

Rapid Biosynthesis of Silver Nanoparticles Using *Cymbopogon Citratus* (Lemongrass) and its Antimicrobial Activity

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Abstract: The present study deals with the rapid green synthesis of silver nanoparticles using fresh leaves of *Cymbopogon Citratus* (Lemongrass). Silver nanoparticles were formed within 8~10 minutes by microwave irradiation using aqueous solution of AgNO_3 (1 mM) with fresh leaves extract of *Cymbopogon Citratus*. The synthesized silver nanoparticles were characterized by using UV-visible spectrophotometer analysis, nanoparticle tracking analyzer, transmission electron microscope and energy dispersive X-ray spectra. The antibacterial activity of these nanoparticles was studied against multiple drug resistant hospital isolates of *E.coli*, *S.aureus*, *P.mirabilis* and hospital isolates of *S. typhi*, *K.pneumoniae*. Also, the antifungal activity of these nanoparticles was studied against *C.albicans* (hospital isolate) and *A.niger* (NCIM 616). The synergistic effect of silver nanoparticles along with antibiotics was also studied against multiple drug resistant hospital isolates and found to be effective. The extracellular synthesis of Silver nanoparticles using leaves of *Cymbopogon Citratus* appears to be rapid and eco-friendly.

Keywords: Green synthesis; *Cymbopogon Citratus*; Nanoparticle tracking analyzer; Transmission electron microscope; Energy dispersive X-ray spectra

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Introduction

Nanoparticles are being considered to be the fundamental building blocks of nanotechnology. Nanotechnology is interdisciplinary which includes physics, chemistry, biology, material science and medicine. An important aspect of nanotechnology deals with the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. Nanoscale materials and structures are usually ranging from 1~100nm and is emerging area of nanoscience and nanotechnology. Synthesis of noble nanoparticles for the applications such as catalysis, electronics, environmental and biotechnology is an area of constant interest [1,2].

Generally metal nanoparticles are synthesized and stabilized by using physical and chemical methods: the chemical methods, such as chemical reduction [3,4], electrochemical techniques [5], and now a day via green chemistry route [6]. The biological methods are preferred than chemical methods because of the use of toxic chemicals on the surface of nanoparticles and non-polar solvents in the synthesis procedure limits their applications in clinical fields [7]. Therefore, development of clean, biocompatible, non-toxic and eco-friendly methods for nanoparticles synthesis has great importance. These biological methods are regarded as safe, cost-effective, sustainable and environment friendly as well as it do not require any special culture preparation and isolation techniques [8].

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Till now various plants have been used for synthesis of silver nanoparticles like *Pongamia pinnata* (*L*) *pierre* [9], *Allium cepa* [10], *Gliricidia sepium* [11], *Azadirachta indica* [12], *Carica papaya* [13], *Phyllanthus amarus* [14]. Shankar et al. [15] used lemongrass and geranium plant extracts to induce the formation of gold nanoparticles.

In present study, the fresh leaves extract of *Cymbopogon citratus* (Lemongrass) obtained by boiling the leaves in distilled water and it was used to synthesize silver nanoparticles from silver nitrate. The synthesized silver nanoparticles were characterized by using UV-visible spectrophotometer, nanoparticles tracking analyzer (NTA) and transmission electron microscopy (TEM) and energy dispersive X-ray spectra (EDX). After characterization silver nanoparticles were used for antibacterial and antifungal testing.

Materials and Methods

Preparation of leaf extract

Fresh leaves of *Cymbopogon citratus* (Lemongrass) were collected from local farm of Loni village, MS, India. The leaves were washed thoroughly with distilled water. About 50 gm of leaves were cut into small pieces. Finely cut leaves were dipped into a beaker containing 200 ml distilled water. After that the mixture was boiled for 10~12 minutes. The extract was filtered using Whatmann filter paper and filtrate was collected.

Synthesis of silver nanoparticles using extract of *Cymbopogon citratus* (Lemongrass) leaves

The extract of *Cymbopogon citratus* (Lemongrass) leaves was mixed with aqueous solution of 1 mM Silver nitrate (99.99%) in 1:4 ratio in conical flask under aseptic conditions. The pH was adjusted to 8.0. The conical flasks were then incubated at 37°C for 24 hours. A change in the color of the solution was observed.

