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Biosynthesis of silver nanoparticles using *Cymbopogon citratus* and their antimicrobial activity

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Abstract

The rise of antibiotic-resistant bacteria highlights the urgent need for alternative antimicrobial strategies. This study focuses on the eco-friendly biosynthesis of silver nanoparticles (AgNPs) using an aqueous extract of *Cymbopogon citratus* (lemongrass) and assesses their antibacterial efficacy against various pathogenic bacterial strains. Through a green synthesis approach, silver ions were successfully reduced to nanoparticles, facilitated by the natural phytochemicals in *Cymbopogon citratus*. Characterization techniques, including UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and Particle Size Analysis (PSA), confirmed the formation of spherical AgNPs with a size range of 40-60 nm. Antibacterial assays revealed that the synthesized AgNPs exhibited potent antimicrobial activity against the tested bacterial strains i.e. 8.6 mm for *E. coli*, 9.3 mm for *S. aureus*, 8.0 mm for *T. viride* and 8.2 mm for *A. awamori*. These findings suggest that *Cymbopogon citratus* not only acts as a natural reducing and stabilizing agent for the synthesis of AgNPs but also enhances their antibacterial properties, offering a promising alternative in the battle against bacterial infections.

Keywords: *Cymbopogon citratus*, green synthesis, biosynthesis, antimicrobial activity, UV-Vis spectroscopy

Introduction

Textiles often referred to as the 'second skin,' play a crucial role in our daily lives, continuously interacting with the human body. As our lifestyle trends evolve, there is a growing need to enhance the properties of traditional fibers, incorporating innovative features to meet new demands and improve functionality (Kilinc-Balci, 2011 [3]; Syduzzaman *et al.*, 2016) [12]. Among these innovations, the development of antimicrobial textiles has gained significant attention due to their ability to inhibit the growth of bacteria, which can thrive in moist and warm conditions. These microorganisms are typically classified into Gram-positive and Gram-negative bacteria, each posing unique challenges to human health and hygiene (Sathianarayanan *et al.*, 2011) [8, 9].

The environmental impact of the textile industry remains a critical concern, particularly due to the discharge of high levels of chemical pollutants into natural ecosystems. To address these challenges, the industry is increasingly focusing on sustainable alternatives, including the development of antimicrobial finishing techniques to mitigate the harmful effects of microorganisms on textile materials (Sathianarayanan *et al.*, 2010) [8, 9]. These antimicrobial finishes not only enhance the hygiene and longevity of textile products but also reduce the dependence on chemical treatments that can be detrimental to the environment. In recent years, protective textiles have become a pivotal aspect of contemporary fabric applications, providing essential defense against hazardous environmental conditions and ensuring safety for individuals exposed to potential health risks (Zayed *et al.*, 2022) [14].

The application of nanoparticles in textiles, especially inorganic metal nanoparticles like silver, gold, zinc, copper, titanium, magnetite, and nickel, has revolutionized the approach to antimicrobial and protective fabric technologies. Silver nanoparticles (AgNPs), in particular, have garnered considerable interest due to their unique properties, such as low toxicity, long-term stability, versatile shapes (including spheres, prisms, or plates), and exceptional antibacterial and antifungal efficacy (Ghosh *et al.*, 2012; Huang *et al.*, 2019) [1, 2].

The antibacterial properties of AgNPs are largely influenced by their size, shape, and high surface-to-volume ratio, which significantly enhance their catalytic activity and interaction with microbial cells (Morones *et al.*, 2005) [5].

Among the natural biocides used in antimicrobial textile finishing, plant extracts and essential oils have proven to be highly effective. They are known for their broad-spectrum bactericidal and fungicidal properties, making them ideal candidates for sustainable and eco-friendly antimicrobial treatments. *Cymbopogon citratus*, commonly known as lemongrass, is a fragrant perennial plant with significant economic and medicinal value. Beyond its widespread use in culinary applications for its sharp lemon flavor, lemongrass is recognized for its potent bioactive compounds, particularly citral, which exhibits strong antimicrobial properties (Korenblum *et al.*, 2013) [4]. These properties make lemongrass an excellent candidate for green synthesis approaches in developing antimicrobial agents.

Lemongrass not only serves as a natural remedy with anti-spasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic, and sedative properties but also finds application in various forms, including fresh leaves, dried powder, concentrated extract, or essential oil, depending on its intended use. The extraction of these bioactive compounds from lemongrass can be achieved through both traditional and modern techniques, optimizing their use in diverse industrial applications (Zinoviadou *et al.*, 2015) [15].

