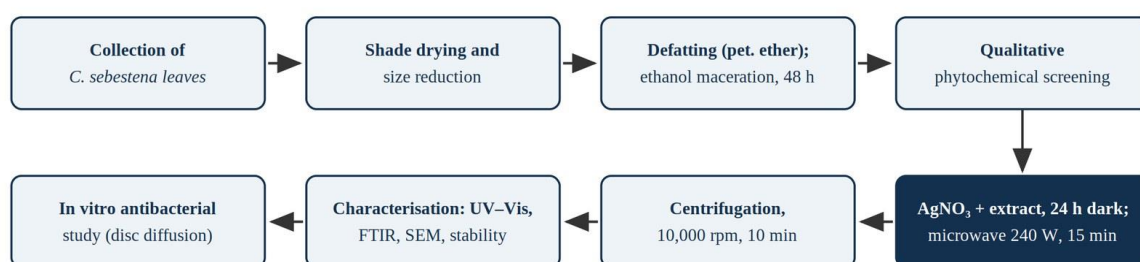


Fig. 1: Schematic of the experimental workflow for the microwave-assisted green synthesis, characterisation and antibacterial evaluation of *C. sebestena* AgNPs.

2.4. Qualitative phytochemical screening

The ethanolic extract was screened for major classes of secondary metabolites using standard qualitative procedures.^[43,44] Alkaloids were detected by treatment of the extract in chloroform with three to four drops of Wagner's reagent, a reddish-brown colouration indicating a positive result. Tannins were detected by boiling the extract (5 g) in distilled water (10 mL), filtering, and treating the filtrate with 0.1% iron(III) chloride, a blue-green precipitate indicating their presence. Terpenoids were detected by the Salkowski test, adding chloroform (2 mL) and concentrated sulphuric acid (3 mL) to the extract (5 mL), a reddish-brown interface indicating a positive result. Saponins were detected by the froth test on warming the extract (5 g) in distilled water (10 mL). Steroids were detected by adding chloroform (2 mL) and sulphuric acid to the extract, a bluish-green interface indicating their presence.

Flavonoids were detected by the Shinoda test, using magnesium turnings and concentrated hydrochloric acid, a yellow colouration indicating a positive result. Phlobatannins were tested by adding 1% hydrochloric acid, a reddish-brown precipitate indicating their presence. Anthraquinones were tested by Bornträger's method, using benzene extraction followed by addition of ammonia, a greenish-yellow colouration indicating a positive result. Coumarins were detected by adding 10% sodium hydroxide (3 mL) to the extract (2 mL), a yellow colouration indicating their presence.^[45]

2.5. Microwave-assisted green synthesis of AgNPs

The ethanolic leaf extract (200 mL) was mixed with aqueous silver nitrate solution (1000 µg/mL, 300 µL). The mixture was incubated in the dark at room temperature for 24 h to prevent photo-induced reduction of silver, and was then irradiated in a domestic

microwave oven at 240 W for 15 min. The mixture was filtered through Whatman No. 1 filter paper and the development of colour was monitored visually. The resulting colloid was centrifuged at 10,000 rpm for 10 min, the supernatant was discarded, and the pellet was collected. Residual water was removed by washing the pellet with petroleum ether, and the dried AgNPs were retained for characterisation and antibacterial evaluation.^[27,42]

2.6. Characterisation of the AgNPs

UV–visible spectroscopy. Reduction of silver ions was monitored using a Shimadzu double-beam UV–visible spectrophotometer over the range 200 to 800 nm, using distilled water as the blank. Aliquots of the reaction mixture were withdrawn at 30 min, 210 min and 24 h to follow the progress of AgNP formation through the development of the surface plasmon resonance band.^[46,47]

Fourier transform infrared spectroscopy. To identify the biomolecules responsible for reduction and capping, the AgNP sample was dried on a glass plate at 150 °C, collected as a powder, mixed with potassium bromide and analysed by FTIR spectroscopy. Absorption bands were assigned to functional groups by reference to standard correlation tables.^[47]

Scanning electron microscopy. The surface morphology, shape and size of the AgNPs were examined by field emission scanning electron microscopy at magnifications of 50,000× and 180,000×. The particle size distribution was determined from the micrographs and presented as a histogram, from which the mean particle size was calculated.

