

Facile one-pot green synthesis, characterisation, and antibacterial activities of silver nanoparticles using *Camellia chrysantha* leaf extract

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Abstract:

Green synthesis is a promising strategy for addressing pathogenic microorganisms. This study investigated the green synthesis of silver nanoparticles using *Camellia chrysantha* leaf extract (CC-AgNPs) and evaluated their antibacterial efficacy in wound treatment. CC-AgNPs were synthesised with ultrasonic assistance at 37 kHz, employing 4 mM silver nitrate (AgNO₃), an extract-to-silver salt ratio of 1:10, a pH of 8, and a temperature of 60°C for 60 min. Characterisation revealed a spherical morphology with an average transmission electron microscopy diameter of 18.53±5.66 nm, hydrodynamic size of 46 nm, polydispersity index of 0.457, and zeta potential of -25.7 mV. X-ray diffraction and selected area electron diffraction analyses confirmed a face-centred cubic crystalline structure, while energy-dispersive X-ray spectroscopy and Fourier-transform infrared spectroscopy analyses indicated that plant-derived biomolecules facilitated the reduction and stabilisation of nanoparticles. The CC-AgNPs demonstrated strong antibacterial activity, achieving complete inhibition of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 at a 256-fold dilution within 24 h. These findings underscore the significant potential of *C. chrysantha* leaf extract-mediated green synthesis of AgNPs as novel antibacterial agents. The unique phytochemical

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composition of *C. chrysantha* leaves, combined with an ultrasonic-assisted green chemistry approach, supports its potential application in wound healing.

Keywords: antibacterial activity, *Camellia chrysantha*, green synthesis, silver nanoparticles.

Classification numbers: 2.2, 3.2, 3.3

1. Introduction

Nanomaterials, defined as materials with dimensions ranging from 1 to 100 nm, manifest in various forms, including particles, fibres, tubes, and thin films, and are integral to advancements in information technology, materials science, and biotechnology. Metallic nanoparticles are particularly noteworthy due to their distinctive physicochemical properties, such as adjustable morphology, size, and crystallinity. Among them, silver nanoparticles (AgNPs) are extensively utilised in the fields of pharmaceuticals, cosmetics, and antimicrobial coatings due to their potent antibacterial properties, including their efficacy against multidrug-resistant microorganisms. Given that the size, shape, and uniformity of these particles significantly affect their biomedical performance, it is imperative to develop synthesis methods that can precisely control the characteristics of nanoparticles. Conventional chemical and physical synthesis methods frequently involve the use of toxic reagents, high energy consumption, or complex processing techniques, thereby necessitating a transition towards more environmentally friendly and sustainable approaches. Green synthesis techniques utilise biological agents such as microorganisms, plant extracts, or natural polymers, wherein phytochemicals function as reducing and stabilising agents. Plant-mediated synthesis is capable of producing silver nanoparticles with a range of sizes and bioactivities. Numerous studies have underscored their potent antimicrobial and biofilm-inhibitory properties, as well as their broader biomedical potential [1-3].

Camellia chrysantha (Hu) Tuyama (commonly known in Vietnamese as Trà hoa vàng or Kim hoa trà), also referred to as the golden camellia or golden tea, is a shrub or small tree bearing distinctive yellow flowers and classified within the family Theaceae [4]. This species is predominantly distributed in Asia, particularly in China and Vietnam. In

Vietnam, it has been documented in the mountainous district of Ba Che in Quang Ninh Province [5]. The plant is regarded as a highly valuable medicinal resource, containing over 400 bioactive constituents, including polyphenol saponins, trace elements [6]. The potential of *C. chrysanthra* for the green synthesis of silver nanoparticles is attributed to its abundance of natural bioactive compounds. In plant-mediated biosynthesis, these compounds can function as reducing agents, donating electrons to convert silver ions from a silver salt precursor into elemental silver, thereby initiating the nucleation and growth of nanoparticles. Additionally, they can bind to the surface of newly formed AgNPs, preventing aggregation and ensuring their stability in solution [7].

Although several studies have reported the green synthesis of AgNPs from the *Camellia* family [8-10], further investigation is necessary to elucidate species-specific variations and optimise synthesis conditions. This study aimed to synthesise CC-AgNPs and to evaluate their antibacterial activity against several common wound-infecting bacterial strains. The novel aspects of this study include the investigation and selection of extraction conditions based on achieving the highest total reducing capacity, as well as the ultrasonic-assisted synthesis parameters for producing AgNPs with enhanced antibacterial efficacy. The unique phytochemical composition of *C. chrysanthra* leaves, combined with a green chemistry approach, collectively advance the potential application of AgNPs in wound treatment. The overall scheme of the ultrasonic-assisted green synthesis, physicochemical characterisation, and antibacterial evaluation of CC-AgNPs is illustrated in Fig. 1.

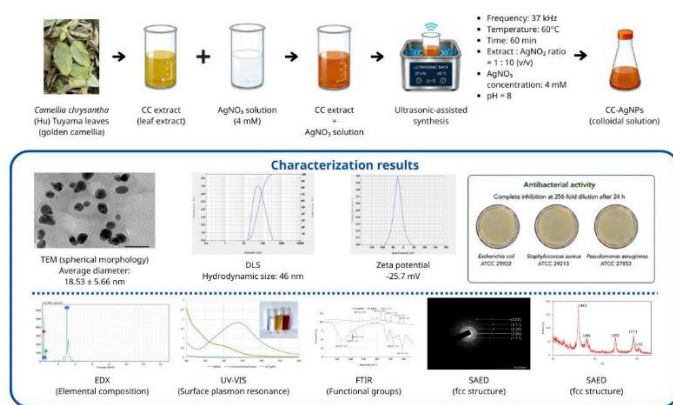


Fig. 1. Schematic diagram of green synthesis and characterisation of CC-AgNPs.

