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Green Synthesis of Silver Nanoparticles using Daucus Carota Leaves and Their in Vitro Anti-Urolithiatic Activity Against Struvite Crystals

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***Abstract*—** The green synthesis of silver nanoparticles (AgNPs) provides an eco-friendly and sustainable alternative to conventional chemical and physical methods, which often pose environmental risks. In this study, AgNPs were synthesized using an ethanolic leaf extract of *Daucus carota* as a natural reducing and stabilizing agent. A 1 mM aqueous solution of silver nitrate ($AgNO_3$) was treated with the extract, resulting in nanoparticle formation within one hour. The synthesized AgNPs were characterized by UV-Visible spectroscopy (showing a peak at 434.75 nm), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Spectroscopy (EDX). FTIR analysis confirmed the presence of functional groups involved in nanoparticle formation, SEM revealed spherical and cubic morphology, XRD confirmed a face-centered cubic crystalline structure, and EDX validated the elemental composition of silver. Phytochemical screening of the *Daucus carota* extract indicated the presence of bioactive constituents including terpenoids, flavonoids, tannins, alkaloids, saponins, steroids, coumarins, emodin, anthraquinones, phenols, and xanthoproteins, with quantitative analysis highlighting significant levels of key compounds such as flavonoids, phenols, and alkaloids. The in vitro anti-urolithiatic activity of the synthesized AgNPs against struvite crystals demonstrated a significant percentage of inhibition, suggesting their potential therapeutic application in the management or prevention of struvite kidney stones.

Keywords— silver nanoparticles (AgNPs); anti-urolithiatic activity; struvite crystals; eco-friendly synthesis; kidney stones

I. Introduction

Daucus carota (carrot) has long been recognized in traditional medicine for its benefits in urinary health. Wild carrot is considered to possess antilithic (stone-dissolving) and diuretic properties and has been used to manage cystitis and kidney/bladder disorders [1]. Modern phytochemical studies have confirmed that carrot leaves are rich in bioactive compounds such as vitamin C, phenolic antioxidants, flavonoids, tannins, and saponins, which can offer antioxidant and anti-inflammatory protection to renal tissues [2].

In vitro studies have demonstrated that hydroalcoholic extracts of *D. carota* can significantly inhibit calcium oxalate (CaOx) crystal nucleation, growth, and aggregation [3]. These extracts can also influence crystal morphology, promoting the formation of calcium oxalate dihydrate (COD), which is less adherent and less damaging to renal tissues than the monohydrate form (COM). The inhibitory effects are attributed to the phytochemicals present, which can chelate calcium ions and suppress crystal formation [4].

In vivo studies using rat models of ethylene glycol-induced urolithiasis have shown that *D. carota* extracts can normalize urinary biochemistry, reduce serum creatinine and BUN levels, and decrease renal crystal deposition [5]. These effects could be linked to both the anticrystallization properties and the antioxidant capacity of the extract [6].

Although most research has focused on calcium oxalate stones, the potential of *D. carota* against struvite stones (magnesium ammonium phosphate) is promising. These stones are often caused by infections with urease-producing bacteria[7]. Since *D. carota* extracts exhibit antibacterial and diuretic activities, they could help prevent the formation of struvite stones by reducing urinary tract infections and promoting urine flow [8].

The antiurolithiatic properties of *Daucus carota* leaf extract are supported by multiple lines of evidence, including phytochemical analysis**,** in vitro inhibition of crystallization**,** and in vivo renal protection**.** Further research focusing specifically on struvite crystallization is warranted to explore its full therapeutic potential [9].

II. Materials and Methods

A. Plant Material Collection

The leaves of *Daucus carota* were collected from the Kolli Hills region in Namakkal district [10].

B. Preparation of Plant Extract of Leaves of Daucus carota

The collected leaves were cut into small pieces, thoroughly washed with tap water, and shade-dried to avoid sunlight

exposure [11]. Once dried, they were crushed using a mortar and pestle. The resulting powder was stored in airtight containers in a cool, dark, and dry place.
For extraction, 10 g of the powdered leaf sample was soaked in 100 ml of ethanol for 24 hours. The extract was then subjected to hot percolation [12].

C. Phytochemical Analysis (Qualitative Tests)

Phytochemical tests were conducted based on precipitation and color reactions [13].