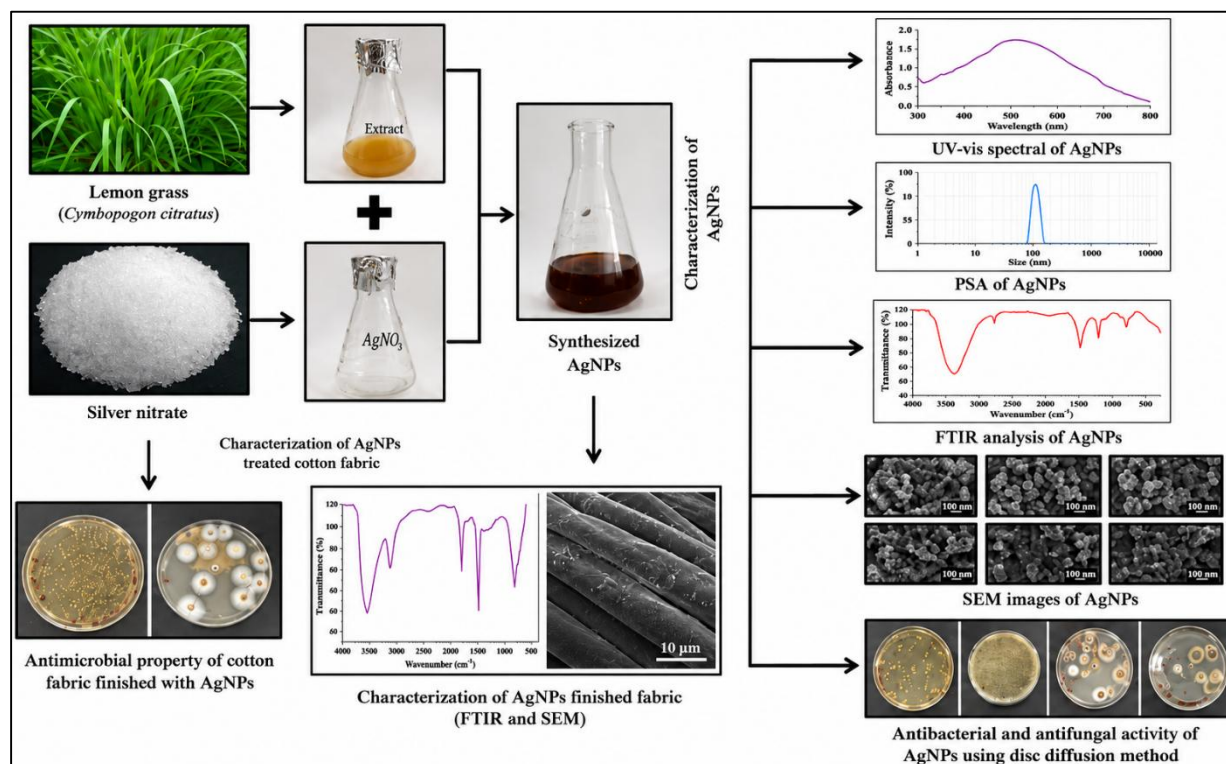
Nanotechnology has opened new avenues in the production of nanoparticles, defined as particles with a diameter of less than 100 nm. These nanoparticles exhibit unique and enhanced properties due to their small size, distinct morphology, and higher surface-to-volume ratio, which significantly boost their catalytic and antibacterial activities

(Rakib-Uz-Zaman and Khan, 2022) [7]. Several methods, including chemical, physical, and biological approaches, have been developed for nanoparticle synthesis. However, biological methods, also known as "green synthesis," have gained preference due to their eco-friendliness, cost-effectiveness, and reduced reliance on toxic chemicals and non-polar solvents, which are typically associated with chemical synthesis. This approach aligns with the growing demand for sustainable and safe production techniques suitable for clinical and environmental applications.

The current study focuses on the biosynthesis of silver nanoparticles (AgNPs) using *Cymbopogon citratus* as a natural resource. This plant-based method not only supports the principles of green chemistry but also enhances the value of textile materials by integrating eco-friendly and biodegradable antimicrobial finishes. The ultimate goal is to create functional textiles that not only meet environmental standards but also provide effective protection against harmful microorganisms. This innovative approach aims to harness the natural properties of *Cymbopogon citratus* to develop sustainable solutions for the textile industry, thereby contributing to a cleaner and safer environment.

2. Materials and Methods

The silver nanoparticles (AgNPs) using the aqueous extract of *Cymbopogon citratus* were synthesized and characterization of the synthesized AgNPs was performed using UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and Particle size analysis (PSA). The evaluation of the antibacterial activity of the synthesized silver nano particles against several pathogenic bacterial strains was done following material and methods:



2.1 Preparation of plant extract

Fresh green leaves of lemon grass were collected and washed thoroughly to get rid of all dust and unwanted particles. The lemon grass leaves were kept for drying for two hours on the water absorbent paper at room temperature. The dried plant leaves were ground to make paste.

For extraction, four grams of lemon grass leaves were added to 40 ml of distilled water (1:10 ratio). Solution was boiled for 15-20 minutes at 100 °C. After cooling, Whatman No. 1 filter paper (pore size: 11 μm) was employed for removing debris from the extract solution and stored at 4 °C for further use.

2.2 Synthesis of silver nanoparticles using lemon grass leaves extract

The synthesis of silver nanoparticles was done by mixing of 1mM silver nitrate (AgNO_3) solution with lemon grass leaves extract in 1:5 proportions having 7 pH in a clean sterilized conical flask. The mixture was kept under dark condition for 24 hours at room temperature. A change in colour was observed which confirmed the synthesis of nanoparticles.

