



Eco-Friendly Techniques Based Nanoparticles of Fig Fruit (*Ficus carica*) Mediated Synthesis of AgCl and Their Biological Evaluation

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ABSTRACT:

In recent years, green synthesis of nanoparticles using plant-based resources has gained significant attention due to its eco-friendly, cost-effective, and sustainable approach. This study focuses on the biosynthesis of silver chloride (AgCl) nanoparticles mediated by aqueous extract of *Ficus carica* (fig fruit), a rich source of natural antioxidants and bioactive compounds. The phytochemicals present in the fig extract act as both reducing and stabilizing agents in the nanoparticle formation process. The synthesized AgCl nanoparticles were characterized using UV-Vis spectroscopy, FTIR, XRD, and SEM to confirm their size, shape, and crystalline structure. Furthermore, the biological efficacy of the synthesized nanoparticles was evaluated for antimicrobial and antioxidant activities. The results demonstrated significant inhibitory effects against both Gram-positive and Gram-negative bacterial strains, along with promising free radical scavenging activity. This study highlights the potential of *Ficus carica*-mediated AgCl nanoparticles as a novel, green alternative for biomedical and environmental applications.

Keywords: Green Synthesis, Nanoparticles, Silver, Micro-organism

INTRODUCTION

Nanoparticles exhibit unique properties that are strongly influenced by their size, shape, and morphology, which in turn enable them to interact effectively with plants, animals, and microorganisms [1, 2]. Among these, silver nanoparticles (AgNPs) have demonstrated remarkable bactericidal activity against a wide range of microorganisms [3, 4]. They are synthesized using various methods, often to study their morphology or physical characteristics. Some studies have incorrectly labeled chemically synthesized nanoparticles as “green synthesis,” although this was done inadvertently [5]. AgNPs have found applications in electronics, catalysis, pharmaceuticals, and microbial control, contributing to their recognition as eco-friendly nanomaterials [6]. Biogenic synthesis of AgNPs can be achieved using bacteria, fungi, yeast, actinomycetes, and plant extracts [7]. Recently, various plant parts, including flowers, leaves, and fruits, as well as enzymes, have been utilized for the synthesis of gold and silver nanoparticles [1]. The size, morphology, and stability of nanoparticles are influenced by factors such as the synthesis method, nature of the solvent, concentration and strength of the reducing agent, and reaction temperature [8, 9].

Ficus carica Linn, the most widely known member of the genus *Ficus*, is recognized by over 135 names. Although native to the Sub-Himalayan tract, Bengal, and central India, it has been extensively cultivated worldwide. [10] This temperate species originates from southwest Asia and the Mediterranean region (from Afghanistan to Portugal) and has been cultivated since ancient times for its nutritious fruits, commonly known as figs. [11] *Ficus carica* has also attracted global research attention due to its diverse biological activities. Traditionally, it has been used in systems such as Ayurveda, Unani, and Siddha to treat disorders of the endocrine system (diabetes), respiratory system (liver diseases, asthma, cough), gastrointestinal tract (ulcers, vomiting), reproductive system (menstrual pain), and various infectious diseases (skin disorders, scabies, gonorrhea) [12]. Ongoing research continues to validate these traditional medicinal uses, and this study provides a detailed overview of its pharmacological potential. Previous reviews have highlighted the pharmacological activities of *Ficus carica* [13-16], though only a few studies appear across all three reviews. This study reports on the eco-friendly green syntheses of silver nanoparticles (Ag NPs) using *Ficus carica* extract. In our study, the role of the extracts in reducing Ag⁺ to Ag⁰ has been investigated through spectroscopic and microscopic analyses emphasizing the antibacterial efficacy.

**MATERIALS AND METHODS**

Collection and Procurement of *Ficus Carica* Fruit: The plant *Ficus carica*, belonging to the family Moraceae, was collected and thoroughly washed with tap water to remove dirt and impurities. The fruit parts were air-dried at room temperature for 20 days and then ground into a fine powder using a pestle and mortar. From this, 60 grams of the powdered fruit were weighed and dissolved in 150 mL of methanol, followed by maceration at room temperature for 8 days to ensure efficient extraction of phytochemicals. [17] After maceration, the mixture was filtered through Whatman filter paper to separate the methanol extract (filtrate) from the plant residue. The residue was then re-extracted with chloroform using the same procedure. Both methanol and chloroform filtrates were concentrated by evaporating the solvents under reduced pressure at 50°C using a rotary evaporator. The resulting crude extracts were collected and stored in airtight flasks at 5°C for further analysis. The percentage yield of each extract was determined using the formula:

$$\text{Extract yield \%} = \frac{\text{Weight of extracted plants residues}}{\text{weight of plant raw sample}} \times 100$$