- **Saponins**: Formation of stable foam after shaking indicates presence.
- **Tannins**: Ferric chloride reaction shows blue-black or brownish-green coloration.
- **Terpenoids**: Sulfuric acid reaction gives reddish-brown color.
- **Phlobatannins**: Red precipitate formed with HCl.
- **Flavonoids**: Yellow color with sulfuric acid.
- **Proteins**: White color with sulfuric acid indicates presence.
- **Anthraquinones**: Violet/pink/red with benzene and ammonia.
- **Cardiac glycosides**: Brown/violet ring with glacial acetic acid, ferric chloride, and sulfuric acid.
- **Carbohydrates**: Reddish violet ring with sulfuric acid and naphthol.
- **Xanthoproteins**: Blue/black color with ferric chloride.
- **Leucoanthocyanin**: Red organic layer with isoamyl alcohol.
- **Phenols**: Orange/red color with ammonia.
- **Emodin**: Crimson with ammonia and benzene.
- **Steroids**: Crimson ring with chloroform and sulfuric acid.
- **Anthocyanins**: Pink/crimson/violet with HCl and ammonia.
- **Alkaloids**: Yellow color with glacial acetic acid and ammonia.
- **Glycosides**: Violet/blue/green color with chloroform and glacial acetic acid.
- **Coumarins**: Yellow color with NaOH.

D. Quantitative Analysis of Phytochemicals

Quantification showed the presence of flavonoids, phenols, terpenoids, alkaloids, saponins, and tannins in mg/g [14].

- **Alkaloids**: 0.5 g powder was extracted using acetic acid in ethanol, followed by precipitation with ammonium hydroxide and drying.
- **Phenols**: 0.5 g powder was boiled in diethyl ether, treated with ammonia and amyl alcohol, then dried.
- **Terpenoids**: 0.5 g powder soaked in ethanol, filtered, dissolved in petroleum ether, and dried.
- **Tannins**: 0.5 g powder was shaken with water, filtered, and reacted with ferric chloride and potassium ferrocyanide, then dried.
- **Flavonoids**: 0.5 g powder dissolved in methanol, filtered, and dried after evaporation.
- **Saponins**: 0.5 g powder extracted with ethanol, filtered, then reacted in a separating funnel with diethyl ether and n-butanol, and dried.

E. Synthesis of Silver Nanoparticles

20 ml of silver nitrate was dissolved in 50 ml of distilled water under continuous stirring. The mixture was left at room temperature, allowing silver ions to reduce. Color change from transparent white to dark brown confirmed the formation of silver nanoparticles [15].

F. Characterization of Silver Nanoparticles

Various instruments were used to analyze the properties of synthesized nanoparticles [16]:

- **UV-Visible Spectroscopy**: Silver nanoparticles were scanned after 24 hours in the 350–500 nm range.
- **X-Ray Diffraction (XRD)**: Analyzed crystal size and structure.
- **FT-IR**: Identified functional groups; spectra recorded in the 4000–400 cm^{-1} range.
- **SEM and EDX**: SEM analyzed surface morphology; EDX confirmed elemental composition.
- **DLS**: Assessed particle size distribution.

G. Struvite Crystal Growth and Characterization

Single diffusion reaction was used. 15 ml each of ammonium dihydrogen phosphate and sodium metasilicate (1.04 g/cm^3, pH 9.4) were mixed in test tubes. After gel formation (4–5 days), 1 M magnesium acetate was added carefully. FTIR (HITACHI 570) was used to characterize the crystals [17].

H. Effect of Silver Nanoparticles on Struvite Crystals

Ethanolic extract of *Daucus carota* was used to synthesize AgNPs and study their effect on struvite growth using the gel method [18].
Different concentrations (1–5%) of silver nanoparticles were added to the supernatant. Crystal growth was measured based on the average weight [19].

% Inhibition was calculated as:

$$\text{Inhibition (\%)} = [(TSI - TAI)/TS1] \times 100$$

Where,
TSI = Total number of crystals without inhibitor
TAI = Total number of crystals with inhibitor [20].

III. Results and Discussion

A. Qualitative phytochemical analysis

The qualitative phytochemical analysis of *Daucus carota* extract revealed the presence of a wide range of bioactive constituents, confirming the therapeutic potential of the plant. Figure 1, the ethanol extract tested positive for major phytochemicals such as flavonoids, tannins, saponins, alkaloids, phenols, terpenoids, steroids, glycosides, anthraquinones, and resins. Among them, saponins, tannins, alkaloids, steroids, glycosides, proteins, anthraquinones, anthocyanins, carbohydrates, cardiac glycosides, xanthoproteins, and phenols were strongly present (+++), indicating their high concentration [21]. Flavonoids, phlobatannins, coumarins, emodin, and leucoanthocyanins were moderately present (++ to +), supporting their moderate occurrence. These results are consistent with previous findings by Abdul Rasheed Hadiza Haruna et al. (2020), who reported similar compounds including alkaloids, flavonoids, glycosides, terpenes, steroids, anthraquinones, and resins in *Daucus carota* leaves. Similarly, the study by Anak Agung Putu Putra Wibawa et al[22]. (2021) confirmed the presence of phenolic compounds such as tannins, saponins, flavonoids, steroids, terpenoids, and significant beta-carotene content (908.75 mg/100g leaves) in *Daucus carota* leaves. These findings collectively affirm that the ethanol extract of *Daucus carota* is rich in pharmacologically important phytochemicals, potentially contributing to its antimicrobial and therapeutic properties[23].