2.3 Characterization of synthesized nanoparticles

Characterization of silver nanoparticles synthesized from lemon grass leaves extract was done by using UV visible (UV-vis) spectrophotometer, Particles Size Analyzer (PSA), Fourier-Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).

2.3.1 UV-Visible spectral analysis

The reduction of silver nanoparticles from lemon grass leaves extract in reaction mixture was monitored by using UV-Visible double beam spectrophotometer (Model- eppendorf Biospectrometer basic) at a wavelength of 300-600 nm. UV-vis spectral analysis of synthesized silver nanoparticles was got done at Department of Microbiology, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar to confirm the formation of silver nanoparticles.

2.3.2 Particle size analysis (PSA)

The particle size range along with polydispersity of the silver nanoparticles synthesized from lemon grass leaves was determined using a Particle Size Analyser (Model- Microtrac). The PSA of the synthesized silver nanoparticles was tested by Center for Bio Nanotechnology, CCSHAU, Hisar.

2.3.3 Fourier transform infrared spectral (FTIR) analysis

The residual solution containing nanoparticles was centrifuged at 4800 rpm for 10 minutes and the resulting suspension was redispersed in 20 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was completely dried at 60 °C. Finally, the dried nanoparticles were analysed by Nicolet-is 50 (Thermo Scientific) to determine the functional groups present in the lemon grass extract that led to the formation of silver nanoparticles. The absorption bands were observed in the regions of 500-4000 cm^{-1} . The extract and synthesized silver nanoparticles were sent to Guru Jambheshwar University of Science and Technology, Hisar, Haryana (India) for the FTIR analysis.

2.3.4 Scanning electron microscopy analysis (SEM)

The nanoparticles synthesized from lemon grass extract were subjected to Scanning Electron Microscopy analysis in order to study the surface morphology. The sample was sent to GJU University, Hisar for the SEM analysis. The SEM images of the silver nanoparticles were captured using Scanning Electron Microscope (SEM) (7610F Plus/JEOL-model) at different magnifications, according to test standard ISO 17751:2007. The samples were coated with a Gold-Palladium alloy and positioned on a flat platform beneath the electron gun. The electron gun emitted electrons that penetrated the sample surface, producing numerous low-energy secondary electrons. These secondary electrons' intensity depended on the sample's surface topography.

Consequently, an image of the sample surface was generated by recording the secondary electron intensity while scanning the primary electron beam across its positions.

2.4 Analysis of antimicrobial activity of synthesized silver nanoparticles

The antimicrobial efficacy of the synthesized silver nanoparticles was assessed against microbial growth using disc agar diffusion method. The experiment was conducted in Laminar Air Flow Chamber under controlled conditions.

2.4.1 Selection of test microorganisms

Pure cultures of test organisms viz., one Gram negative bacteria (*E. coli*); one Gram positive bacteria (*S. aureus*); one unicellular fungi (*Trichoderma viride*) and onefilamentous fungi (*A. awamori*) were used for testing the antimicrobial activity of synthesized silver nanoparticles. The bacterial and fungal strains were obtained from the Department of Microbiology, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Nutrient Agar medium was used for bacterial growth. Potato Dextrose Agar was used for fungal growth. Inoculums were prepared by aseptically transferring 1 ml of microbial culture in conical flasks (100 ml) having 50 ml of respective broth media (Nutrient broth for bacterial culture and Potato Dextrose broth for fungal culture). The inoculated conical flasks were incubated in orbital incubator shaker for 24 hrs at 37 °C for bacteria and 28 °C for fungal strains. After subsequent hours, the microbial growth in the broth flasks was appeared in the form of turbidity and the flasks were stored at 4 °C for further research work.

2.4.2 Preparation and composition of media

All the components of media which were used to test the antimicrobial activity of silver nanoparticles against various microorganisms were dissolved in distilled water and the volume was made one litre in a conical flask and mixed properly to get a uniform mixture. The solution was sterilized in autoclave at 15 lbs pressure at 121 °C temperature for 20 minutes for absolute suspension. The prepared media was allowed to cool and poured into sterile petri plates and agar medium was left to solidify in Laminar Air Flow Chamber. The petri plates containing medium were kept in incubator for 24 hours to check any type of microbial growth. The sterile medium petri plates were stored at 2-8 °C.

2.4.3 Disc agar diffusion method

The antimicrobial susceptibility tests were carried out using disc diffusion assay. From the bacterial cultures, 0.1 ml of culture was taken with the help of micropipette and uniformly spread on Nutrient agar plates using sterile glass spreader. Then cultured petri plates were kept for 20 minutes for drying. The sterile filter paper discs (Whatman no. 1, 6 mm in diameter) were impregnated with different concentrations (10 μl , 20 μl , 30 μl and 40 $\mu\text{l/ml}$) of synthesized silver nanoparticles (from lemon grass leaves) and were placed on the surface of agar plates. Plates were incubated in inverted position at 37 °C for 48 hours in a BOD incubator to analyse the microbial growth. The antimicrobial agent diffused into the agar, inhibiting germination and growth of the test microorganisms. Then the diameters of the zone of inhibition produced by silver nanoparticles were measured by measuring scale in

millimeter (mm). All the experiments were performed in triplicate to confirm the reproducibility and reliability. Similarly, antifungal activity of silver nanoparticles was screened on Potato Dextrose Agar plates using disc agar diffusion method and plates were incubated at 28 °C for 36–48 hrs. After incubation period, zones of inhibition were measured in millimeter (mm) (Nasr *et al.*, 2019) [6].