2.7. Stability study

A nanosuspension was prepared by dispersing the AgNPs in de-ionised water. The suspension was stored and its UV–visible spectrum was recorded at 15-day intervals from day 0 to day 60. Samples were also

inspected visually for sedimentation, aggregation or change in colour at each interval.^[48]

2.8. In vitro antibacterial activity

Test organisms. Antibacterial activity was evaluated against two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and two Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*. [Insert the source of each strain, including MTCC or ATCC accession numbers.] Amoxicillin was used as the reference standard and distilled water as the solvent.

Disc diffusion method. Nutrient agar medium was prepared by dissolving the dehydrated medium in purified water with heating, dispensed into 100 mL conical flasks, plugged with cotton and sterilised by autoclaving at 121 °C (15 lb) for 45 min. Sterile agar medium (25 mL) was poured into flat-bottomed sterile Petri dishes placed on a level surface to ensure uniform depth. Each bacterial strain was grown in nutrient broth on a rotary shaker at 37 °C for 24 h. After serial dilution, 100 µL of each overnight culture was spread uniformly over the surface of freshly prepared agar using a sterile glass spreader. Sterile Whatman filter paper discs (6 mm diameter) impregnated with the *C. sebestena* leaf extract or the AgNPs at 250, 500 and 1000 µg/mL were placed on the inoculated plates using sterile forceps, alongside the amoxicillin standard. The plates were incubated at 37 °C for 24 h and the diameter of the zone of inhibition was measured in millimetres.^[49,50]

3. RESULTS

3.1. Phytochemical screening

Qualitative screening of the ethanolic leaf extract indicated the presence of alkaloids, terpenoids, flavonoids, coumarins, steroids, phenolics and tannins, and saponins. Anthraquinones and phlobatannins were absent (Table 2; Fig. 2). This profile agrees with previous phytochemical investigations of *C. sebestena*.^[35,37]

Table 2: Qualitative phytochemical screening of the ethanolic leaf extract of *C. sebestena*.

S. No.	Phytoconstituent	Ethanolic extract
1	Alkaloids	+
2	Terpenoids	+
3	Flavonoids	+
4	Coumarins	+
5	Steroids	+
6	Anthraquinones	–
7	Phlobatannins	–
8	Phenolics and tannins	+
9	Saponins	+

(+) present; (–) absent.



Fig. 2: Qualitative phytochemical screening of the ethanolic leaf extract of *C. sebestena*.

3.2. Synthesis of AgNPs

On addition of silver nitrate to the leaf extract and subsequent incubation and microwave irradiation, the reaction mixture changed in colour from pale yellow to brown (Fig. 3A). This colour change is the first visual

indication of AgNP formation and arises from excitation of the surface plasmon resonance of the colloidal silver.^[5,46] Centrifugation yielded a dark pellet of AgNPs (Fig. 3B).



(A)



(B)

Fig. 3: (A) Reaction mixture of *C. sebestena* leaf extract and silver nitrate after synthesis. (B) AgNP pellet obtained after centrifugation.

3.3. UV-visible spectroscopy

The UV-visible spectra recorded at 30 min, 210 min and 24 h are shown in Fig. 4. A characteristic surface plasmon resonance band developed with a maximum near 430 nm, which lies within the range of 400 to 450

nm typically reported for spherical AgNPs.^[46,48] The position of the band confirmed the reduction of Ag^+ to metallic silver, and the progressive development of the band over the sampling period reflected the continued formation of nanoparticles.