B. Quantification of phytoconstituents

In the extract of *Daucus carota* revealed measurable concentrations of key bioactive compounds. The extract contained flavonoids (0.036 mg/g), tannins (0.03 mg/g), saponins (0.485 mg/g), alkaloids (0.087 mg/g), phenols (0.452 mg/g), and terpenoids (0.215 mg/g). These values indicate a notable presence of saponins and phenols compared to other constituents [24]. Supporting this, Moragot Chatatikun and Anchalee Chiabchalard (2013) reported that petroleum ether and ethanolic extracts from baby carrots and carrots exhibited high total phenolic and flavonoid contents. Specifically, the total phenolic content was 35.9 ± 4.0 mg GAE/g and 30.7 ± 3.1 mg GAE/g dry plant material, while the total flavonoid content was 35.3 ± 6.8 mg QE/g and 20.4 ± 2.8 mg QE/g for baby carrots and carrots, respectively. These comparative studies affirm the presence of significant amounts of phenolics and flavonoids in *Daucus carota*, supporting its potential antioxidant and therapeutic properties [25].

C. AgO Nanoparticle Synthesis

The green synthesis method was employed for the preparation of silver oxide (AgO) nanoparticles using the ethanolic extract of *Daucus carota*. When silver nitrate solution was mixed with the extract and exposed to light (photosynthesis), a visible reddish-brown precipitate formed, indicating the reduction of silver nitrate to silver oxide nanoparticles. This colour change confirmed the successful synthesis of AgO nanoparticles, as shown in Figure 1. This finding aligns with the report by Nida Fareed *et al* [26]. (2023), where a similar synthesis method resulted in a blackish fine powder of silver nanoparticles, which was stored in falcon tubes at room temperature for subsequent characterization and evaluation of bioactivities [27].

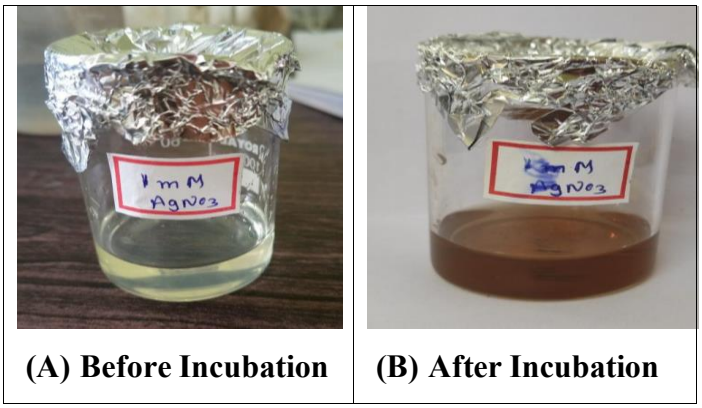

Fig. 1: Visual observation of synthesized silver nanoparticle

D. Visual Colour Change and UV-Visible Spectroscopy

Upon the addition of ethanolic extract of *Daucus carota* to a glass vial containing $AgNO_3$, a visible colour change from colourless to reddish-brown was observed, indicating the formation of silver nanoparticles (Fig. 2). This change is attributed to surface plasmon resonance (SPR), a phenomenon confirmed by the UV-Visible absorption spectrum, which showed a prominent peak at 428 nm—consistent with literature reports for silver nanoparticles [28]. UV–Visible spectrophotometry is a rapid and effective technique for the preliminary identification and characterization of silver nanoparticles. The nanoparticles formed were predominantly spherical and polydisperse, suggesting the presence of multiple phytochemical constituents that act as reducing and stabilizing agents, influencing the growth rate and shape of the nanoparticles. A similar observation was reported by M.R. Suchithra et al. (2021), where brown coloration upon exposure of Hybanthus enneaspermus extract to Ag^+ ions confirmed nanoparticle biosynthesis, with absorption peaks around 439 nm [29].

