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Green Synthesis of Silver Nanoparticles Using *Trachyspermum Ammi* (Ajwain) Leaf extract and its Catalytic, Biological Activities

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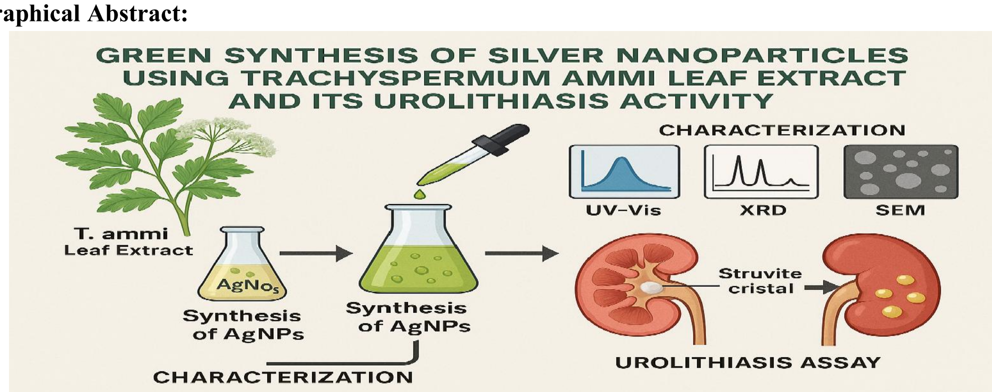
Abstract: *Nanotechnology has modernized material science, leading to the expansion of innovative solutions across multi-disciplines. The synergistic collaboration between nanoscale metals and biomolecules plays a vital role in current medicine. This study explores the microwave-assisted in situ synthesis of silver nanoparticles (AgNPs) using Trachyspermum ammi leaf extract and evaluates their potential applications in urolithiasis treatment, antibacterial activity, and catalysis. This study describes the eco-friendly synthesis of silver nanoparticles (AgNPs) utilizing aqueous leaf extract of Trachyspermum ammi (ajwain) as a reducing and stabilizing agent. A distinct brown coloration and in a UV Spectrum characteristic surface plasmon resonance (SPR) peak near 415 nm confirmed nanoparticle formation.The synthesized silver nanoparticles were analysed by XRD and SEM. The silver nanoparticles had an approximate size of 45 nm and exhibited a FCC crystalline structure. To examine their distribution on cotton fabric, a scanning electron microscope. The results indicate that the AgNP exhibits excellent antibacterial, urolithiasis & catalytic activity, making it a potential candidate for biomedical applications.*

Graphical Abstract:



Keywords: AgNPs, *Trachyspermum*, Microwave, urolithiasis, catalytic, antibacterial

I. INTRODUCTION

Traditional antimicrobial treatments often involve synthetic chemicals, which may pose environmental and health risks. Hence, there is a growing need for sustainable and eco-friendly alternatives for imparting antimicrobial properties [1]. Nanotechnology is one of the revolutionary fields of research that focuses on the creation and applications of nano particles in various domains such as catalysis, electrochemistry, biomedicine, pharmaceuticals, sensors, food technology and cosmetics etc. [2-5]. Al-doped-ZnO/Ag and Al-doped-ZnO/Cu multilayer-coated polyester fabrics exhibit strong hydrophobic properties, high infrared reflectivity, and superior UV protection while maintaining air permeability [6,7]. Ag-coated polyester fabrics also offer durability, water repellency, UV protection, and a comfortable texture while effectively reducing air permeability [8].

Urolithiasis, or kidney stone disease, involves the formation of crystalline deposits in the urinary system, leading to pain and renal dysfunction. A significant subset of these calculi, known as infection stones, comprises struvite ($MgNH_4PO_4 \bullet 6H_2O$) and typically develops in alkaline urine due to urease-positive bacterial infections [9]. Conventional management methods, including surgical interventions and pharmacotherapy, may carry risks of recurrence and side effects, underscoring the need for alternative strategies [10].