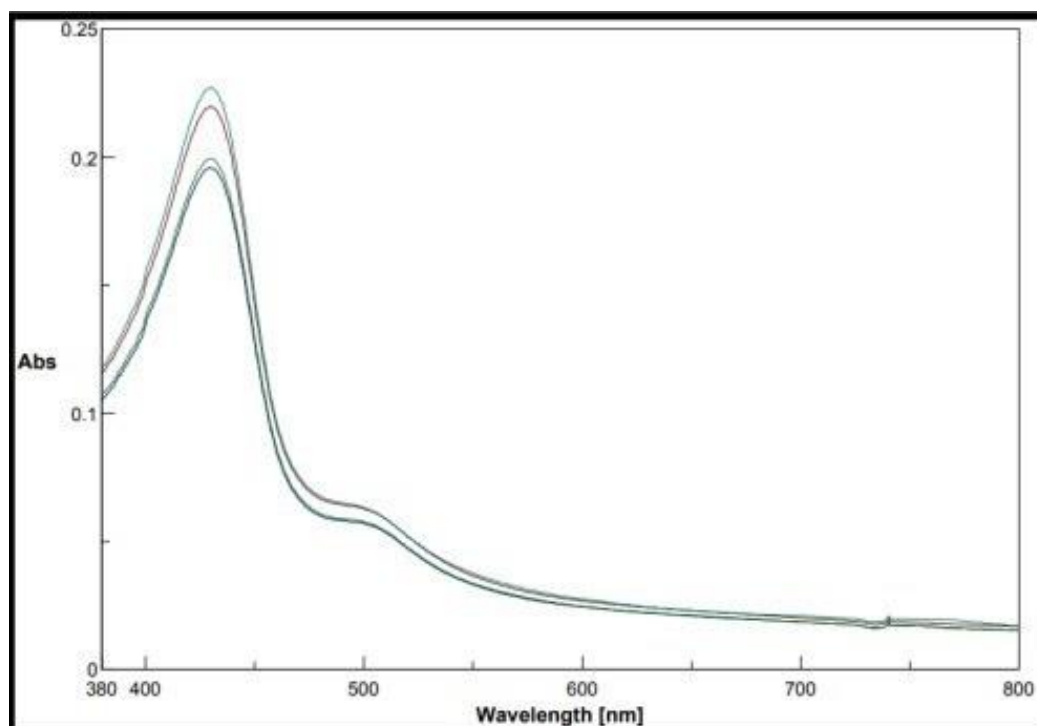


Fig. 4: UV-visible spectra of the green-synthesised AgNPs at different time intervals, showing the surface plasmon resonance band near 430 nm.

3.4. FTIR spectroscopy

The FTIR spectrum of the AgNPs is shown in Fig. 5, and the principal band assignments are summarised in Table 3. Bands were observed at approximately 2900 cm^{-1} (C–H stretching of aliphatic and aldehyde groups), 1600

cm^{-1} (C=C stretching of alkenes), 1400 cm^{-1} (aromatic ring vibrations), 1300 cm^{-1} (C–N stretching of amines) and 1025 cm^{-1} (C–O stretching of alcohols, ethers, esters and carboxylic acids).

Table 3: FTIR band assignments for the green-synthesised AgNPs.

Wavenumber (cm^{-1})	Assignment
2900	C–H stretching (aliphatic, aldehyde)
1600	C=C stretching (alkene)
1400	Aromatic ring vibration
1300	C–N stretching (amine)
1025	C–O stretching (alcohol, ether, ester, carboxylic acid)

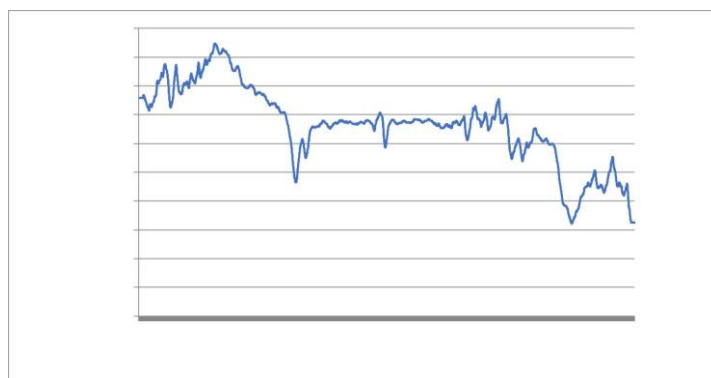


Fig. 5: FTIR spectrum of the AgNPs synthesised using *C. sebestena* leaf extract.

