

# LEAF EXTRACT MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *CYMBOPOGON CITRATUS* FOR TREATMENT OF SKIN INFECTIONS

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## Abstract

Skin infections are caused by variety of germs, and symptoms vary from mild to serious. Bacterial and Fungal infections require the special attention as they affect 80% of serious skin infections. Development of specific consortium between antibiotic and microorganism during repeated treatment develops antibiotic resistance. Treatment of skin infections are considered to be as a major research area in personal cosmetic care industries and pharmaceutical. Nanotechnology has played vital role in the search for new ways to prevent and treat infections, including metallic nanoparticles with antimicrobial properties. Green synthesis of silver nanoparticles (AgNPs) has gained a vital role in society. In this concern, Indian flora has yet to reveal innumerable roots of cost effective non-hazardous reducing and stabilizing compound utilized in preparing AgNPs. This study investigates an efficient and sustainable route of AgNPs preparation using leaf extracts of *Cymbopogon citratus* well adorned for their wide availability and medicinal property. The AgNPs were duly synthesized and characterized. The biosynthesized AgNPs exhibited antibacterial activity against gram negative and gram positive bacteria. This study also aims at evaluating the antifungal properties of AgNPs.

Keywords: Silver nanoparticles; *Cymbopogon citratus*; Antibacterial; Antifungal

## 1. Introduction

The development of green synthesis of nanoparticles is evolving into a vital part of nanotechnology. The research on synthesized nanomaterials and their characterization is an emerging field of nanotechnology from the past two decades, due to their huge applications in the fields of physics, chemistry, biology and medicine. Plant mediated nanoparticle synthesis has been achieved using environmentally acceptable, ecofriendly reducing and capping agents. Biologically mediators are presently used for nanoparticle synthesis. The use of plants for synthesis of nanoparticles is expeditious, cost effective, eco-friendly, safe for human therapeutic use and a single-step method for biosynthesis process<sup>[1]</sup>.

Silver nanoparticles (AgNPs) assuring utilization in wide range fields from engineering to applied sciences because of its high surface to volume ratio. AgNPs is non-toxic so it is used for curing different types of diseases causing due to bacterial as well as fungal infections. Silver nanoparticles showed antimicrobial property by attaching with highly reactive faces of the bacterial cell wall and inhibit their metabolism<sup>[2]</sup>. Plant extracts acts as reducing and capping agent for bioprocessing of AgNPs, as extract comprised of biomolecules such as vitamins, polysaccharides, proteins, amino acids, enzymes, and organic acids<sup>[2]</sup>.

*Cymbopogon citratus* is an aromatic, evergreen, cluster-forming, continual grass producing numerous rigid stems originating from a short rhizomatous rootstock, and growing approximately 1.5 metres tall. In Some regions it grows below 500 metres. The plant is widely cultivated in the tropical regions, both on a commercial scale as well as in gardens. *Cymbopogon citratus* exhibited high *in vitro* antiplasmodial activity, which may be due to the presence of compounds such as alkaloids, terpenoids and flavonoids that were previously isolated from the plant. Lemon grass is a bitter, aromatic, cooling herb that increases sweating and relieves muscle spasms. The essential oil obtained from the plant is an effective antimicrobial<sup>[3]</sup>. The

essential oil contains about 70% citral, plus citronellal - both of these are markedly depressant. Internally, the plant is used chiefly as a tea in the treatment of digestive problems, where it relaxes the muscles of the stomach and gut, relieving localized pains and food intolerance. It is particularly useful for children, for whom it is also used to treat minor febrile illnesses<sup>[3]</sup>. Externally, the plant is a very effective treatment for wide range of skin impetigo and candidiasis.

## 2. Material and Methodology

### 2.1 Preparation of plant extract

*Cymbopogon citratus* leaf extract was used to prepare silver nanoparticles on the basis of cost- effective, ease of availability and its medicinal property. Fresh leaves were collected from Kardehalli, South Solapur. They were surface cleaned with running tap water to remove the dirt and other contaminated organic contents, followed by double distilled water. Take 10 gm fresh leaves of *Cymbopogon citratus* and crush finely in mortar - pestle then add 100mL of deionized water to it. The extract was filtered through Whatman filter paper no 1 and cooled down. The prepared solution then stored at 4°C for further use<sup>[5]</sup>.