3. Results and Discussion

The biosynthesis process of silver nanoparticles using *Cymbopogon citratus* leaves extract was characterized by a noticeable color change in the solution, indicating successful reduction of silver ions to nanoparticles. This was further confirmed through various analytical techniques, including UV-Vis spectroscopy, Particle size analysis and Fourier-Transform Infrared spectroscopy, SEM, which provided insights into the size and morphology of the synthesized nanoparticles. The antibacterial efficacy was assessed against

common pathogens, namely *Escherichia coli* and *Staphylococcus aureus*, demonstrating significant inhibition zones.

3.1 Green synthesis of silver nanoparticles

The silver nanoparticles were synthesized with optimized variables *i.e.* concentration of lemon grass leaves extract (5 ml) with 1mM concentration of AgNO₃ solution at 7.0 pH for 24 hours at room temperature (Table 8). The lemon grass leaves (LGL) extract was used as reducing as well as capping agent. It was observed that the light yellow colour of lemon grass extract was changed to dark brown colour, indicating the formation of silver nanoparticles (AgNPs). But no colour change was observed in control (without addition of lemon grass leaves extract in the silver nitrate solution) as reflected in Fig. 7. Ag⁺ ions were reduced into Ag⁰ nanoparticles when lemon grass leaves extract was mixed with silver nitrate (AgNO₃) solution.

Table 8: Optimized variables for green synthesis of silver nanoparticles from lemon grass

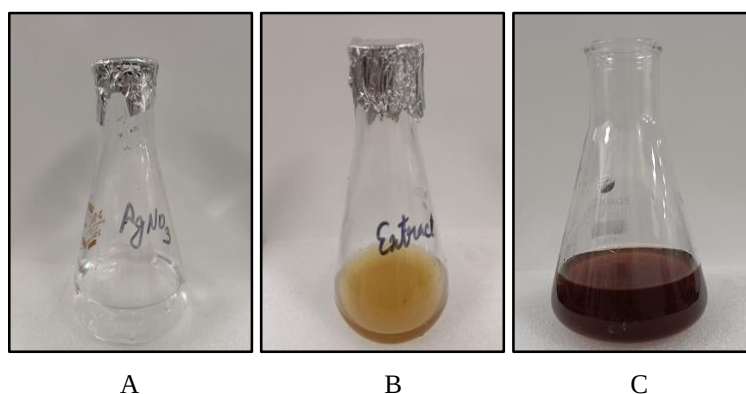
Variables	Optimized concentration/conditions
Concentration of extract	5 ml
Concentration of silver nitrate	1 mM
pH	7.0
Synthesis time	24 hours

The reduction in pH and colour of reaction mixture (lemon grass extract + AgNO₃) during the synthesis of silver nanoparticles was recorded (Table 9 and Fig. 7). Initially, colour of the lemon grass extract was very light yellow and pH was 5.76 respectively. The silver nitrate solution was colourless and pH was 5.08. When lemon grass leaves

extract was mixed with silver nitrate solution, the reduction in reaction mixture took place and a change of colour from light yellow to dark brown was observed. The pH of reaction mixture also reduced to 5.24 that indicated the synthesis of silver nanoparticles.

Table 9: Changes in synthesis parameters during formation of silver nanoparticles from lemon grass

Reaction mixture	Initial colour of extracts	Initial pH of extracts	Colour after reduction (AGNPS)	pH after reduction (AGNPS)
Silver Nitrate (AgNO ₃)	Colourless	5.08	-	-
LGL	Light yellow	5.76	Dark brown	5.24



A-Silver nitrate solution, B- lemon grass plant extracts and C- synthesized silver nanoparticles

Fig 7: Synthesis of silver nanoparticles from lemon grass under optimized conditions

3.2 Characterization of synthesized silver nanoparticles

The synthesized silver nanoparticles were characterized by using various physico-chemical techniques such as UV–vis Spectroscopy, Particle Size Analyzer (PSA), Fourier-Transform Infrared Spectroscopy (FTIR) analysis and Scanning Electron Microscopy (SEM).

3.2.1 UV-vis spectral analysis

UV–vis spectroscopy was used to examine size, shape and stability of silver nanoparticles in aqueous suspensions. The

bio reduction of silver ions (Ag⁺) to silver nanoparticles (Ag⁰) using lemon grass leaves extract was characterized by using UV-Vis spectrophotometer at the range of 300–600 nm, maximum deflection was observed at 430 nm of the silver nanoparticles synthesized from lemon grass extract (Fig. 8). The UV–vis absorbance of the reaction mixture (lemon grass extract + silver nitrate solution) was recorded after 24 hrs of reaction. The UV–vis absorbance of 1.929 for lemon grass leaves (LGL) was observed.