3.5. Scanning electron microscopy

SEM showed that the AgNPs were predominantly spherical and polydisperse, with phytoconstituents associated with the particle surfaces (Fig. 6A). The

particles were well separated and showed no evident agglomeration. The size distribution histogram (Fig. 6B) gave a mean particle size of 13 ± 0.63 nm.

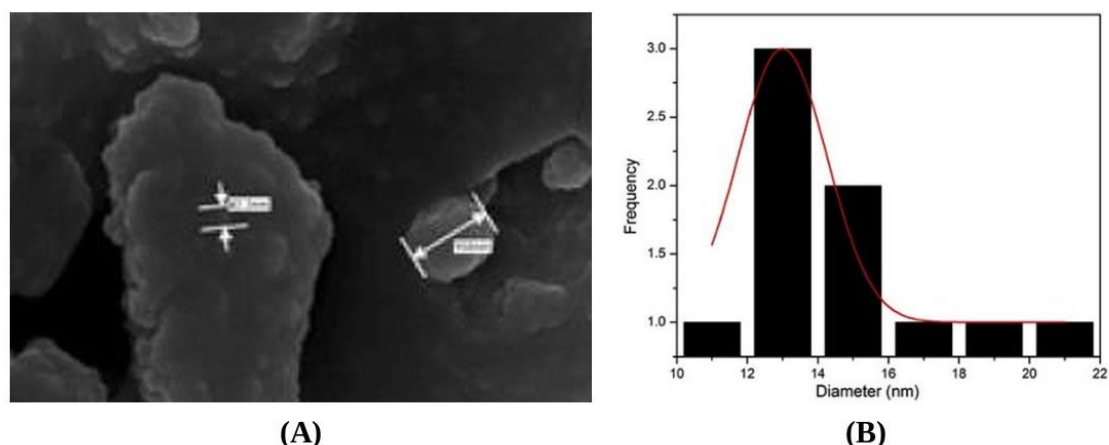


Fig. 6: (A) SEM micrograph of the green-synthesised AgNPs. (B) Particle size distribution histogram.

3.6. Stability study

The AgNP nanosuspension showed no sedimentation and no visible change in colour over the 60-day observation period (Fig. 7), and no change in absorbance was

observed across the stability intervals. The green-synthesised AgNPs were therefore considered stable under the storage conditions employed.



Fig. 7: Appearance of the AgNP nanosuspension at day 0, 15, 30, 45 and 60 of the stability study.

3.7. *In vitro* antibacterial activity

The zones of inhibition produced by the crude extract and by the AgNPs are presented in Tables 4 and 5 and Figs. 8 and 9. Both preparations inhibited all four test organisms, and in both cases the diameter of the zone of inhibition increased with concentration from 250 to 1000 $\mu\text{g/mL}$.

The crude extract produced zones of 12 to 23 mm against *S. aureus*, 14 to 21 mm against *B. subtilis*, 14 to 18 mm against *E. coli* and 13 to 16 mm against *K. pneumoniae*

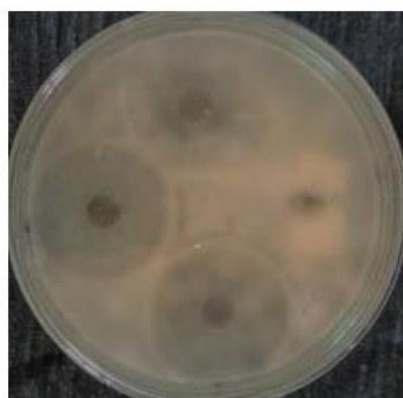
across the concentration range (Table 4). The AgNPs produced larger zones than the extract at every concentration and against every organism: 17 to 24 mm against *S. aureus*, 16 to 22 mm against *B. subtilis*, 15 to 21 mm against *E. coli* and 16 to 20 mm against *K. pneumoniae* (Table 5). At 1000 $\mu\text{g/mL}$ the AgNPs produced zones approaching those of the amoxicillin standard. Activity of the AgNPs was greatest against the Gram-positive organism *S. aureus*.