2. Materials and methods

2.1. Chemicals and materials

C. chrysantha leaves were obtained from Quang Ninh Province, Vietnam. The plant material was taxonomically identified as *Camellia chrysantha* (Hu) Tuyama at the Herbarium of the Department of Botany and Plant Science, Institute of Ecology and Biological Resources, VAST (voucher specimen No. BTTH-QY 001). Silver nitrate (AgNO_3 , >99.8%) was purchased from Guanhao High-Tech Co., Ltd. (China). All chemicals and reagents were of analytical grade and used without further purification. Three bacterial strains, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213, were obtained from the Military Hospital 103, Hanoi, Vietnam. All working solutions were prepared using double-distilled water.

2.2. Preparation of *C. chrysantha* leaf extract

Fresh green leaves of *C. chrysantha* were collected and authenticated in the laboratory. After removing diseased, damaged, or contaminated leaves, the remaining leaves were air-dried for 12 h to reduce moisture content and then oven-dried at 60°C for an additional 24 h. The dried material was ground into a fine powder, placed in sealed plastic zip bags, and stored for further experimentation.

Dried *C. chrysantha* leaf powder was extracted with distilled water at a ratio of 1:20 (w/v) in an Erlenmeyer flask using ultrasonic assistance (Elmasonic, USA). Extraction times were set at 0.5, 1, 1.5, 2, 2.5, 3 h and extraction temperatures were 25, 40, 60, 80°C, respectively. Aqueous extracts of *C. chrysantha* were obtained by filtration through Whatman No. 1 filter paper. The extracts were then stored in a refrigerator for subsequent experiments.

2.3. Phytochemical screening

Phytochemical screening was performed on the plant extracts to determine the presence of phenols, tannins, saponins, triterpenes, flavonoids, alkaloids, and steroids [11].

2.4. Determination of total phenolic and flavonoid content

*C. chrysanth*a leaf extract was analysed for its total phenolic and flavonoid content using the Folin-Ciocalteu method and the aluminium chloride colourimetric assay, respectively.

To determine the total phenolic content, a gallic acid standard solution (1000 µg/ml) was prepared in methanol/water (50:50 v/v). Briefly, 0.5 ml of the sample, blank, or standard was added to separate test tubes. Subsequently, 2.5 ml of Folin-Ciocalteu reagent (10%, v/v) was added and vortexed. After 5 min of incubation, 2.5 ml of Na₂CO₃ (7.5%) was added, followed by vortexing and incubation in the dark at room temperature for 2 h. The absorbance of the samples was measured at 765 nm using a UV-Vis spectrophotometer (V730, Jasco, Japan). A standard calibration curve of gallic acid was constructed and used to determine the total phenolic content, expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g) [12-14].

For flavonoid content, a quercetin standard solution (1000 µg/ml) was prepared in 100% methanol. Then, 0.5 ml of the sample, blank, or standard was added to each test tube. This was followed by the addition of 1.5 ml of methanol, 0.1 ml of aluminium chloride (10%), 0.1 ml of sodium acetate (1 M), and 2.8 ml of distilled water. The mixtures were vortexed and incubated in the dark for 30 min before measuring the absorbance at 415 nm using the same UV-Vis spectrophotometric method described above. A quercetin calibration curve was generated and used to determine the total flavonoid content, expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g) [15-17].

2.5. Synthesis of CC-AgNPs

Predetermined volumes of *C. chrysanth*a extract were prepared in separate Erlenmeyer flasks. A silver nitrate solution (1-10 mM) was gradually added to the flasks. The reaction was carried out with the assistance of ultrasound at a frequency of 37 kHz (Ultrasonic bath Elma S30H, Germany) under dark conditions at room temperature, 40, 60, and 80°C for predetermined time intervals. The progress of the reaction was monitored by observing the colour change from colourless or pale yellow to brown.

After completion of the reaction, the silver nanoparticles were washed and collected by repeated centrifugation of the reaction mixture with distilled water at 13,000 rpm for 20 min (Hettich Mikro 220R, Germany). The nanoparticle suspension was then centrifuged again at 10,000 rpm for 10 min. The supernatant was discarded, and the nanoparticles were re-dispersed in distilled water. This washing process was repeated three times to remove impurities and any unreacted biomaterials. The purified CC-AgNPs were subsequently dried in an oven at 60°C until a constant weight was reached to obtain a dry powder, which was then utilised for further physicochemical characterisation (FTIR, XRD, EDX). The as-synthesised CC-AgNPs colloidal solution was used for antibacterial assays against *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853.

2.6. Silver nanoparticle characterisation

2.6.1. UV-Vis absorption characteristics of silver nanoparticles

UV-Vis spectroscopy was used as a preliminary technique to confirm the formation of CC-AgNPs. A UV-Vis spectrophotometer (Jasco V730, Japan) was employed to record the extinction spectra of the AgNPs in the wavelength range of 300-600 nm at a resolution of 1 nm, using a quartz cuvette with a 1 cm path length.

2.6.2. Particle size distribution by dynamic light scattering

To determine the particle size and zeta potential, the CC-AgNP suspension was subjected to dynamic light scattering (DLS) analysis. A Zetasizer (Horiba SZ100, Japan) was used to measure both the particle size distribution and zeta potential of the CC-AgNPs.