Thin Layer Chromatography (TLC): Thin Layer Chromatography (TLC) is a simple, rapid, and cost-effective technique used to separate and identify compounds in plant extracts. A small spot of the extract is applied near the bottom of a silica gel-coated TLC plate, which is then placed in a solvent system (mobile phase) that ascends the plate by capillary action. The components in the extract move at different rates depending on their polarity and interaction with the stationary phase, resulting in distinct spots. To perform TLC, a baseline is drawn about 1 cm from the bottom of the plate, and small spots of methanol and chloroform extracts are applied using a capillary tube or micropipette. The plate is then developed in an appropriate solvent system—ethyl acetate: methanol:water (6:4:1) for the polar methanol extract, and hexane:ethyl acetate: formic acid (7:3:0.5) for the less polar chloroform extract. Once the solvent front reaches about 1 cm from the top, it is marked and the plate is dried. The separated compounds are visualized under UV light (254 nm or 366 nm) or with specific spray reagents such as ferric chloride (for tannins and phenolics), anisaldehyde-sulfuric acid (for terpenoids), natural product + AlCl₃ (for flavonoids), or iodine vapors (for general organics). [18, 19] Finally, the retention factor (R_f) value for each spot is calculated using the formula: $R_f = (\text{distance moved by the compound}) / (\text{distance moved by the solvent front})$, which helps identify and compare the compounds present in the extracts.

Table 1: Phytochemical detection of Methanol extract

Phytochemical	Explanation	Detection in TLC
Polyphenols	Broad class including flavonoids, tannins, etc.	Seen as distinct spots, often reactive with Folin–Ciocalteu reagent.
Flavanols	A type of flavonoid (e.g., catechins).	Detected with UV light or natural product/AlCl ₃ spray.
Terpenoids	Aromatic plant compounds; bioactive.	Detected with anisaldehyde-sulfuric acid spray – gives purple/blue spots.

Table 2: Phytochemical detection of chloroform extract

Phytochemical	Explanation	Detection in TLC
Terpenoids	Lipophilic bioactive compounds.	Detected by anisaldehyde-sulfuric acid – spots may be purple, blue, or green.
Flavonoids	Broad group of polyphenolic plant compounds.	Detected using UV light or NP/AlCl ₃ reagents for fluorescence.

Biosynthesis Of Ag/AgCl NPs: The methanolic extract of *Ficus carica* fruit was employed as both a reducing and stabilizing agent in the green synthesis of silver nanoparticles (AgNPs). Analytical-grade silver nitrate (AgNO₃) (A.R., 99.2%, molecular weight 169.87 g/mol) obtained from Merck, South Africa, served as the silver precursor. A 0.1 mM aqueous solution of silver nitrate was prepared by dissolving the required amount of AgNO₃ in 1 liter of distilled water. To initiate nanoparticle synthesis, 50 mL of the *Ficus carica* methanolic extract was added dropwise to 450 mL of the AgNO₃ solution under continuous stirring at room temperature. The reaction mixture was maintained in a dark chamber throughout the process to prevent photoreduction of silver ions, ensuring controlled formation of silver nanoparticles. [20]

The successful bio-reduction of silver ions (Ag⁺) to elemental silver (Ag⁰) was visually indicated by a distinct color change in the reaction mixture from colorless to dark brown, confirming the formation of silver/silver chloride nanoparticles (Ag/AgCl NPs). To isolate the synthesized nanoparticles, the mixture was centrifuged at 15,000 rpm for 20 minutes at 15°C. A 2 mL aliquot of the supernatant was reserved for UV–Visible spectroscopic analysis to monitor nanoparticle synthesis. The resulting pellet was subsequently washed three times with distilled water to eliminate any remaining impurities and unreacted biomolecules. Finally, the purified nanoparticles were oven-dried overnight at 80°C and stored for further characterization and potential applications. [21]