Table 4: Zones of inhibition (mm) produced by the ethanolic leaf extract of *C. sebestena*.

Concentration ($\mu\text{g/mL}$)	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
250	12	14	14	13
500	18	19	15	16
1000	23	21	18	16
Amoxicillin (250)	23	20	18	17

Table 5: Zones of inhibition (mm) produced by the AgNPs synthesised using *C. sebestena* leaf extract.

Concentration ($\mu\text{g/mL}$)	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
250	17	16	15	16
500	20	21	18	19
1000	24	22	21	20
Amoxicillin (250)	25	22	21	20

*S. aureus**B. subtilis**E. coli**K. pneumoniae*Fig. 8: Zones of inhibition produced by the ethanolic leaf extract of *C. sebestena* against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae*.

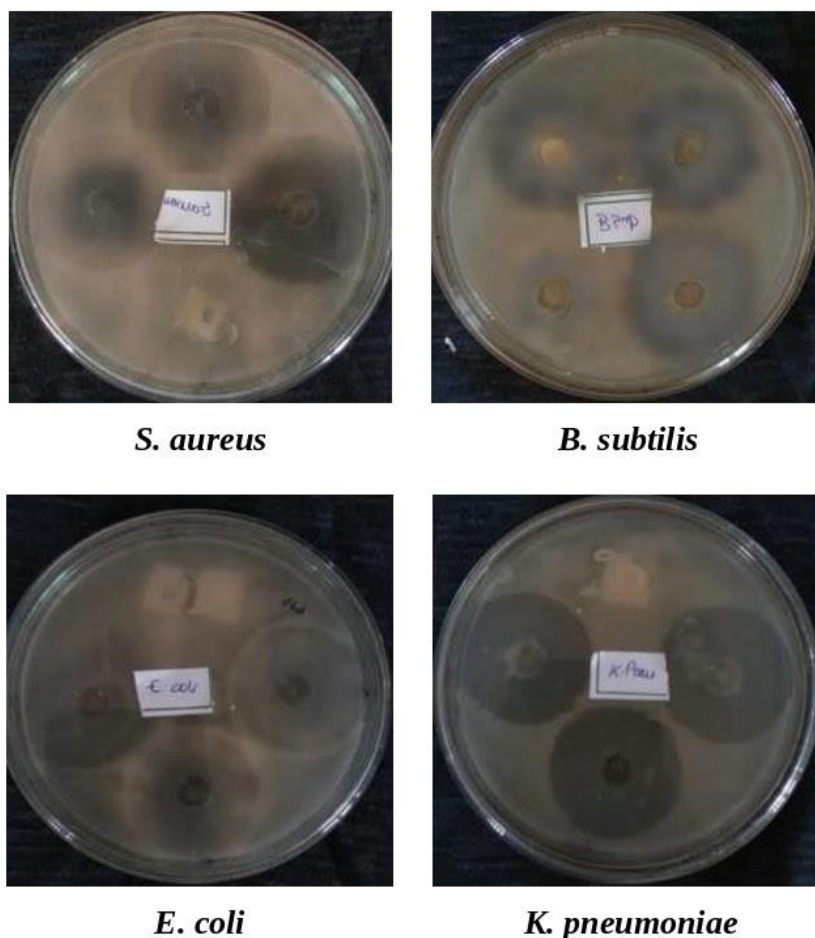


Fig. 9: Zones of inhibition produced by the green-synthesised AgNPs against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae*.