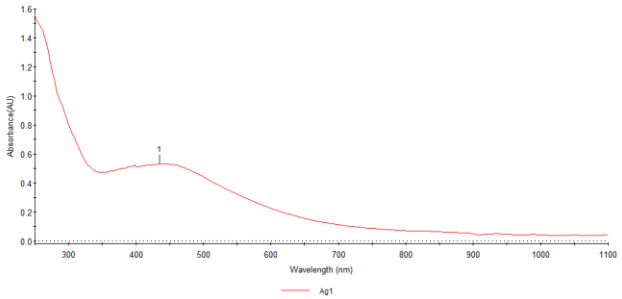

Fig : 2 UV-Vis's spectrum of synthesized silver nanoparticles of *Daucus Carota*

E. FT-IR Spectroscopy for Functional Group Identification

Fourier-transform infrared (FT-IR) spectroscopy of the synthesized silver nanoparticles revealed key functional groups involved in the reduction and stabilization of nanoparticles, as shown in Figure 3. Peaks observed at 3498.23 cm^{-1} (O–H stretching, H-bonded), 2375.47 cm^{-1} (C–N stretching in aliphatic amines), 1645.62 cm^{-1} (C–O stretching in ethers), 1067.24 cm^{-1} (C=C in alkenes), and 677.58 cm^{-1} (C=C stretching in conjugated alkenes) indicate the presence of phytochemicals acting as reducing and capping agents. This observation is supported by the findings of S. Vidhya et al. (2021), who reported that such phytoconstituents play a critical role in nanoparticle synthesis[30].

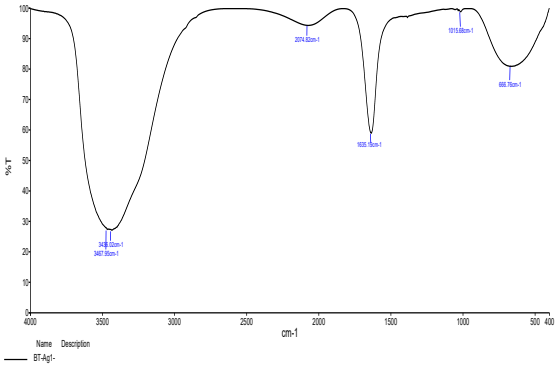

Fig. 3: FT-IR Spectrum of Synthesized Silver Nanoparticles

F. Scanning Electron Microscopy (SEM)

SEM analysis was used to determine the morphology and size distribution of the synthesized silver nanoparticles. The particles were predominantly spherical and cubic in shape, with sizes ranging from 86.15 to 104.5 nm. The average size was approximately 200 nm (Fig. 4)[31].

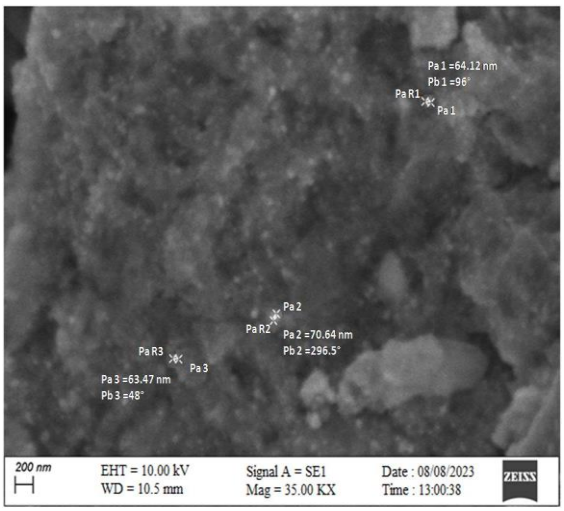

Fig. 4: SEM Image of Synthesized Silver Nanoparticles

G. EDX (Energy Dispersive X-ray) Analysis

EDX spectroscopy confirmed the elemental composition of the synthesized silver nanoparticles (Fig. 5). The major elemental peaks corresponded to silver and a secondary chlorine peak, likely due to phytochemical capping agents. The silver nanoparticles showed a silver weight percentage of 67.36%, with AgCl contributing 32.64% [32].