Plants rich in bioactive phytochemicals have been explored for their stone-inhibiting potential. Trachyspermum ammi, widely used in traditional medicine, contains compounds such as thymol, phenolics, and terpenoids, known for diverse bioactivities including antimicrobial and antilithiatic effects [11]. Parallelly, silver nanoparticles (AgNPs) synthesized via green methods have gained attention for their biomedical and antibacterial properties. By employing plant extracts, these methods circumvent the use of toxic chemicals and capitalize on phytoconstituents for reduction and capping, resulting in biocompatible nanomaterials [12]. While previous studies have demonstrated the inhibitory effects of various plant-mediated AgNPs on calcium oxalate and struvite crystal formation, investigations utilizing T. ammi leaf extract specifically remain limited [13]. This study aims to synthesize AgNPs using T. ammi leaf extract and evaluate their in vitro efficacy in preventing struvite crystallization, hypothesizing a dual mechanism involving surface interaction and ion chelation [14].

However, chemical synthesis often involves toxic substances that may pose environmental and health risks. To mitigate this issue, alternative coating methods such as using plant extracts which is a green and safe approach [15]. Recent research has highlighted the green synthesis of silver nanoparticles using extracts from plants such as moringa [16], cassia [17], Azadirachta [18], and Geranium leaf [19] etc. Trachyspermum ammi (Ajwain) is known for its rich phytochemical content, which can act as a natural reducing and stabilizing agent for the synthesis of AgNPs. T. ammi is a medicinal herb that has antimicrobial, antipyretic, carminative, anti-inflammatory, antipyretic and anti-oxidant stuffs [20].

Struvite, scientifically known as ammonium magnesium phosphate, forms rapidly due to urinary tract infections caused by urease-producing bacteria [21]. These stones present a treatment challenge due to their rapid growth, persistence, and high recurrence rate of approximately 50%. Struvite stones account for around 17% of all urinary calculi. Additionally, studies indicate that calcium oxalate stones constitute approximately 79% of kidney stone cases worldwide [22]. The formation of calcium oxalate stones follows a series of physicochemical processes, including crystal nucleation, growth, aggregation, and eventual retention on renal tubular epithelial linings [23].

Our study explores the potential of natural resources in promoting sustainability by synthesizing silver nanoparticles (AgNPs) through both chemical and green approaches. The synthesized nanomaterial is characterized using various analytical techniques, and its antibacterial activity, Silver nanoparticles in vitro anti-urolithiasis and methylene blue dye reduction catalytic activity was studied. This research contributes to the advancement of green nanotechnology while emphasizing the significance of eco-friendly solutions in addressing bacterial infections, urolithiasis. The findings highlight the potential of environmentally sustainable nanoparticles to transform antibacterial, urolithiasis treatments in the future.

II. RESEARCH METHODOLOGY

2.1. Synthesis of AgNPs:

Trachyspermum ammi leaves were collected and washed properly with tap water followed by distilled water. After air drying in the shade they were crushed into fine powder. The powdered leaf extract (0.5gr) was added to 50 ml double distilled water and kept for boiling in a household conventional microwave oven for 10 min to obtain the extract. It is the plant extraction that was used for the preparation of Silver nanoparticles. The *in situ* generation of AgNPs by 0.01N $AgNO_3$ solution and kept in microwave oven for ten minutes. The formation of the AgNPs was confirmed from the observed colour change of solution from colourless to brown colour shown in Fig.1.

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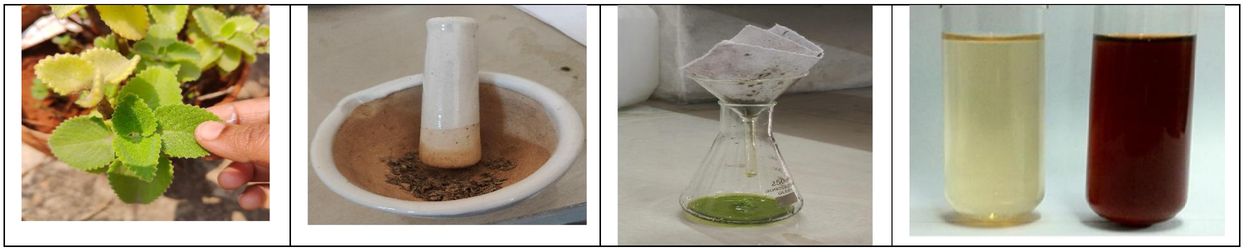

Fig.1. Synthesis & Formation of AgNPs

2.2 Antimicrobial Activity of Ag Nanoparticles:

The antibacterial activity of silver nanoparticle (Ag NP)-coated cotton fabric was evaluated using the disk diffusion method. This study aimed to assess the efficacy of Ag Naanoparticles inhibiting bacterial growth. A Gram-positive bacterium, *Staphylococcus aureus* (*S. aureus*), was selected as the test organism due to its clinical significance in causing infections. Pure cultures of *S. aureus* were subcultured in Muller-Hinton broth and incubated at 37°C for 24 hours to ensure active bacterial growth. The subcultured organism was then uniformly swabbed onto solidified agar plates using sterile cotton swabs.