2.6.3. Transmission electron microscopy (TEM)

The morphology and size of the CC-AgNPs were examined using a JEM-2100 transmission electron microscope (JEOL, Japan) operating at an accelerating voltage of 100 kV. Size distribution was analysed using ImageJ software by measuring the diameters of nanoparticles in the TEM images.

2.6.4. Fourier-transform infrared spectroscopy

The major functional groups present in the CC-AgNPs were identified using FTIR spectroscopy. FTIR spectra were recorded with a Cary 630 FTIR spectrophotometer (Agilent, USA) in the range of 4000-400 cm⁻¹.

2.6.5. X-ray diffraction analysis

X-ray diffraction (XRD) analysis was performed to determine the crystalline structure of AgNPs. XRD patterns were recorded in the 2θ range of $10-90^\circ$ using a powder diffractometer (X'Pert Pro, PANalytical) equipped with Cu-K α radiation ($\lambda=1.54 \text{ \AA}$) at a step size of 0.05° per second.

2.6.6. Energy-dispersive X-ray spectroscopy

Elemental composition was analysed using energy-dispersive X-ray spectroscopy (EDX, TM400 Plus, Hitachi).

2.7. Antibacterial activities

All procedures were performed under aseptic conditions, using a Class II biosafety cabinet. *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were cultured on Mueller-Hinton (MH) agar. Bacterial suspensions were prepared in sterile physiological saline and adjusted to 10^8 CFU/ml using a turbidity meter, then diluted to obtain a working inoculum of 10^6 CFU/ml, following established broth dilution guidelines [18-20].

Serial tenfold dilutions (10^8 - 10^2 CFU/ml) were prepared by transferring 100 μ l of the bacterial suspension to tubes containing 0.9 ml sterile saline. These dilutions were streaked onto MH agar plates (seven streaks per plate) to verify viable counts, in accordance with the standard MIC determination procedures [18, 19].

The test compound was prepared by twofold serial dilution in sterile distilled water to obtain concentrations ranging from 1/2 to 1/128 (1 ml per tube). Each tube was then mixed with 1 ml of the CFU/ml bacterial suspension, yielding final concentrations ranging from 1/4 to 1/256. The tubes were vortexed and incubated at 37°C .

At 2, 6, and 24 h, aliquots from each drug-bacteria mixture were streaked onto MH agar plates (seven streaks corresponding to the seven drug concentrations). Plates were incubated at 37°C for 24 h, after which bacterial growth was recorded following CLSI recommendations [19].

3. Results and discussion

3.1. Phytochemical compounds in the plant extract

Table 1. Phytochemical screening results of *C. chrysanth* leaf extract.

Phytochemicals	Presence/absence
Alkaloids	–
Phytosterols	+
Saponins	+++
Flavonoids	+++
Coumarins	–
Tannins	+++
Anthraquinones	–
Amino acids	+
Reducing sugars	+
Cardiac glycosides	–

(+) present; (-) absent, (+++) present with strong signal.

Table 1 presents a summary of the phytochemical constituents identified in the leaf extract of *C. chrysanth*. The analysis revealed the presence of flavonoids, tannins, saponins, amino acids, phytosterols, and reducing sugars, while alkaloids, cardiac glycosides, coumarins, and anthraquinones were not detected. Notably, flavonoids, tannins, and saponins exhibited the most pronounced positive reactions. Previous studies [21, 22] have indicated that these phytochemical groups are instrumental in the reduction of silver ions and the facilitation of AgNPs formation.

3.2. Total phenolic and flavonoid contents

The phenolic and flavonoid contents of *C. chrysanth* leaf extract were evaluated to ascertain the optimal conditions for extraction temperature and duration. Extracts with higher concentrations of phenolic and flavonoid compounds are typically considered to possess superior overall reducing capacity [22, 23]. The spectrophotometric assays were validated for sensitivity, showing an LOD of 5.42 µg/ml and LOQ of 16.40 µg/ml for polyphenol, and 3.15 and 9.54 µg/ml for flavonoid, respectively.

As shown in Fig. 2A, both the total phenolic and flavonoid contents increased as the extraction temperature increased from 25 to 60°C. The highest values were obtained at

60°C, reaching 54.19 ± 0.77 and 34.67 ± 3.20 mg QE/g, respectively. At temperatures above 60°C, a slight decrease in phenolic and flavonoid content was observed, likely due to the thermal degradation of phytochemicals in aqueous conditions. As shown in Fig. 2B, the phenolic content gradually increased and reached its maximum (56.93 ± 1.35 mg GAE/g) when the extraction time increased from 0.5 to 1.5 h, followed by a slight decline at longer extraction times. Similarly, the flavonoid content peaked at 40.65 ± 1.56 mg QE/g at 1.5 h and remained relatively stable between 2 and 3 h. Based on these results, the optimal extraction temperature and time were determined to be 60°C and 1.5 h, respectively. These optimised conditions ensured maximum recovery of the reducing agents, which is crucial for the subsequent efficient synthesis of AgNPs with controlled characteristics.