### 2.2 Synthesis of Silver nanoparticles

The filtrate was treated with aqueous 1 mM AgNO<sub>3</sub> solution in an Erlenmeyer flask and incubated at room temperature in dark condition for 6 hours. The setup was incubated in a dark room to minimize photo-activation of silver nitrate at room temperature. Reduction of Ag<sup>+</sup> to Ag<sup>0</sup> was confirmed by the colour change of solution from colourless to yellowish brown. Its development was also confirmed by using UV-Visible spectroscopy<sup>[1,5]</sup>.

### 2.3 Characterization of synthesised silver nanoparticles

Synthesis of silver nanoparticles solution with leaves extract may be easily detected by ultraviolet-visible (UV-Vis) spectroscopy. UV-Vis spectra of aliquots were monitored as a function of time of reaction on spectrophotometer in 400–600 nm range operated at a resolution of 1 nm. FT-IR spectra of were recorded on Perkin Elmer 1750 FTIR Spectrophotometer. X-Ray diffraction(XRD) analysis was performed by Xpert-pro using monochromatic Cu K $\alpha$  radiation ( $\lambda=1.5406\text{\AA}$ ) operated at 40kV and 30mA at a 2 $\theta$  angle pattern<sup>[5]</sup>.

### 2.4 Antimicrobial activity

*Staphylococcus aureus*, *Bacillus subtilis*, *Cutibacterium acne*, *Escherichia coli*, *Proteus vulgaris*, *Kleibseilla pneumoniae* and fungal strain *Candida albicans* were chosen based on their clinical and pharmacological importance. The bacterial and fungal stock cultures were cultured for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium, respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C, whereas the yeast was grown on Sabouraud dextrose agar at 28°C. The stock cultures were maintained at 4°C.

*In vitro* antibacterial and antifungal activities were examined for AgNPs. Antibacterial and antifungal activities of plant part extracts against six pathogenic bacteria (three Gram-positive and negative) and one pathogenic fungi were investigated by the agar disk diffusion method. All the extracts were screened for their antibacterial and antifungal activities against the *Escherichia coli*, *Proteus vulgaris*, *Kleibseilla pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Cutibacterium acne* and the fungi *Candida albicans*. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using tetracycline for antibacterial activity and nystatin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 24 hours of incubation at 37°C for bacteria and 48 hours for fungi at 28°C. The sensitivities of the microorganism species to the AgNPs were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks<sup>[5,6]</sup>.

## 2.5 Haemolytic assay

*In vitro* haemolytic activity was performed by spectroscopy method<sup>[7]</sup>. A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the AgNPs. The reaction mixture were incubated for 30 min at 37°C in an incubator. The reaction mixture was centrifuged at 1500 rpm for 10 min in a cooling centrifuge. The suspended haemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as negative and positive haemolytic controls. Each experiment was performed in triplicates.

The level of percentage hemolysis by the extracts was calculated from following formula:

$$\% \text{ Hemolysis} = \frac{A_t - A_n}{A_c - A_n} \times 100$$

Here:  $A_t$  = absorbance of test sample.

$A_n$  = absorbance of the control (saline control)

$A_c$  = absorbance of the control (water control)

## 2.6 Free radical scavenging activity

A 1ml of 0.1 mM 2,2-diphenyl-1-picryl-hydrazyl(DPPH) was added to 3ml of AgNP solution. Ascorbic acid was used as positive control. After incubation for 30 mins in the dark, the discolouration was measured at 517 nm<sup>[8]</sup>. The capacity toto scavenge the DPPH radical was calculated and expressed as inhibition percentage using the following equation:

$$I\% = (\text{absorbance of control} - \text{absorbance of test} / \text{absorbance of control}) \times 100$$

## 3. Result And Discussion

### 3.1 Synthesis of AgNPs

Addition of leaf extract of *Cymbopogon citratus* into the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution to yellow to yellowish brown within reaction duration due to excitation of surface plasmon vibrations in silver nanoparticles.



Fig. 1 Photographs showing colour changes after adding AgNO<sub>3</sub> before reaction (a) and after reaction time of 6 h (b)

### 3.2 Characterization of AgNPs

#### 3.2.1 Ultraviolet visible spectroscopy

The silver nanoparticles are synthesized and characterized by UV-Vis. Absorbance at 445nm of silver nanoparticles synthesized by *Cymbopogon citratus*. The UV-Vis spectra and visual observation revealed that formation of silver nanoparticles occurred. AgNPs were ascertained by distinctive peak observed at 445 nm  $\lambda$  in the pilot range of 300-900 nm  $\lambda$ .