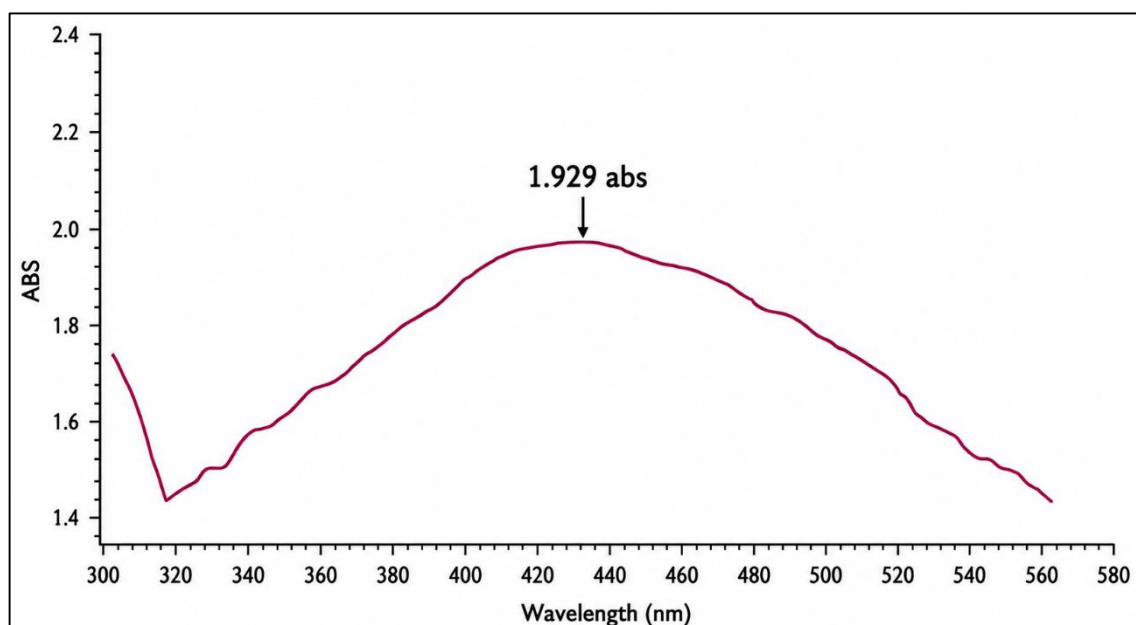


Fig 8: UV-Vis spectral analysis of synthesized silver nanoparticles

3.2.2 Particle size analysis (PSA)

The size of the synthesized silver nanoparticles (AgNPs) was measured by particle size analyzer. The particle size distribution curve showed that the average size of silver nanoparticles synthesized from lemon grass leaves extract was 43.00 nm in diameter (Fig. 9). The polydispersity

indexes (PDI) of the silver nanoparticles suspension were 0.17, which indicated that synthesized particles are more or less homogeneous. The PDI value “0” represents monodisperse distribution, whereas the value “1” represents polydisperse distribution.