Ag NP,s samples were carefully placed onto the inoculated agar plates, the plates were subsequently incubated at 37°C for another 24 hours. The antibacterial efficacy of the AgNP's determined by examining the presence or absence of a clear zone of inhibition around AgNP's samples, indicating bacterial growth suppression. The size of the inhibition zone, if present, was recorded and analyzed using digital photography to ensure accurate documentation. The results of this study provide valuable insights into the potential application of Ag NP's in hygiene-related fields.

2.3. Catalytic degradation assay

Synthesized AgNPs underwent tests of catalytic degradation against MB (Methylene Blue) and EV (Ethyl Voilet) solutions in both H_2O_2 affected and unadjusted conditions. The experimental method to measure degradation followed this standard procedure: 0.5 mL of solution (5mg·mL-1) was combined with 1.0 mL of organic dye aqueous solution (50 mg·L-1) in a 25 mL conical flask containing H_2O_2 or a H_2O_2-free environment with continuous stirring for 30 min. The reaction progression could be monitored through spectral absorption changes analyzed in a UV-vis spectrophotometer during set time points. The degradation of the binary system and dye adsorption by testing solution without A and solution without H_2O_2 separately.

$$\textbf{Decolorization \%} = (A_0 - A)/A_0 \times 100\%$$

Where $\mathbf{A_0}$ and **A** are the absorbance of the dyes solution at times corresponding to 0 and t at the characteristic wavelength, respectively.

2.4 Synthesis of struvite:

The hydrogel medium needed for crystal growth was attained by employing a solution containing 1M Sodium Meta Silicate ($NaSiO_3.9H_2O$) and 0.5M Ammonium dihydrogen phosphate ($NH_4H_2PO_4.2H_2O$) as well as a solution composed of 1M Magnesium acetate ($Mg\ (CH_3COO)_2.4H_2O$). Following the 24-hour duration the struvite composition of the crystals that had formed was examined.

III. RESULTS AND DISCUSSION

3.1 Characterization of the AgNPs:

UV-Visible:

Initially the produced Silver nanoparticles are spotted by visible colour changing from colourless to dark brown colour. UV Spectral data revealed the absorption peak at 415 nm confirming the presence of Silver nanoparticles Fig.3. The green reduction of Ag^+ ion to Ag^0 by the phytochemicals present in the *Trachyspermum ammi* leaf extract caused this.

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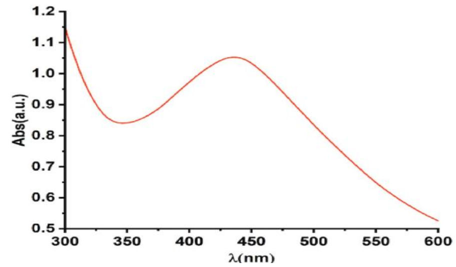

Fig.3. Uv Spectra of Ag NPs

3.2. XRD Analysis:

Newly crystallized silver nanoparticle was confirmed by Bruker 5000 the Bxrd Pattern analysis given the plane indices of 111,220,311 as metallic silver XRD values are coincided. The excellent XRD spectral values promising the Silver nanoparticles formation in dissimilar concentrations of starting materials were taken shown in Fig.4

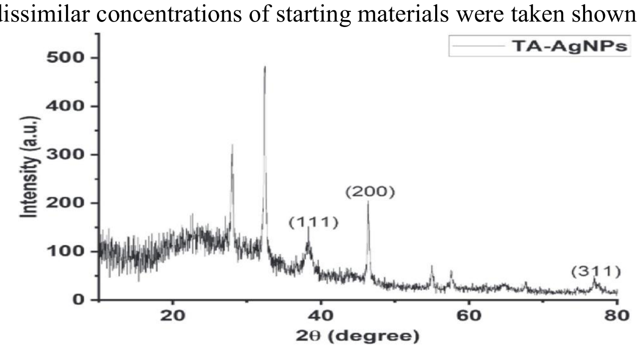

Figure 4. XRD Spectrum of TA-AgNPs.