4. DISCUSSION

The results show that ethanolic leaf extract of *C. sebestena* functions effectively as both reducing agent and stabiliser in the microwave-assisted synthesis of AgNPs, yielding small, spherical and stable particles with antibacterial activity exceeding that of the extract alone.

The phytochemical profile of the extract is directly relevant to its function in synthesis. Flavonoids and phenolic compounds are the constituents most consistently implicated in plant-mediated reduction of silver ions.^[21,22] Their hydroxyl groups can donate electrons to Ag^+ , and the resulting oxidised forms, together with other extract constituents, can adsorb to the metal surface and cap the growing particle.^[17,19] Terpenoids, alkaloids and proteins have also been proposed to contribute to reduction and stabilisation.^[18] The presence of all of these classes in the extract (Table 2) is therefore consistent with its observed capacity to reduce silver ions and to stabilise the resulting particles.

The development of a surface plasmon resonance band near 430 nm is the principal spectroscopic evidence of AgNP formation. Surface plasmon resonance arises from the collective oscillation of conduction electrons at the

particle surface in resonance with incident light, and its position depends on particle size, shape and the surrounding dielectric environment.^[5,46] A single band in the region of 400 to 450 nm is characteristic of approximately spherical AgNPs, whereas anisotropic shapes typically give rise to additional or broadened bands at longer wavelengths.^[51] The single band observed here is therefore consistent with the spherical morphology subsequently confirmed by SEM, and comparable absorption maxima have been reported for AgNPs synthesised using other plant extracts.^[48,49,52]

The FTIR spectrum provides evidence of the involvement of extract constituents in capping. The bands attributed to C–O, C–N, C=C and aromatic vibrations correspond to functional groups present in polyphenols, flavonoids, terpenoids and proteins, and their retention in the spectrum of the purified particles indicates that these biomolecules remained associated with the nanoparticle surface after centrifugation and washing.^[53,54] Such capping layers are considered to be responsible for the colloidal stability of green-synthesised AgNPs, preventing aggregation through steric and electrostatic effects.^[54] This interpretation is supported by the SEM observation that the particles were well separated and associated with phytoconstituents,

and by the stability study, in which no sedimentation or change in absorbance occurred over 60 days. Long-term stability of this kind is a practical advantage of green synthesis, since chemically synthesised AgNPs frequently require the addition of synthetic stabilisers.^[15]

The mean particle size of 13 nm is small relative to many reports of plant-mediated AgNP synthesis at ambient temperature, and this is plausibly attributable to the use of microwave irradiation. Microwave heating is volumetric and rapid, so that the reaction mixture reaches the nucleation threshold uniformly and quickly; a burst of simultaneous nucleation favours the formation of many small nuclei, and rapid consumption of silver ions then limits subsequent growth.^[27,28] Reduced reaction times and smaller, more uniform particles relative to conventional heating have been reported repeatedly for microwave-assisted synthesis.^[30,31,32] Particle size is of direct relevance to antibacterial activity, since smaller particles offer a larger surface area for ion release and for contact with bacterial cells, and size-dependent antibacterial activity of AgNPs has been demonstrated.^[13,55]

The antibacterial results show three features. First, activity was concentration-dependent for both the extract and the AgNPs, which is consistent with a dose-related mechanism. Second, the AgNPs were more active than the extract at every concentration and against every organism. The antibacterial activity of the crude extract is presumably attributable to its phenolic and flavonoid constituents, which are known to possess intrinsic antimicrobial activity;^[41] the greater activity of the AgNPs indicates that the silver itself makes a substantial contribution beyond that of the capping phytochemicals. Third, the AgNPs inhibited both Gram-positive and Gram-negative organisms, confirming the broad-spectrum activity characteristic of silver.^[11,56]