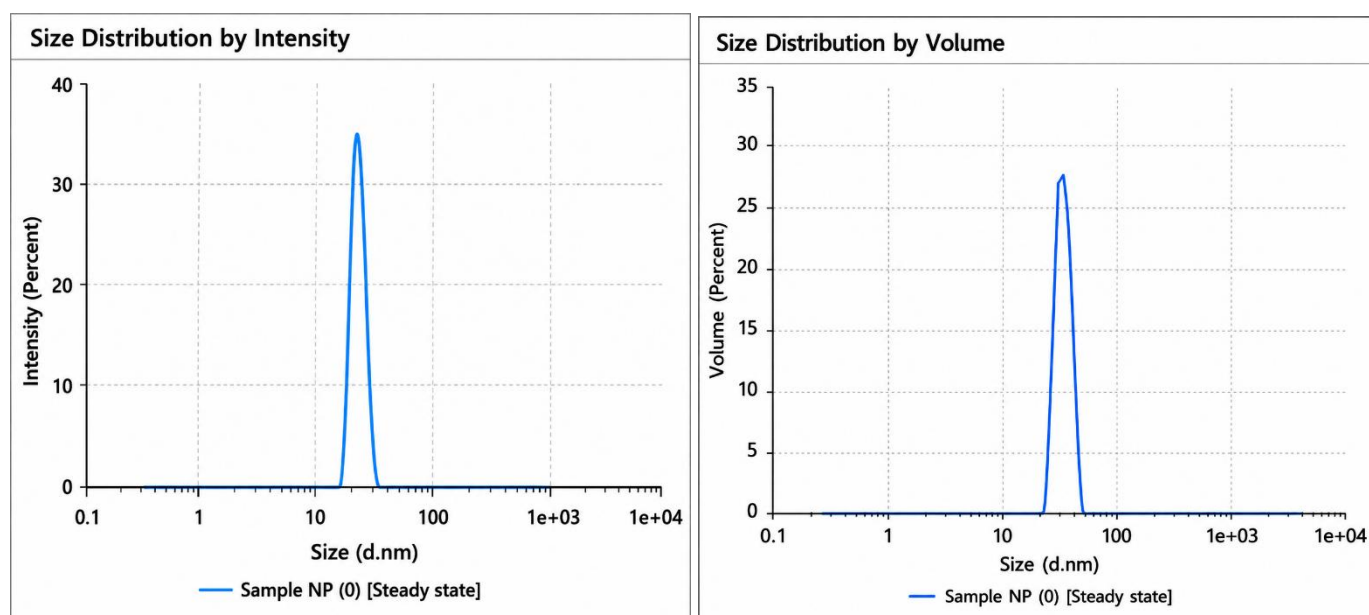


Fig 9: Particle size analysis of synthesized silver nanoparticles

3.2.3 Fourier-Transform Infrared spectroscopy (FTIR) analysis

FTIR analysis was carried out for the identification of possible functional groups in bio molecules, present in the lemon grass leaves extract responsible for the reduction of the silver ion (Ag^+) into silver nanoparticles (AgNPs). FTIR spectrum of the silver nanoparticles is shown in Fig. 10. In the synthesized nanoparticles, two main peaks can be allocated to the stretching (3451.07 cm^{-1}) which was assigned to $-\text{OH}$ stretching of alcohol and bending (1634.56 cm^{-1}) denotes the $\text{C}=\text{O}$ and weak $\text{C}=\text{C}$ stretching of aldehyde and ketone. The peak allocated at 2925.9 cm^{-1} shows the $\text{C}-\text{H}$ stretching of alkanes. Peak at 2850 related to symmetric and asymmetric stretching of CH_2 , presence of

similar carbon stretch. The peak attributed at 1470.7 cm^{-1} associated the $\text{C}-\text{H}$ and $\text{N}-\text{O}$ stretching of alkane and nitro group.

Observed spectra within $1000-1400$ range correspond to $\text{N}-\text{H}$ stretch of primary and secondary amines and $\text{C}-\text{O}$ stretch (groups of polyphenols like flavonoid, terpenoids and polysaccharides) (Vankar and Shukla, 2012) [13].

The FTIR results confirmed the presence of $-\text{N}-\text{H}$, $-\text{OH}$, $\text{C}=\text{C}$ and $\text{C}-\text{H}$ groups, which indicated that the plant extract containing the hydroxyl and amine groups that evidence the presence of flavonoids and terpenoids. Further, presence of flavonoids was responsible for reducing Ag^+ to Ag^0 and an amino group stabilized the synthesized nanoparticles.

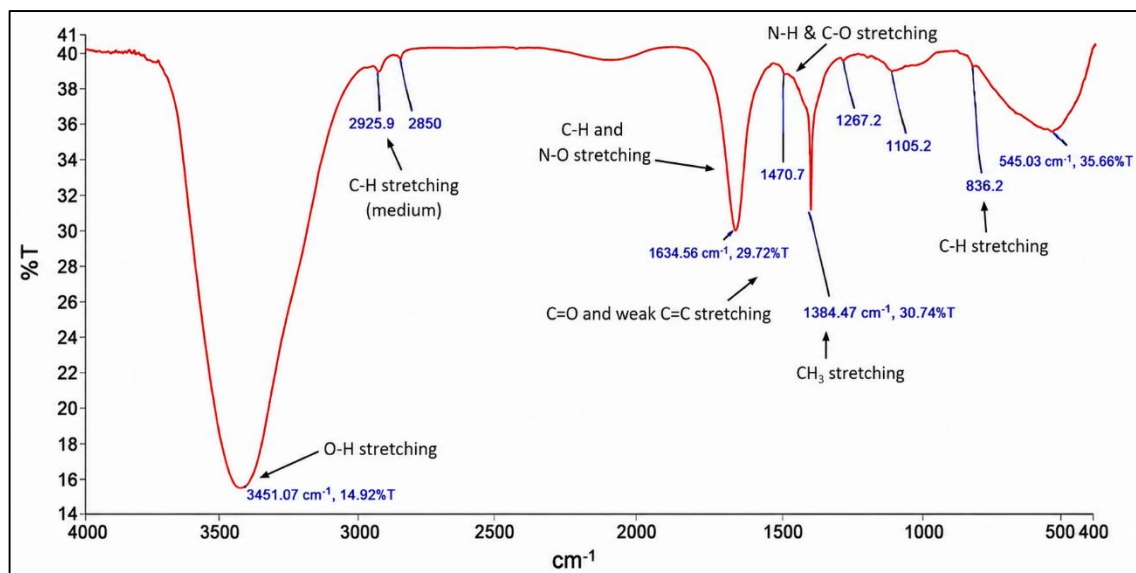


Fig 10: FTIR spectrum of synthesized silver nanoparticles

3.2.4 Scanning electron microscopy (SEM)

The shape of the synthesized silver nanoparticles was analyzed by SEM. Figure 11 show the result of surface morphological and nanostructural studies using SEM images at 10,000, 20,000, 30,000, 50,000, 80,000 and 100,000x.