Characterization Techniques: The synthesized silver nanoparticles (AgNPs) were characterized using a range of analytical techniques to assess their optical, structural, and surface properties. UV-Visible spectroscopy was used to confirm nanoparticle formation and optical behavior, recording absorbance spectra from 300 to 600 nm in a 2 mL quartz cuvette with a 1 cm path length using a PerkinElmer spectrophotometer at 1 nm resolution. Fourier Transform Infrared (FT-IR) spectroscopy identified functional groups on the AgNP surface derived from the plant extract, with spectra recorded on a Shimadzu FTIR spectrometer over 4000–400 cm^{-1} at 4 cm^{-1} resolution, using dried nanoparticles dispersed in KBr discs and KBr as a reference. Morphology and surface features were examined via Scanning Electron Microscopy (SEM) using a JEOL JSM-6480 LV, with samples coated with platinum to enhance conductivity. Dynamic Light Scattering (DLS) and zeta potential analysis measured the hydrodynamic particle size distribution and surface charge using a Malvern Zetasizer, with samples diluted tenfold in 0.15 M phosphate-buffered saline (pH 7.4) and sizes recorded from 0.1 to 10,000 nm. Finally, X-Ray Diffraction (XRD) analysis using an X'Pert Panalytical diffractometer with Cu $\text{K}\alpha$ radiation at 40 kV and 30 mA assessed the crystalline nature and phase of the nanoparticles, scanning dried samples on holders to obtain characteristic diffraction patterns. [22-27]

Antimicrobial Activity: The antimicrobial activity of the synthesized silver nanoparticles and plant extracts was evaluated against selected bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*) and fungal strains (*Aspergillus niger* and *Aspergillus oryzae*) [97]. For inoculum preparation, a loopful of each microorganism was cultured in 5 mL of nutrient broth and incubated at 37°C for 24 hours, after which 0.2 mL of the culture was transferred into 20 mL of sterile saline solution. The turbidity was adjusted to an absorbance of 580 nm, corresponding to approximately 10⁷ colony-forming units (CFU)/mL, equivalent to 0.5 McFarland standards, and used within 15 minutes to maintain uniform cell density. Antimicrobial susceptibility was assessed using the well diffusion method, where sterile Muller-Hinton agar plates were uniformly inoculated with 100 μL of the standardized microbial suspension. [28] Four wells of 6 mm diameter were created in each plate using a sterile cork borer, and 100 μL of plant extract or nanoparticle suspension (500 mg/mL) was added to each well, with dimethyl sulfoxide (DMSO) serving as the negative control. Plates were incubated at 37 ± 2°C for 24–48 hours, and antimicrobial activity was determined by measuring the diameter of the zones of inhibition (in mm) surrounding each well [29].

RESULTS AND DISCUSSION

Plants Extraction Yield: The table illustrated that 1-4% yield was obtained from chloroform extract and 2-5% yield was obtained from methanolic extract of fruit. The crude extract of plant parts indicated that percentage yield is directly proportional to the polarity of solvents. Polarity indices of methanol were 5.1 and chloroform polarity indices was 4.1, moreover difference in % yield depends upon the solubility of different constituents of plant material in different solvents Table 3.

Table 3: % Extraction Yield Of Plant Parts Fruits

Plant Part	% Extraction Yield (Chloroform)	% Extraction Yield (Methanol)
Fruit	1.13%	2.79%

Thin Layer Chromatography: The retention factors (R_f) of chloroform and methanol extracts in different solvent systems are shown in table 2. The chromatogram revealed 3 spots and 6 spots for chloroform and methanol extracts, respectively.

Table 4: TLC profile of chloroform and methanol extract of *Ficus carica*

Extract	Solvent System	Detecting Reagent	No. of Spots	Rf Values
Chloroform	Hexane : Ethyl acetate : Formic acid (7:3:0.5)	Vanillin–Sulphuric Acid	3	0.43, 0.65, 0.87
Methanol	Ethyl acetate : Methanol : Water (6:4:1)	anisaldehyde-sulfuric acid	6	0.19, 0.38, 0.43, 0.56, 0.65, 0.87

TLC profiling of chloroform and methanol extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals give different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extract can only be achieved by analyzing the R_f values of compounds in different solvent system.