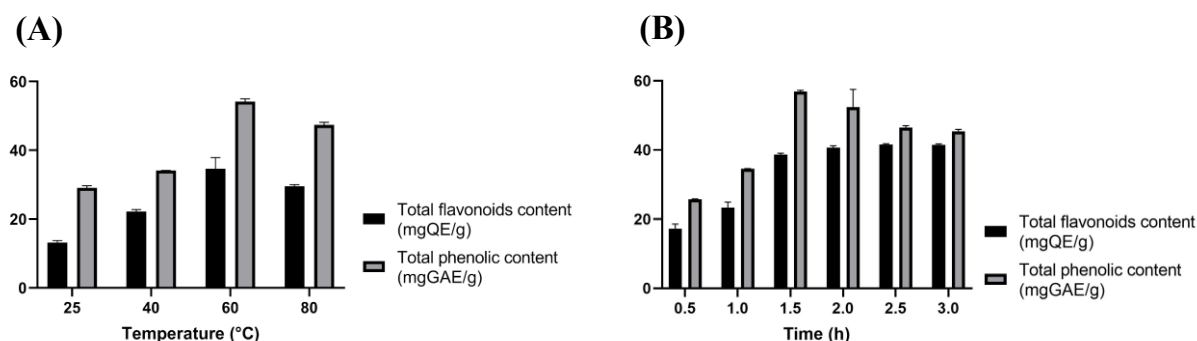


Fig. 2. Total phenolic (mg GAE/g) and total flavonoid (mg QE/g) content of the *C. chrysanthea* leaf extract (A) at a constant time of 1.5 h and (B) at a constant temperature of 60°C.

According to previous studies, the hydroxyl (-OH) groups of flavonoids play a key role in the reduction of Ag^+ ions to AgNPs, likely through a tautomeric shift from the enolic to the keto form. This transformation may release reactive hydrogen atoms that facilitate the reduction. In general, antioxidant compounds are recognised as efficient metal ion reducers, and phenolic substances, owing to their strong antioxidant capacity, can act as effective reducing agents in the green synthesis of nanoparticles [24]. Density functional theory (DFT) indicated that the epigallocatechin gallate (EGCG) in *C. chrysanthea* (Hu) Tuyama is mainly responsible for stabilising AgNPs [25].

3.3. Characterisation of CC-AgNPs

The factors influencing the synthesis of AgNPs were investigated, and the reaction conditions were optimised to obtain a nanoparticle solution with the highest absorbance at the maximum wavelength and the smallest particle size. CC-AgNPs were synthesised using ultrasonic assistance at a frequency of 37 kHz, with 4 mM AgNO₃, an extract-to-silver salt ratio of 1:10, pH 8, at 60°C for 60 minutes. The physicochemical properties of CC-AgNPs were characterised as follows.

3.3.1. UV-Vis spectroscopic study

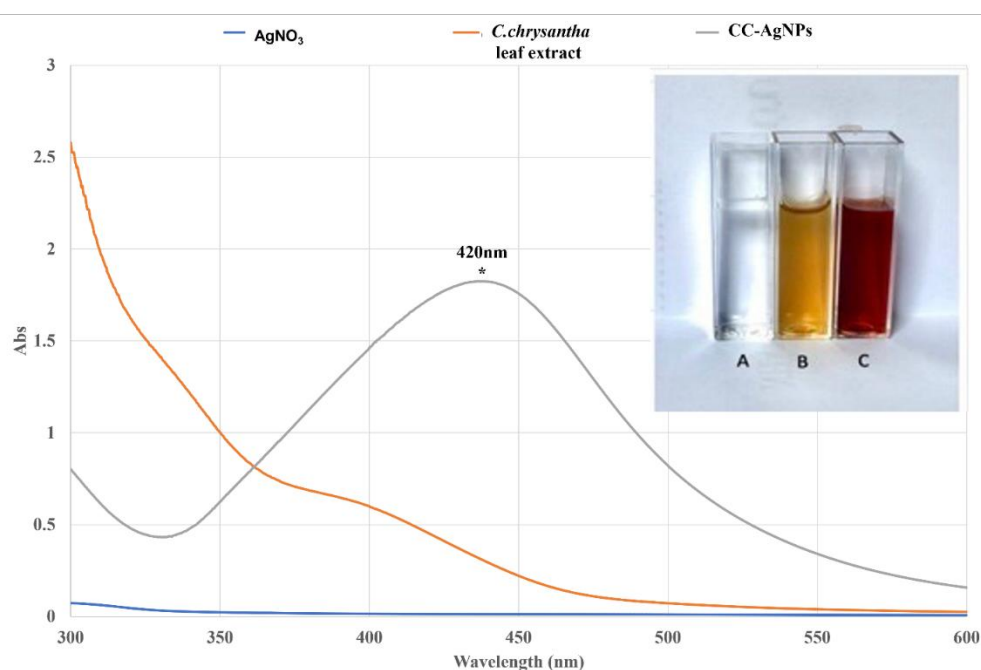


Fig. 3. UV-Vis absorption spectra of (A) silver nitrate, (B) *C. chrysanthra* leaf extract and (C) CC-AgNPs.

Ultraviolet-visible (UV-Vis) spectroscopy was utilised to examine the formation of AgNPs. The observed colour change in the reaction mixture provided an initial indication of the reduction of silver nitrate to CC-AgNPs (Fig. 3). This colour transition is attributed to the development of nanoparticles, which is due to the oscillation of conduction electrons on the nanoparticle surface in resonance with the incident electromagnetic radiation. Metallic nanoparticles typically exhibit a characteristic surface plasmon resonance (SPR) absorption band within the range of 370-470 nm [26].