The results showed monodispersed spherical silver nanoparticles of varying sizes and shapes. Overall the synthesized lemon grass silver nanoparticles are spherical in shape, and well dispersed with low agglomeration.

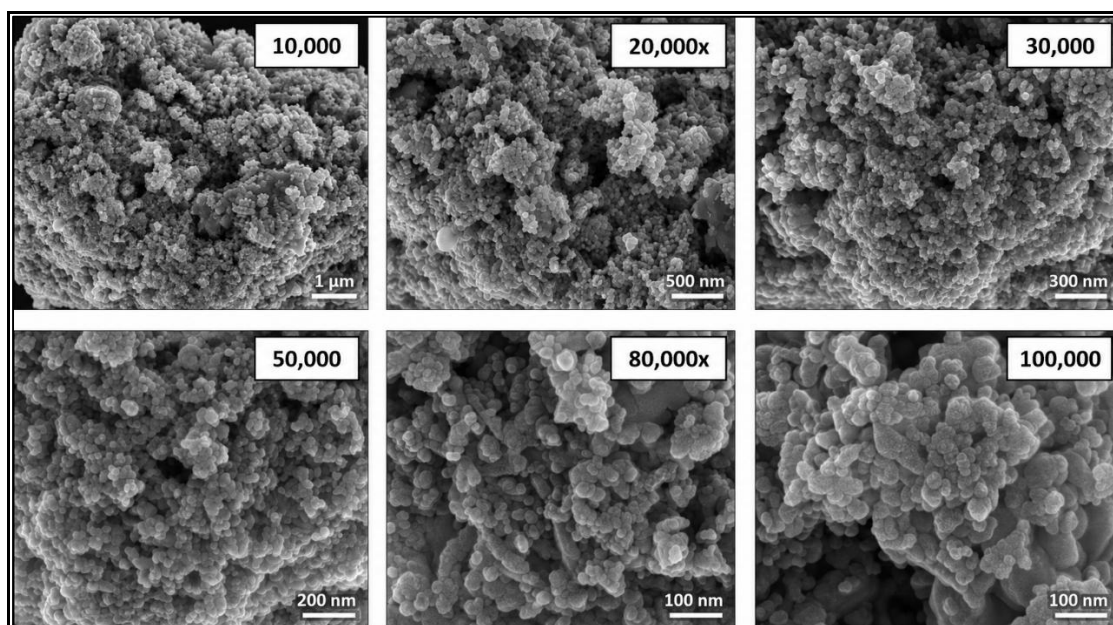


Fig 11: SEM images of synthesized silver nanoparticles

3.3 Antimicrobial activity of silver nanoparticles against selected microorganisms

The antimicrobial activity of silver nanoparticles (AgNPs) synthesized from lemon grass leaves extract were tested

using disc diffusion method against common human pathogenic micro-organisms i.e. *E. coli*, *S. aureus*, *T. viride* and *A. awamori*.

Table 10: Antimicrobial activity of Lemon grass extract against selected microorganisms using disc diffusion method

Microorganisms	Lemon grass extract zone of inhibition (MM)*
Gram negative bacteria	
<i>E. coli</i>	8.6 ± 0.16
Gram positive bacteria	
<i>S. aureus</i>	9.3 ± 0.06
Unicellular Fungi	
<i>T. viride</i>	8.0 ± 0.04
Filamentous Fungi	
<i>A. awamori</i>	8.2 ± 0.11

Table 10 showed that, silver nanoparticles synthesized from lemon grass extract showed inhibition zone of 8.6 mm, 9.3 mm, 8.0 mm and 8.2 mm for *E. coli*, *S. aureus*, *T. viride* and *A. awamori*, respectively. Thus, it was found that, silver nanoparticles synthesized from lemon grass leaves extract has showed maximum zone of inhibition for gram positive bacteria as compared to other tested microorganisms.

3.3.1 Antimicrobial activity of silver nanoparticles synthesized from lemon grass extract

The antimicrobial activity of silver nanoparticles (AgNPs) synthesized from lemon grass leaves extract at different concentrations were tested using disc diffusion method against common human pathogenic microorganisms *i.e.* *E. coli*, *S. aureus*, *T. viride* and *A. awamori*. The concentrations taken were 10 μ L, 20 μ L, 30 μ L and 40 μ L/ml. The results attained were calculated in terms of zone of inhibition by performing triplicate experiments.

The data in Table 11 revealed that, silver nanoparticles synthesized from lemon grass extract showed inhibition zone of 8.3 mm, 9.6 mm, 10.6 mm and 8.8 mm against *E. coli* whereas 7.8 mm, 8.9 mm, 11.9 mm and 9.1 mm at 10, 20, 30 and 40 μ L/ml, respectively against *S. aureus*.