The antibacterial mechanism of AgNPs is generally considered to be multifactorial.^[12,14] AgNPs can adhere to the bacterial cell wall and membrane, altering permeability and disrupting the respiratory chain; they release Ag⁺ ions that bind to thiol groups of membrane and cytoplasmic proteins and inactivate enzymes; and they promote the generation of reactive oxygen species that cause oxidative damage to lipids, proteins and nucleic acids.^[10,12] Silver ions may also interact with phosphorus- and sulphur-containing biomolecules, including DNA, interfering with replication.^[13] Differences in susceptibility between Gram-positive and Gram-negative bacteria are commonly attributed to differences in cell wall architecture, the thick peptidoglycan layer of Gram-positive organisms and the lipopolysaccharide-rich outer membrane of Gram-negative organisms presenting different barriers to particle and ion penetration.^[11,14] The direction of this difference varies between studies and depends on particle size, surface chemistry and test conditions.^[56]

Two features of the synthesis protocol deserve comment. The 24 h incubation in the dark before irradiation was adopted to prevent photochemical reduction of silver nitrate, which would otherwise proceed in parallel with the phytochemical reduction and produce particles of uncontrolled size.^[15,42] The subsequent microwave step was performed at a moderate power of 240 W, since excessive microwave power promotes rapid local heating, Ostwald ripening and aggregation, whereas moderate power sustains uniform nucleation while limiting growth.^[27,28] The combination of a slow, low-temperature pre-incubation that allows complexation of silver ions with extract constituents, followed by a short burst of uniform heating that drives reduction to completion, is a plausible explanation for the small mean size and narrow size spread observed here. Systematic variation of irradiation power, irradiation time, extract-to-precursor ratio and pH would be needed to establish optimal conditions, and has been used successfully to tune particle size in other microwave-assisted plant systems.^[30,32]

The present findings are consistent with a previous report of AgNP synthesis using *C. sebestena*^[42] and with the substantial body of work on plant-mediated AgNPs with antibacterial activity.^[49,50,57] The use of the ethanolic extract after defatting, the microwave-assisted procedure, the 60-day stability assessment and the direct comparison of extract with nanoparticle activity extend that earlier work.

Several limitations should be noted. The disc diffusion method provides a qualitative to semi-quantitative measure of activity, and determination of minimum inhibitory and minimum bactericidal concentrations by broth microdilution would allow more rigorous comparison with the standard and with other reports. Characterisation could be extended by X-ray diffraction to confirm the crystalline face-centred cubic structure of the silver, by energy-dispersive X-ray analysis to confirm elemental composition, and by dynamic light scattering and zeta potential measurement to characterise hydrodynamic size and surface charge in suspension. The cytotoxicity of the particles towards mammalian cells was not assessed, and this is an essential step before any biomedical application, given the documented potential for AgNP toxicity.^[58,59]

5. CONCLUSION

Silver nanoparticles were successfully synthesised by a rapid, simple and eco-friendly microwave-assisted route using the ethanolic leaf extract of *C. sebestena* as both reducing and capping agent. The particles showed a surface plasmon resonance band near 430 nm, were spherical, polydisperse and non-agglomerated with a mean size of 13 ± 0.63 nm, were capped by extract-derived biomolecules, and remained stable for at least 60 days. The AgNPs exhibited concentration-dependent antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae* that exceeded the activity of the

crude extract and approached that of amoxicillin at the highest concentration tested. *C. sebestena* leaf extract is therefore a promising bioresource for the green synthesis of antibacterial AgNPs. Further work should establish minimum inhibitory concentrations, complete the structural characterisation, and evaluate cytotoxicity before therapeutic application is considered.

CRediT authorship contribution statement

Sneka C: Investigation, Methodology, Formal analysis, Data curation, Writing – original draft. **P. Perumal:** Resources, Supervision, Writing – review and editing. **K. Amuthavalli:** Conceptualisation, Methodology, Supervision, Validation, Writing – review and editing. [Confirm these contributions with all authors.]

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on reasonable request.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used a large language model in order to restructure the dissertation into journal article format and to edit the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication. [Amend to reflect actual use, in line with the target journal's policy.]

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