The appearance of a distinct SPR band in the UV-Vis spectrum further corroborated the successful formation of the CC-AgNPs. The SPR phenomenon is highly sensitive to various physicochemical parameters, including particle size, morphology, surface charge, and the surrounding dielectric environment. As the nucleation and growth of CC-AgNPs advanced, the collective oscillation of surface electrons became more pronounced, resulting in a sharper and more intense SPR peak, as widely reported in plant-mediated AgNP synthesis [27]. Several other investigations have reported the formation of AgNPs using different plant aqueous extracts, such as flower [10], peel [28], and branch [29] extracts.

3.3.2. Particle size distribution

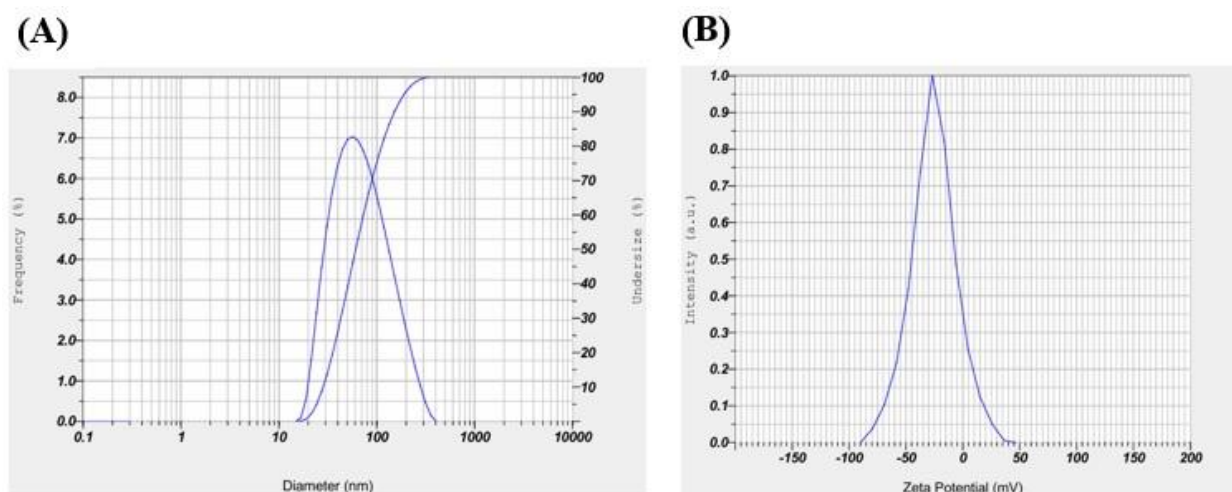


Fig. 4. (A) Particle size distribution and (B) zeta potential distribution of CC-AgNPs.

The particle size distribution of CC-AgNPs was assessed using DLS, with the results depicted in Fig. 4A. The CC-AgNPs demonstrated a polydisperse distribution, characterised by an average hydrodynamic diameter of 46 nm and a polydispersity index (PDI) of 0.457. A PDI value exceeding 0.4 typically signifies moderate polydispersity, a common occurrence in green-synthesised nanoparticles due to the heterogeneous nature of plant extracts and the presence of multiple reducing and stabilising agents. Zeta potential measurements were conducted to further assess the colloidal stability of AgNPs. As illustrated in Fig. 4B, the synthesised nanoparticles exhibited an average zeta potential of -25.7 mV.

Zeta potential values ranging from -20 to -30 mV are generally indicative of moderate stability, where electrostatic repulsion is adequate to prevent rapid aggregation of

nanoparticles. Previous studies have shown that nanoparticles with high positive or negative surface charges tend to repel each other, resulting in stable suspensions with low aggregation tendencies [30]. In plant-based synthesis, stability is often enhanced by a combination of electrostatic and steric effects, known as electrosteric stabilisation, provided by the adsorbed phytochemicals [31].