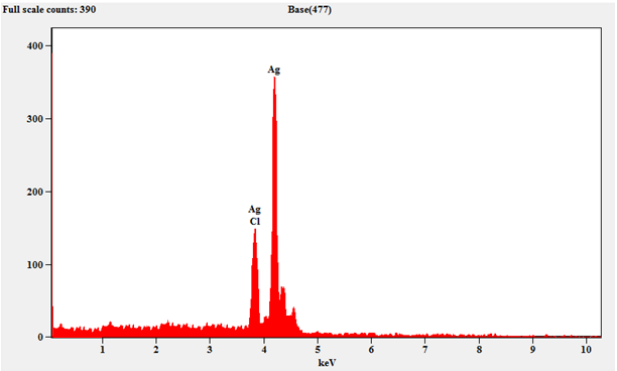

Fig. 5: EDX Spectrum of Synthesized Silver Nanoparticles

H. X-ray Diffraction (XRD) Analysis

XRD analysis confirmed the crystalline nature of the synthesized silver nanoparticles. The diffractogram displayed prominent peaks at 2θ values of 38.34°, 45.58°, 65.16°, and 78.45°, corresponding to the (111), (200), (220), and (311) planes of face-centered cubic silver crystals, in accordance with JCPDS File No. 04-0783. Additional unassigned peaks were observed, potentially due to phytochemical residues from the plant extract (Fig. 6)[33].

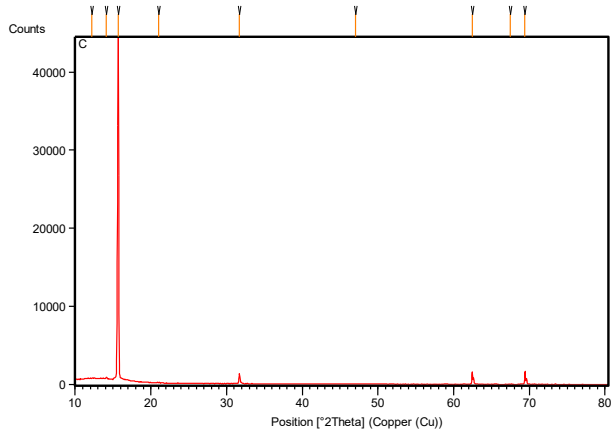

Fig. 6: XRD Pattern of Silver Nanoparticles Synthesized Using *Daucus carota.*

I. Effect of Synthesized Silver Oxide Nanoparticles on Struvite Crystals

The influence of synthesized AgO nanoparticles on struvite crystal growth was assessed using a gel-based crystallization method. Control samples (magnesium acetate without nanoparticles) produced the largest crystals (~4 cm). With increasing concentrations of AgO nanoparticles (1%–5%), a gradual reduction in crystal size was observed, with the smallest crystal (~1 cm) recorded at 5% concentration (Fig.6)[34]. ANOVA statistical analysis revealed a significant correlation ($p < 0.05$) between nanoparticle concentration and inhibition of crystal growth (Table 1). These findings are in agreement with Vidhyashree et al. (2020), who noted that the crystallization mechanism of kidney stones—comprising nucleation, growth, aggregation, and retention—is modulated by inhibitory agents such as silver nanoparticles. Vidhya et al. (2021) also observed similar inhibitory effects in *T. chebula* bark-mediated nanoparticle studies[35].

Table 1: Inhibitory Effect of Synthesized Silver Oxide Nanoparticles on Struvite Crystal Formation

Group	Treatment	Inhibition (%)
A	Control (Magnesium acetate only)	0%
B	Control + Water	2.33%
C	Control + 1% Synthesized Silver Oxide Nanoparticles	61.21%
D	Control + 2% Synthesized Silver Oxide Nanoparticles	68.22%
E	Control + 3% Synthesized Silver Oxide Nanoparticles	75.70%
F	Control + 4% Synthesized Silver Oxide Nanoparticles	82.71%

Fig 7: The inhibition effect of different concentrations of synthesized zinc oxide nanoparticles on struvite crystal formation was evaluated. Group (a) represents the control containing only magnesium acetate, showing no inhibition. Group (b) included water, and group (c) included ethanol, serving as solvent controls. Groups (d) through (h) correspond to increasing concentrations (1%, 2%, 3%, 4%, and 5%) of synthesized zinc oxide nanoparticles. A concentration-dependent inhibition of struvite crystals was observed, with the highest inhibition recorded at 5% nanoparticle concentration.

IV. Conclusion

The present study demonstrates the successful green synthesis of silver oxide (AgO) nanoparticles using ethanolic extract of *Daucus carota*, confirmed through observable color change and supported by previous literature. Qualitative and quantitative phytochemical analysis revealed the presence of major bioactive compounds such as flavonoids, tannins, saponins, alkaloids, phenols, and terpenoids, which likely contributed to the reduction and stabilization of AgO nanoparticles. The synthesized nanoparticles exhibited a significant inhibitory effect on struvite crystal formation, with a dose-dependent response observed — achieving up to 82.71% inhibition at 4% nanoparticle concentration. These findings suggest that *Daucus carota*-mediated AgO nanoparticles hold promising potential as a natural and eco-friendly approach for the prevention or treatment of struvite-related urolithiasis.

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