Fig.4. XRD – Ag NP

3.3. SEM & EDX Spectroscopy

We used JOEL Scanning Electron Microscope (SEM) to analyse the shape and size of the synthesized Silver nanoparticles. The SEM images presented that the AgNPs had smooth surface and a spherical shape, with an average diameter ranging from 17 to 27nm (Fig.5). This method is commonly used to study the surface characteristics of nanoparticles, such as their size, shape, morphology and distribution. The elemental composition of the AgNPs was determined by EDX analysis, which measures the energy and intensity distribution of X-ray signals generated by an electron beam on a specimen. Fig 6. shows the EDX spectra of the AgNPs, which revealed the presence of Agathis Silver nanoparticles coated Cotton may act as capping organic agents that bund to the surface of the Silver nanoparticles.

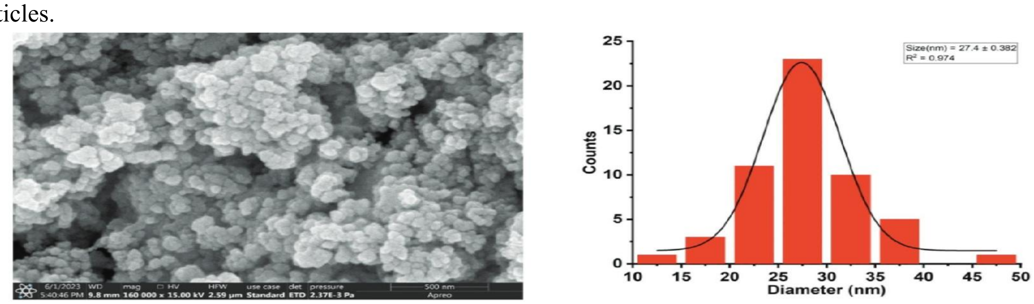

Figure 5. **SEM image of AgNPs at 10µm**

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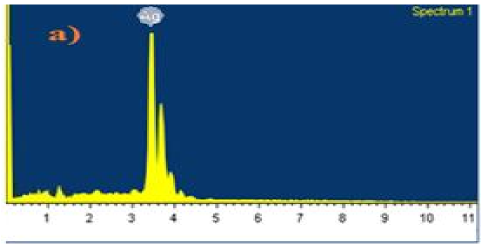

Figure 6..**EDX spectrum of AgNPs**

3.4. FTIR Spectroscopy:

Fourier Transform Infrared spectroscopy (FTIR) is a crucial method in the analysis of Silver nanoparticles using plant extracts. The process described *Trachyspermum ammi* leaf extract can reduce silver nitrate, followed by centrifugation to isolate the nanoparticles. For instance, the peak at 3304 cm-1 suggests the presence of phenols and alcohols, while the peak at 1382 cm-1 is characteristics of aldehyde or ketone.

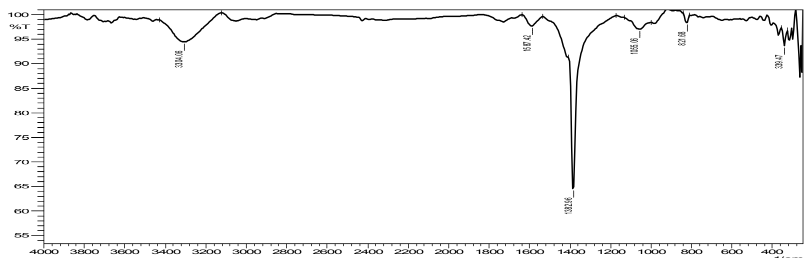

Figure 7. **FT-IR Spectrum of AgNPs**

Catalytic function of AgNPs

As a comparison, the solution was treated by four methods, (1) only MB or EV, (2) MB or EV+H_2O_2, (3) MB or EV+ AgNPs and (4) MB or EV+AgNPs+H_2O_2 prepared. The decrease in MB or EV in solution was determined by measuring the UV-Vis spectrum. The AgNPs as a catalyst, the absorption peaks at 664 and 595 nm for MB and EV decrease slightly when H_2O_2 was added for 20 min, which indicates that the degradation of MB and EV is very slow, even with excessive H_2O_2. However, the absorption peaks for MB and EV decreased obviously while the AgNPs was added in the absence and presence of H_2O_2, and the results show that the percentage of decolorization reaches more than 65%. It can be seen that the AgNPs exhibited efficient catalytic activity in the degradation of MB and EV in the absence and presence of H_2O_2.