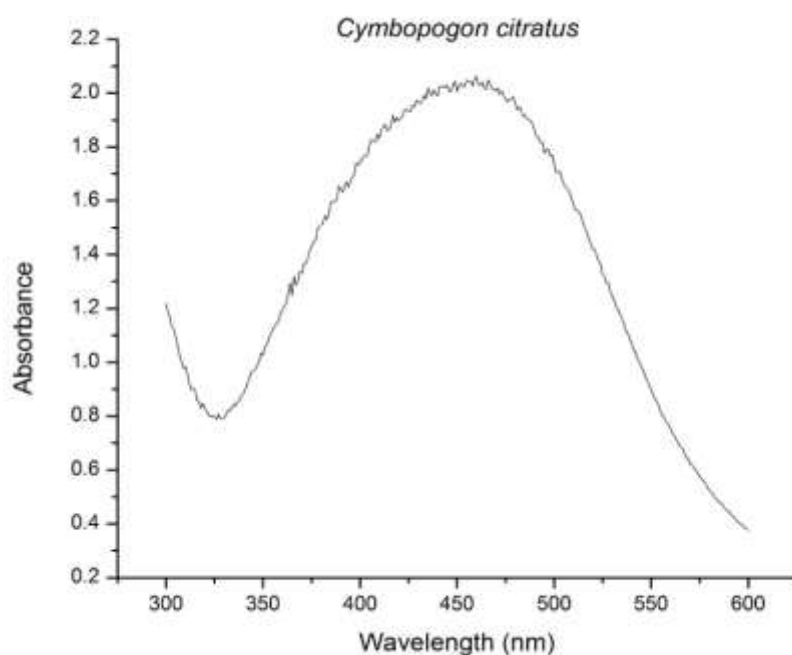


Fig. 2 UV-Vis absorption spectra of AgNPs synthesized from *C. citratus* by reduction of silver ions to AgNPs

#### 3.2.2 FT-IR

FT-IR spectroscopy is important to understand chemical composition of the capping agents on the nanoparticles. The FT-IR spectrum of silver nanopowder is shown in figure. Four main bands could be observed from the figure. The broad band appearing at  $3378\text{cm}^{-1}$  is assigned for O-H stretching vibrations indicating presence of hydroxyl groups in the reducing agents. The strong peaks at  $2916\text{cm}^{-1}$ ,  $1616.40\text{cm}^{-1}$  and  $1275.49\text{cm}^{-1}$  correspond to C-H, C=O and C-N stretch vibrations respectively. It confirms that the capping agents consist of aldehydes, ketones, alcohol, alkanes and amine functional groups. The result of this FT-IR spectroscopic study confirmed that lemongrass leaf extract has ability to perform dual functions of reduction and stabilization of silver nanoparticles.

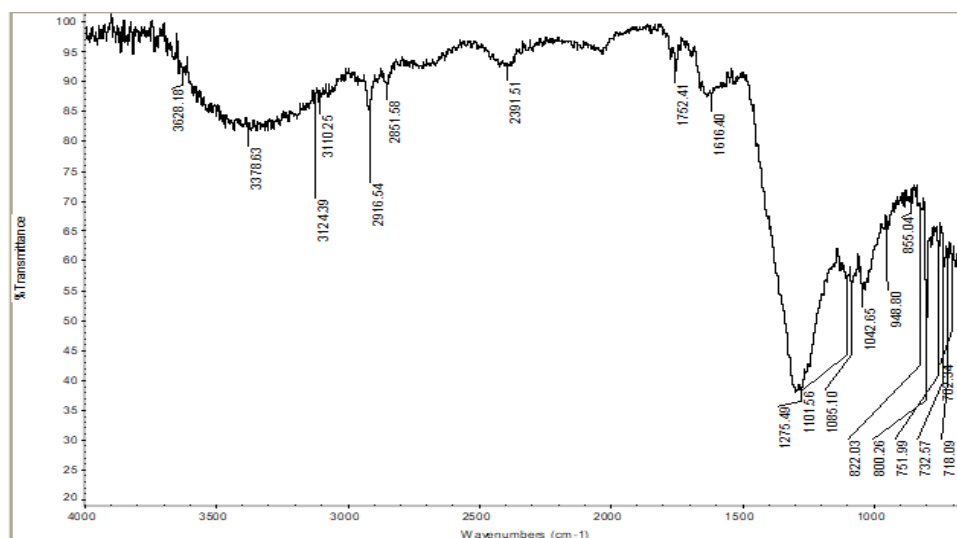


Fig. 3 FT-IR spectrum of synthesised AgNPs using *Cymbopogon citratus*

### 3.2.3 XRD analysis

The XRD pattern of synthesised AgNPs using *Cymbopogon citratus* leaf extract was shown in figure. The XRD was done to determine the crystalline nature of AgNPs and the resulted peaks were found at  $37.90^\circ$ ,  $44.05^\circ$ ,  $64.25^\circ$  and  $77.20^\circ$  corresponding to lattice plane value indexed at (111), (200), (220) and (311) planes of face centred cubic (FCC) silver with the average particle size estimated was approx. 6.45 nm.