The lemon grass leaves extract mediated silver nanoparticles exhibited inhibition zone of 5.3 mm, 6.8 mm, 8.6 mm and 7.8 mm against *T. viride* whereas 6.5 mm, 7.3 mm, 8.8 mm and 7.2 mm at 10, 20, 30 and 40 μ L/ml, respectively against *A. awamori*. Thus, it was found that with an increase in the concentration from 10 μ L to 30 μ L of silver nanoparticles, the zone of inhibition indicating antimicrobial activity against test microorganisms increased. With further increase in concentration *i.e.* from 30 μ L to 40 μ L, slightly decrease in zone of inhibition was observed. The results clearly demonstrate that the silver nanoparticles synthesized from lemon grass leaves extract are promising antimicrobial agents against the pathogens employed.

Table 11: Antimicrobial activity of synthesized silver nanoparticles against selected microorganisms

Bacteria	Control	Concentration of silver nanoparticles (μL/ML)and Zone of inhibition (MM)*			
		10 μl	20 μl	30 μl	40 μl
Gram negative bacteria					
<i>E. coli</i>	No Inhibition	8.3 ± 0.32	9.6 ± 0.41	10.6 ± 0.15	8.8 ± 0.34
Gram positive bacteria					
<i>S. aureus</i>	No Inhibition	7.8 ± 0.40	8.9 ± 0.17	11.9 ± 0.44	9.1 ± 0.13
Unicellular Fungi					
<i>T. viride</i>	No Inhibition	5.3 ± 0.15	6.8 ± 0.43	8.6 ± 0.22	7.8 ± 0.18
Filamentous Fungi					
<i>A. awamori</i>	No Inhibition	6.5 ± 0.15	7.3 ± 0.23	8.8 ± 0.05	7.2 ± 0.04

Values $\pm$ SD indicates the replicates of three experiments

*No activity (-mm), weak (<6 mm), moderate (7-12 mm) and strong activity (>12)

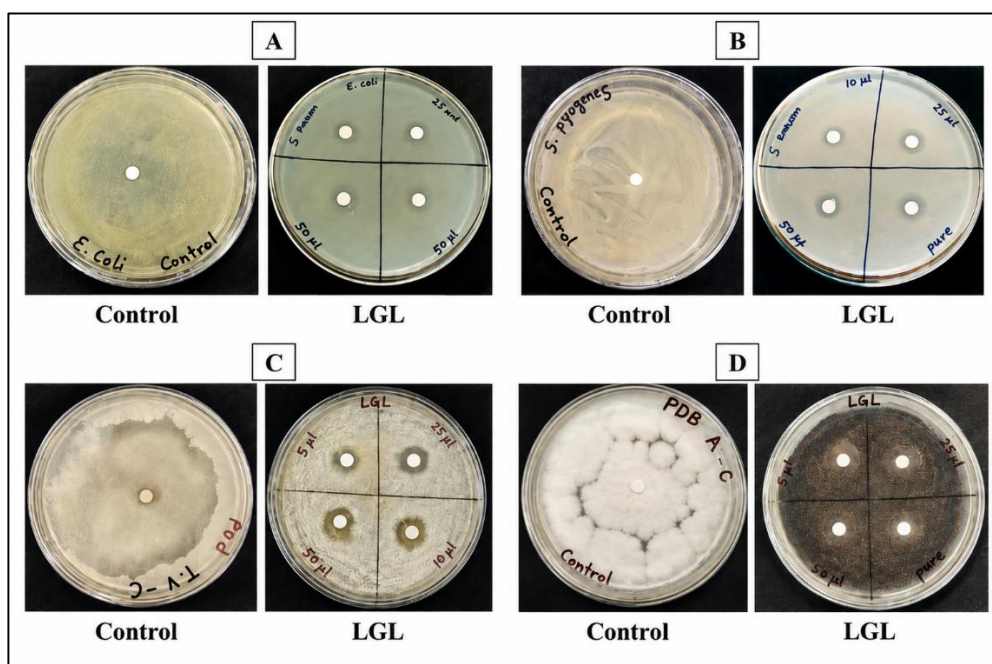


Plate 1: Antimicrobial activity of synthesized silver nanoparticles against *E. coli* (A), *S. aureus* (B), *T. viride* (C) and *A. awamori* (D)

These findings underscore the potential of *Cymbopogon citratus* derived silver nanoparticles as effective antimicrobial agents, highlighting their application in various fields, particularly in enhancing the properties of textiles. The discussion will delve into the implications of these results, exploring the mechanisms underlying the antibacterial activity and the broader significance of using plant-based synthesis methods in nanotechnology.

Conclusion

This study highlights the use of plant materials for synthesizing silver nanoparticles with antibacterial properties. In conclusion, the green-synthesized silver nanoparticles showed remarkable antimicrobial activity against *E. coli* and *S. aureus*. The extraction of silver nanoparticles from lemon grass leaves proved to be a cost-effective, simple, and environmentally friendly approach.

Incorporating these synthesized nanoparticles as an antimicrobial finish could significantly benefit the textile industry by reducing harmful industrial waste that is challenging to manage. Overall, the findings suggest that silver nanoparticles derived from lemon grass leaves extract present a promising solution for enhancing the functionality of cotton fabric while ensuring comfort and sustainability.

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