Characterization of Nanoparticles

UV-Vis Spectroscopy Analysis: Subsequently, UV-Vis spectroscopy was used to confirm the formation of the Ag/AgCl NPs, indicated by the appearance of the dark-brown colloidal solution, as a result of excitation of surface plasmon resonance (SPR) between 290–360 nm (Figure 1). Our findings corroborate with the absorbance value of biosynthesized silver nanoparticles reported from previous studies, and contrary to our present study, some biogenic AgNPs synthesized from other plant extracts had SPR between 400 and 500 nm. The type of biogenic nanoparticles formed depends on the phytochemical compounds in the plant extract. The observed results show that the synthesis of Ag/AgCl NPs through the green approach in this study proved to be ancient with respect to reaction time, as well as having no requirement for the use of toxic chemical reducing or stabilizing agents. Therefore, making it an economical, sustainable, reliable, and an alternative process to chemical or physical methods for the synthesis of silver nanoparticles.

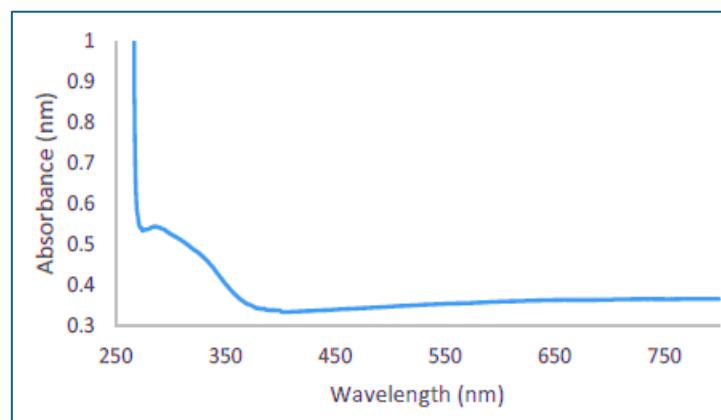


Figure 1: U.V. Spectra of silver nanoparticle

FTIR Analysis: Fourier Transform Infrared (FTIR) spectroscopy is a non-destructive, simple, and essential technique for elucidating the role of plant extracts in the reduction of silver ions to silver nanoparticles. In this study, the FTIR spectra of *Ficus carica* extract and the synthesized Ag/AgCl nanoparticles were analyzed and compared (Figure 2). Vibrational frequencies observed at 1025, 1590, and 2933 cm^{-1} in both the plant extract and Ag/AgCl NPs spectra were attributed to phytochemical compounds present in the extract. These compounds not only facilitated the biosynthesis of Ag/AgCl NPs but also acted as capping and stabilizing agents. The presence of residual phytochemical moieties on the nanoparticle surface confirmed their role in encapsulating the nanoparticles, thereby enhancing stability and preventing aggregation.

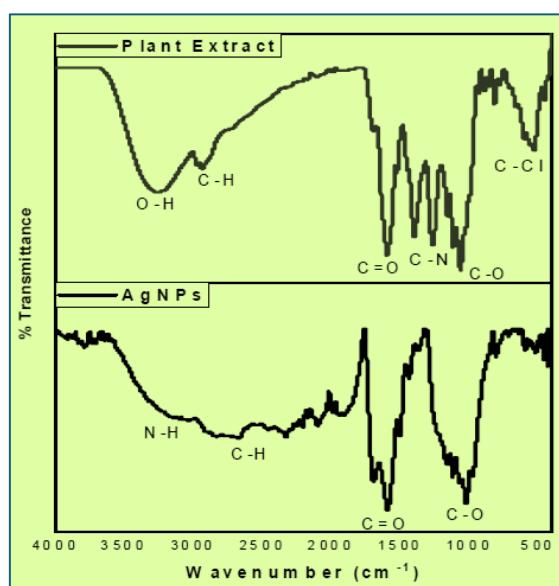


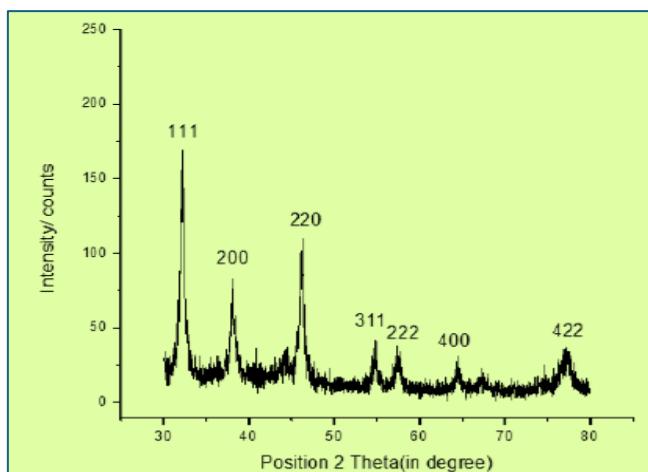
Figure 2: Transform infrared spectroscopy (FTIR) spectra of the methanol extract of *Ficus carica* fruit and Ag/AgCl NPs