3.3.3. Morphological study via transmission electron microscopy (TEM)

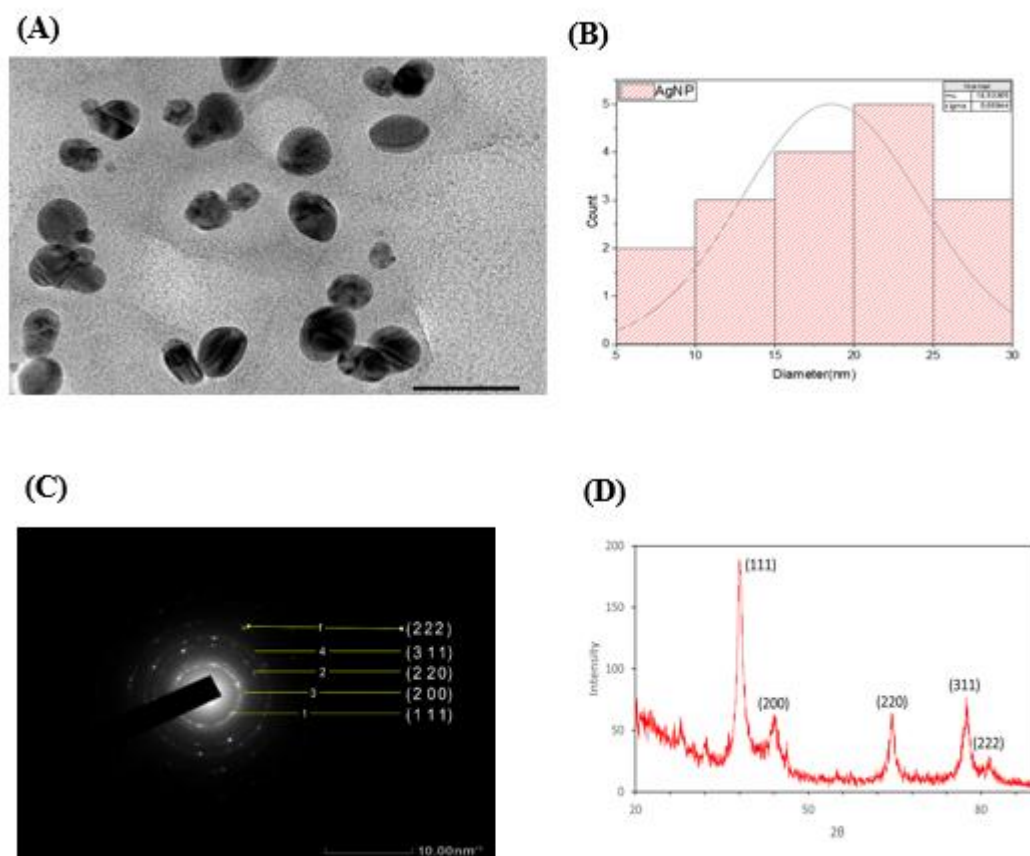


Fig. 5. (A) Transmission electron microscopy (TEM) image, (B) particle size distribution, (C) SAED image, and (D) X-ray diffraction analysis of CC-AgNPs.

Transmission electron microscopy (TEM) was employed to examine the morphology and size of the synthesised silver nanoparticles (CC-AgNPs), with the findings depicted in Fig. 5A. The TEM image reveals that the CC-AgNPs exhibit a variety of shapes and sizes, with smaller particles predominantly spherical. Larger particles appear to result from the aggregation of smaller nanoparticles, leading to less well-defined individual shapes.

Overall, the synthesised CC-AgNPs demonstrated a quasi-spherical morphology, a characteristic commonly observed in green-synthesised nanoparticles. A dark organic layer surrounding the nanoparticle surfaces was also observed, indicating the presence of biomolecules from the *C. chrysanth*a extract. These organic constituents likely function as capping agents, stabilising the nanoparticles and preventing excessive aggregation, which aligns with previous reports on plant-mediated AgNP synthesis [32].

The particle size distribution derived from the TEM images is shown in Fig. 5B. The histogram indicates a predominant frequency peak between 14 and 16 nm, with the majority of particles ranging from 10 to 30 nm. The mean particle size was determined to be 18.53 ± 5.66 nm. This size is smaller than the hydrodynamic diameter measured by DLS, which is anticipated since DLS considers the solvation layer and the adsorbed phytochemicals surrounding the nanoparticles. Such discrepancies between TEM and DLS measurements are well-documented in the literature and are characteristic of nanoparticles synthesised using plant extracts [33]. The crystalline nature of the CC-AgNPs was confirmed through selected-area electron diffraction (SAED) analysis. The SAED pattern exhibited distinct diffraction rings corresponding to the (111), (200), (220), (311), and (222) planes of the face-centred cubic (fcc) structure of elemental silver. The pronounced (111) reflection suggests that this is the most thermodynamically stable and dominant facet, consistent with previous studies on biosynthesised AgNPs [27]. The sharp and well-defined diffraction rings indicate that the nanoparticles are highly crystalline, a property that contributes to their optical, catalytic, and antimicrobial activities.

3.3.4. Fourier-transform infrared spectroscopy

Figure 6 presents the FTIR spectra of CC-AgNPs and the leaf extract from *Camellia chrysanth*a. In the AgNP spectrum, the peak at 2657 cm^{-1} corresponds to O-H stretching vibrations, while the peaks at 2325 and 2361 cm^{-1} are attributed to O=C=O stretching. Peaks at 1712 and 1867 cm^{-1} represent C-H and C=O stretching vibrations, respectively, and peaks at 2092 and 2120 cm^{-1} indicate C≡C stretching. Additional peaks at 1436 , 1168 , and 967 cm^{-1} correspond to the stretching modes of C-H, C-O, and C-C, respectively. These signals corroborate previous findings in plant-mediated AgNP synthesis, confirming

the presence of organic molecules adsorbed on the nanoparticle surface and indicating that components of the plant extract act as stabilising agents [33].