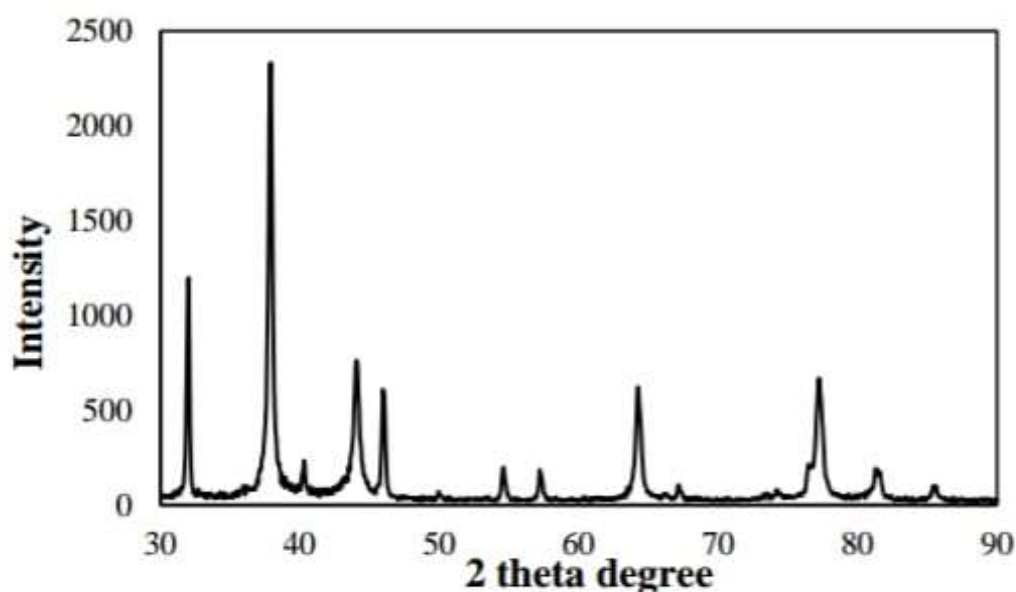


Fig. 4 XRD pattern of synthesised AgNPs from *Cymbopogon citratus*



### 3.3 Antimicrobial activity

The antimicrobial activity of the extracts of AgNPs from *Cymbopogon citratus* were studied against six pathogenic bacterial strains, three Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Cutibacterium acne*) three Gram-negative (*Escherichia coli*, *Proteus vulgaris*, *Kleibseilla pneumoniae* ), and one fungal strain (*Candida albicans*).

Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of microbial growth. As compared with standard drugs, the results revealed that in the AgNPs shows more potent antimicrobial activity. The growth inhibition zone measured ranged from 11 to 20 mm for all the sensitive bacteria, and ranged from 9 mm for fungal strain.



1



2



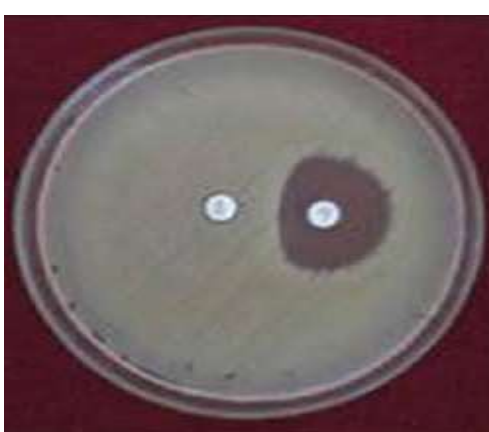
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Fig. 5 : Antimicrobial activity against 1. *Bacillus subtilis* 2. *Candida albicans*  
3. *Staphylococcus aureus* 4. *Klebsiella pneumoniae* 5. *Proteus vulgaris* 6. *Cutibacterium acne*  
7. *Escherichia coli*

### 3.4 Haemolytic activity

The samples shows low haemolytic effect toward human erythrocytes.

$$\% \text{ hemolysis} = (0.0229/2.345) \times 100 \\ = 0.9767\%$$

### 3.5 Free radical scavenging activity

The inhibition percentage of DPPH in presence of aqueous AgNPs is 91.66% at the concentration of 115  $\mu\text{g/mL}$ .



Before Incubation



After Incubation

#### 4. References

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