DLS and Zeta Potential studies: Dynamic light scattering (DLS) is a technique used to determine the size, size distribution profile and poly dispersity index of particles in a colloidal suspension. Fig shows the DLS and zeta potential graph of *H. sinensis* which has an average size of 76.27nm and the particles carry a charge of -7.22 mV. Poly disparity index (PDI) is a measurement for distribution of silver nanoparticle with from 0.000 to 0.5. PDI greater than 0.5 values indicates the aggregation of particles. From the table 3, it was clear that all the AgNPs synthesised from the five plant extracts does not aggregate at all. Zeta potential measures the potential stability of the particles in the colloidal suspension. Silver nanoparticles generally carry a negative charge. All silver nanoparticles synthesized from the five plants showed negative charge and were stable at room temperature.

Table 5: Synthesized silver nanoparticle, PDI and zeta potential

Sl. No.	Plant Sample	DLS Size (nm)	PDI	Zeta Potential (mV)
1	<i>Ficus carica</i>	76.27	0.405	-7.22

XRD Analysis: XRD analysis is used to determine the phase distribution, crystallinity and purity of the synthesised nanoparticles particles. Fig 3 shows the XRD patterns of *Ficus carica*. With reference to the JCPDS data file No. 04-0783 it was concluded that the nanoparticles were crystalline in nature having cubical shape with no such impurities.

**Figure 3: X-RD Image of synthesized nanoparticles**

Zone of Inhibition: Antimicrobial activity of synthesized were investigated against two strains of gram positive bacteria *Staphylococcus aureus*, *Bacillus cereus* and two strains of gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*. While antifungal activity of plant *ficus carica* was tested against two fungal species *Aspergillus niger* and *Aspergillus oryzae*. Well diffusion method was adopted for this purpose. Zone of inhibition was measured by using measuring scale. The antimicrobial activity of silver nanoparticles (AgCl) was assessed by measuring the zone of inhibition (in mm) against various bacterial and fungal strains. The results are summarized below:

Table 6: Antimicrobial Activity (Zone of Inhibition in mm) of *Ficus carica*

Silver nanoparticles	Bacterial strain			
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>
AgCl	12	14	10	17

Table 7: Antimicrobial Activity (Zone of Inhibition in mm) of *Ficus carica*

Silver nanoparticles	Fungal strain	
	<i>Aspergillus niger</i>	<i>Aspergillus oryzae</i>
AgCl	5	13

The results indicate that the AgCl silver nanoparticles exhibited broad-spectrum antimicrobial activity, with varying degrees of inhibition across both bacterial and fungal species.



For bacterial strain: Among bacterial strains, the highest zone of inhibition was observed against *Bacillus cereus* (17 mm), suggesting strong antibacterial potential, particularly against Gram-positive bacteria. *Pseudomonas aeruginosa* and *Escherichia coli* also showed moderate sensitivity with inhibition zones of 14 mm and 12 mm, respectively. *Staphylococcus aureus* showed the least sensitivity among the tested bacteria with a zone of 10 mm, indicating relatively lower susceptibility.

For fungal strains: *Aspergillus oryzae* was more sensitive (13 mm) compared to *Aspergillus niger*, which showed a smaller inhibition zone of 5 mm, suggesting that AgCl nanoparticles have moderate antifungal activity, with some fungi showing resistance. These findings align with previous studies where silver nanoparticles have demonstrated enhanced antibacterial activity, likely due to their small particle size and ability to disrupt microbial cell walls, generate reactive oxygen species, and interfere with DNA replication.

CONCLUSION

Green synthesis of silver nanoparticles using plants offers a cost-effective, safe, non-toxic, and eco-friendly approach suitable for large-scale production. *Ficus carica* efficiently facilitated AgNP synthesis under optimal temperature conditions, with UV-Visible spectroscopy showing a characteristic peak at 421 nm. SEM analyses revealed their morphology and uniform distribution, while DLS and zeta potential confirmed nanoparticle size, charge, and colloidal stability. FTIR spectra indicated the role of phytochemicals in reduction and stabilization, and XRD patterns verified purity and crystalline nature. These AgNPs exhibited potent antimicrobial activity, particularly against Gram-positive bacteria and selected fungal pathogens, consistent with previous reports.

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