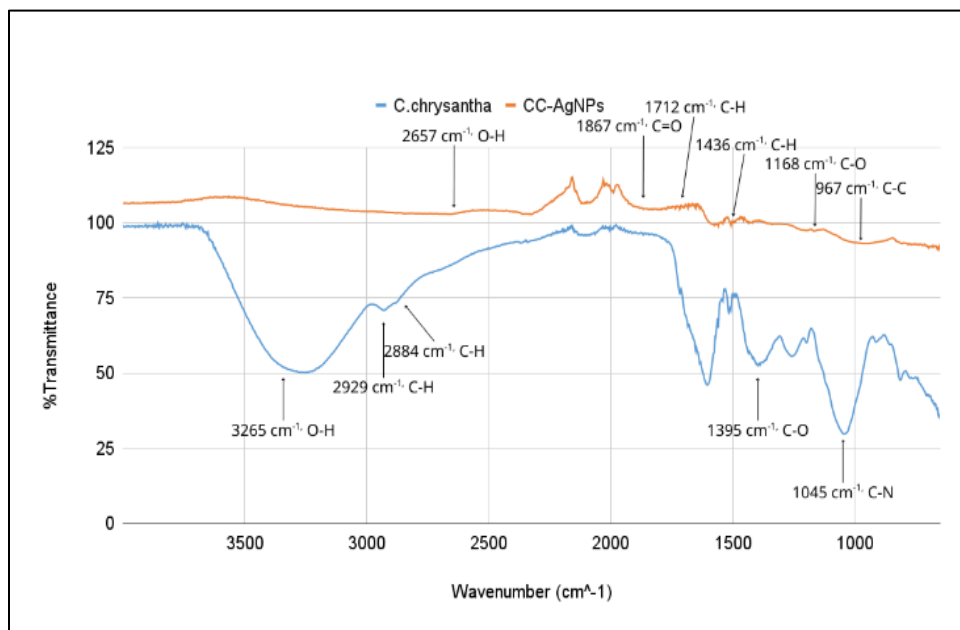


Fig. 6. Fourier transform infrared spectra of CC-AgNPs and *C. chrysantha* leaf extract.

The Fourier transform infrared (FTIR) spectrum of the *C. chrysantha* leaf extract exhibits a broad peak at 3265 cm⁻¹, indicative of O-H stretching, characteristic of phenolic and alcoholic groups. Peaks at 2929 and 2884 cm⁻¹ represent C-H stretching in the -CH₃ and -CH₂ groups, respectively. The peak at 1604 cm⁻¹ is associated with C=O, C-O, and O-H stretching vibrations, while peaks at 1395 and 1045 cm⁻¹ correspond to C-O stretching in carboxyl groups and C-N stretching in amino acid residues.

A comparative analysis of the two spectra reveals that the hydroxyl, carbonyl, carboxyl, and amine groups present in the extract play a pivotal role in the biosynthesis of AgNPs. These functional groups are known to donate electrons during the reduction of Ag⁺ to Ag⁰ and subsequently bind to the nanoparticle surface, forming a stabilising organic layer. This mechanism aligns with previous studies demonstrating that polyphenols, flavonoids, and other secondary metabolites in plant extracts function simultaneously as

reducing and capping agents. The retention of these functional groups on the AgNP surface suggests effective stabilisation, preventing aggregation and enhancing colloidal stability [27].

3.3.5. X-ray diffraction analysis

The X-ray diffraction (XRD) pattern of the synthesised silver nanoparticles (AgNPs) is depicted in Fig. 5D. The diffraction peaks observed at 2θ values of 38.2, 44.3, 64.5, 77.4, and 81.5° correspond to the (111), (200), (220), (311), and (222) crystallographic planes of elemental silver, respectively, as indexed according to JCPDS card No. 04-0783. These reflections confirm that the AgNPs exhibit a face-centred cubic (fcc) crystal structure. Among these peaks, the (111) reflection is the most intense, indicating that this plane is the predominant orientation during nanoparticle growth. Such preferential orientation is commonly reported for green-synthesised AgNPs and is attributed to the thermodynamic stability of the (111) facet [32].

The sharpness and high intensity of the diffraction peaks demonstrate that the nanoparticles are well crystallised and possess nanoscale dimensions. This observation is consistent with the SAED results obtained from the TEM analysis, which also revealed distinct diffraction rings corresponding to the same fcc planes. The absence of additional impurity peaks in the XRD pattern suggests that the synthesised nanoparticles consist solely of metallic silver without detectable silver oxide or other crystalline byproducts. This purity reflects the effectiveness of the *C. chrysanth* extract as a reducing and stabilising agent, enabling the formation of high-quality crystalline AgNPs.

3.3.6. Energy-dispersive X-ray spectroscopy

The energy-dispersive X-ray (EDX) spectrum of the synthesised silver nanoparticles (CC-AgNPs) is shown in Fig. 7. The spectrum exhibits a prominent and well-defined signal corresponding to elemental silver, thereby confirming the presence of metallic Ag as the predominant component of the sample. In addition to the characteristic Ag peak, the EDX profile also displayed signals for carbon (C) and oxygen (O). Quantitative analysis indicated that the sample comprised 74.96, 12.17, and 12.88 wt% of Ag, O, and C, respectively. The detection of C and O is frequently reported in green-synthesised

nanoparticles and is attributed to the presence of phytochemicals and other organic molecules originating from the plant extract used during synthesis [32].

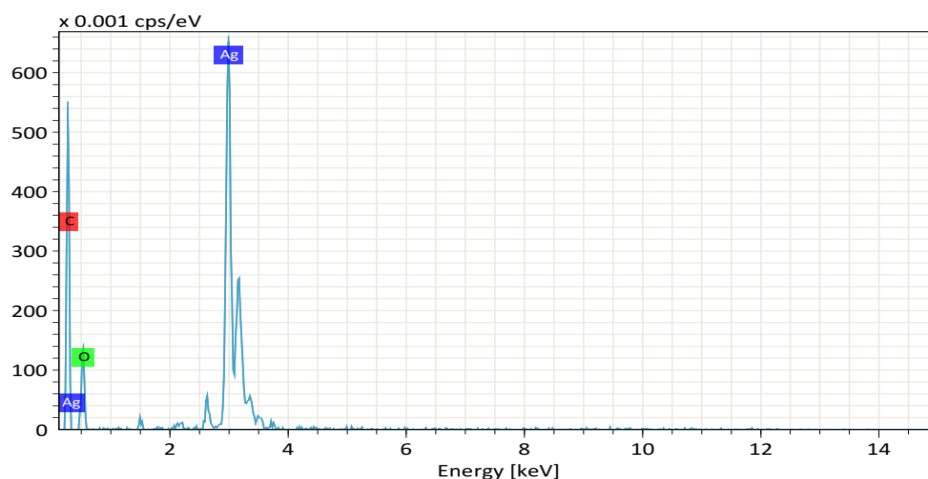


Fig. 7. Energy-dispersive X-ray spectrum of CC-AgNPs.