V. BIOLOGICAL STUDIES

5.1 Anti-urolithiasis:

Silver nanoparticles (AgNPs) were assessed for their potential to prevent urolithiasis through in vitro studies conducted in the laboratory. Struvite crystals were synthesized using sodium metasilicate, ammonium dihydrogen phosphate, and magnesium acetate. Various crystal morphologies, including X-shaped dendritic, rectangular platelet, and coffin-shaped structures, were observed in the gel medium, as shown in Figure 10. To evaluate the dissolution of struvite, the synthesized crystals were placed in a fresh beaker along with AgNPs and left for a day. The presence of AgNPs in the reaction mixture significantly inhibited and prevented struvite formation. These findings confirm that silver nanoparticles synthesized using Trachyspermum ammi exhibit excellent anti-urolithiatic activity.

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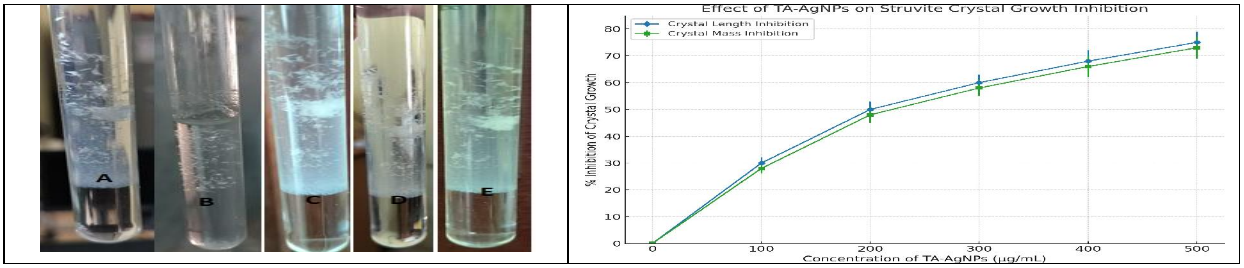

Figure 9. **struvite crystal formation in the gel method**

Antimicrobial studies:

The antibacterial properties of silver nanoparticle (AgNP) were assessed against the Gram-positive *Staphylococcus aureus* strain using the disk diffusion method. The results, illustrated in the figure, demonstrate the antimicrobial efficacy of AgNP's inhibition zone surrounding it, indicating its antimicrobial properties. This zone of inhibition confirms that the AgNP effectively disrupts bacterial membranes, leading to antimicrobial activity.

However, due to the slow diffusion of nanosilver particles contributed to the antibacterial activity. The inhibition zone observed. This increased antibacterial effectiveness is likely attributed to the higher concentration of AgNPs. Moreover, enhancing the coating thickness could further improve the antimicrobial efficiency against *S. aureus*.

SNo	Bacteria	Zone of Inhibition-ZOI (mm)	MIC (µg/mL)
1	Bacillus subtilis	16	8.8
2	Staphylococcus aureus	13	12.5
3	Pseudomonas aeruginosa	19	7.1
4	Escherichia Coli	15	9.4

Table.1.

VI. CONCLUSIONS

This study successfully demonstrated the in situ synthesis of silver nanoparticles using T. ammi leaf extract via microwave irradiation. The green synthesis approach provided an eco-friendly and rapid method for nanoparticle formation, eliminating the need for harmful chemicals. The synthesized Silver nanoparticles exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. The silver nanoparticles act as effective oxidising property in dye reduction. The urolithiasis inhibitory effect might have been due to rich content of bioactive phenols, flavonoid and terpenoid content of the *Trachyspermum ammi* the above studies conclude that the both *Trachyspermum ammi* (aijwan) and AgNPs can be employed as an alternative medicine for the prophylactic treatment of urolithiasis, antimicrobial.

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Conflict of Interest:

As authors, we declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest**.**

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