The combination of a dominant Ag signal and the presence of C and O suggests that the biosynthesis process successfully produced Ag silver nanoparticles with an organic capping layer derived from the *C. chrysantha* extract. This capping layer not only stabilises the nanoparticles but may also enhance their biocompatibility and biological activity, as reported in previous studies on green-synthesised AgNPs [27, 34].

Structures similar to those of the AgNPs were synthesised using *C. chrysantha*, as reported earlier by T.T. Trung, et al. (2026) [25]. Using the same transmission electron microscopy technique, T.T. Trung, et al. (2026) [25] reported an average particle size of 30.71 ± 6.31 nm. In contrast, the incorporation of ultrasonic assistance in the present study resulted in a significantly smaller particle size of 18.53 ± 5.66 nm, indicating the effectiveness of ultrasound in reducing the dimensions of nanoparticles. In addition, T.T. Trung, et al. (2026) [25] did not evaluate the antibacterial activity of the synthesised silver nanoparticles, which is a critical property for their application in wound treatment.

3.4. Antibacterial activities

Table 2. Bacterial growth (CFU/ml) at different drug concentrations and incubation times.

Drug dilution	Bacterial growth (CFU/ml)								
	2 h			6 h			24 h		
	<i>E. coli</i>	<i>S. au</i>	<i>P. ae</i>	<i>E. coli</i>	<i>S. au</i>	<i>P. ae</i>	<i>E. coli</i>	<i>S. au</i>	<i>P. ae</i>
1/4	0	0	0	0	0	0	0	0	0
1/8	0	0	0	0	0	0	0	0	0
1/16	0	0	10 ⁴	0	0	0	0	0	0
1/32	0	10 ²	10 ⁵	0	0	0	0	0	0
1/64	0	10 ⁵	10 ⁶	0	0	0	0	0	0
1/128	0	10 ⁶	10 ⁶	0	0	0	0	0	0
1/256	0	10 ⁷	10 ⁷	0	10 ²	0	0	0	0

E. coli: *Escherichia coli* ATCC 25922, *S. au*: *Staphylococcus aureus* ATCC 29213; *P. ae*: *Pseudomonas aeruginosa* ATCC 27853.

The antibacterial activity of the test compound against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 was assessed using a broth dilution assay. As shown in Table 2, *E. coli* exhibited the highest susceptibility, with complete growth inhibition at all concentrations and time points. This rapid bactericidal effect is consistent with previous findings indicating that Gram-negative bacteria with thinner peptidoglycan layers are more permeable to silver based antimicrobials [18, 19].

P. aeruginosa showed detectable growth at lower concentrations (at dilution of 1/16-1/256) after 2 h; however, no viable cells were observed at 6 or 24 h, demonstrating a clear time- dependent killing pattern. Although *P. aeruginosa* possesses intrinsic resistance mechanisms, such as efflux pumps and a robust outer membrane, AgNPs and Ag⁺ based compounds have been shown to overcome these defences through membrane disruption and oxidative stress induction [35, 36]. The complete inhibition observed after 6 h suggests that the compound exerts cumulative bactericidal activity, similar to previously reported time-kill kinetics [18].

In contrast, *S. aureus* displayed the greatest tolerance, with bacterial survival detected at concentrations $\leq$ a dilution of 1/32 (v/v) during the early stages of incubation. This behaviour aligns with the known resilience of Gram-positive bacteria, whose thick

peptidoglycan layer can hinder nanoparticle penetration and delay the intracellular accumulation of silver ions [19, 31]. Nevertheless, after 24 h, complete inhibition was achieved at concentrations $\geq$ a dilution of 1/32 (v/v), indicating that a sufficient exposure time can overcome this structural barrier. Similar concentration- and time-dependent inhibition patterns have been reported for silver-based antimicrobial agents [31, 35].

4. Conclusions

In this study, an aqueous extract of *C. chrysanth* was successfully prepared and applied as a green reducing agent for the synthesis of silver nanoparticles. CC-AgNPs were synthesised using ultrasonic assistance at a frequency of 37 kHz, with 4 mM AgNO₃, an extract-to-silver salt ratio of 1:10, pH 8, at 60°C for 60 minutes. Under these conditions, the resulting CC-AgNPs exhibited a characteristic SPR peak at ~420 nm, spherical morphology with an average TEM diameter of 18.53±5.66 nm, hydrodynamic size of 46 nm, PDI of 0.457, and zeta potential of -25.7 mV, indicating good colloidal stability. XRD and SAED analyses confirmed the formation of face-centred cubic crystalline AgNPs, while EDX and FTIR results demonstrated the presence of plant-derived biomolecules acting as capping and stabilising agents for the nanoparticles. The synthesised CC-AgNPs exhibited strong antibacterial activity, achieving complete inhibition of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 at a dilution of 1/256 (v/v), after 24 h (Fig 1). These findings highlight the potential of *C. chrysanth* extract as an effective, environmentally friendly reducing agent for producing biofunctional AgNPs with promising antimicrobial and wound treatment applications. Future research will focus on evaluating the cytotoxicity and antifungal properties of these nanoparticles to further confirm their safety and efficacy for advanced wound healing applications.

CRedit author statement

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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