Microwave irradiation mediated synthesis of silver nanoparticles

The extract of *Cymbopogon citratus* (Lemongrass) leaves was mixed with aqueous solution of 1 mM Silver nitrate in 1:4 ratio in conical flask under aseptic conditions. The pH was adjusted to 8.0. The solution was subjected to microwave irradiation (90 watts) till color change was observed.

UV-visible spectrophotometer analysis

After observing color change, the sample was subjected to mild sonication for 10 minutes. The bioreduction of silver ions in aqueous solution was monitored by UV-Vis spectra of the solution between 300~600 nm using Thermo-Biomate 3 UV-visible spectrophotometer.

Distilled water was taken to adjust the baseline.

Nanoparticle tracking analyzer (NTA) measurements

NTA analysis was carried out by using Nanosight-LM20 instrument. 0.3 ml samples were introduced to the viewing unit using a disposable syringe and enhanced by a near perfect black background; particles appear individually as point-scatterers moving under brownian motion.

Transmission electron microscopy (TEM) and energy dispersive X-ray spectra (EDX) analysis

Transmission electron microscopy (TEM) analysis of the sample was done using PHILIPS-CM 200 instrument operated at an accelerating voltage of 200 kV with resolution of 0.23 nm. A drop of solution was placed on carbon coated copper grid and later exposed to infrared light (45 minutes) for solvent evaporation. The EDX analysis was carried out using JEOL JSM 7600F.

Antibacterial Studies

The antibacterial activity of synthesized silver nanoparticles was studied against multiple drug resistant hospital isolates of *E.coli*, *S.aureus*, *P.mirabilis* and hospital isolates of *S.typhi*, *K.pneumoniae* (obtained from Department of Microbiology, Pravara Institute of Medical Sciences, Loni, MS, India).

Antifungal Studies

The antifungal activity was checked against *C.albicans* (hospital isolate) and *A.niger* (NCIM 616).

Well diffusion method

The well diffusion test was performed using Muller Hinton Agar no. 2. (Casein acid hydrolysate 17.50 gm/L, Beef heart infusion 2 gm/L, Starch soluble 1.5 gm/L, Agar 17 gm/L, pH 7.3 ± 0.2). The inoculum was prepared in sterile Nutrient Broth (Peptone 10 gm/L, Beef extract 10 gm/L, Sodium chloride 5 gm/L, pH 7.3) and the tube was incubated at 37°C until the turbidity was achieved up to the 0.5 McFarland standard (usually overnight) [16]. For *A.niger* inoculum preparation, spores were collected from 7 days old culture of *A.niger* with the help of sterile nicrome loop dipped in normal saline (0.85% w/v) containing Tween-20 (0.1% v/v, Sigma Chemicals). Spore count was adjusted in the range of 1×10^6 to 5×10^6 spores/ml by haemocytometer count [17].

The Mueller-Hinton agar no. 2 plate was inoculated with 2 ml of inoculum by streaking the swab over plate. Then, agar was punched with help of sterile borer to create 6 mm well. 30 µl of different concentrations of

antibiotics (Table 1) and nanoparticles solution were added in respective wells. Sterile distilled water and silver nitrate (1 mM) were used as a control. To study synergistic effect of silver nanoparticles and antibiotics, the mixture of 15 µl of respective concentration of silver nanoparticles and 15 µl of antibiotics solution was added into respective wells (Table 2). Plates were incubated at 37°C for 18 hr in upward position. Zone of inhibition was measured after incubation with Hi-Media scale. The whole experiment was performed in duplicates.

Results and Discussion

Synthesis of silver nanoparticles using leaf extract of *Cymbopogon citratus*

No color change was observed upon mixing the leaves

extract of *Cymbopogon citratus* with aqueous solution of 1 mM silver nitrate in 1:4 ratio (pH 8.0), which was incubated at 37°C over for 24 hours. Although, the color change was observed within 8~10 minutes when the mixture of the extract of *Cymbopogon citratus* leaves and aqueous solution of 1 mM silver nitrate was added in 1:4 ratio (pH 8.0) upon microwave irradiation (Fig. 1). It was reported that thermal factors have been demonstrated to affect the size and uniformity of nanoparticles [11] and also increased pH and temperature fastens the rate of synthesis of silver nanoparticles [12]. Debris, if any, was removed by centrifugation at 1500 rpm for 5 minutes. After centrifugation, the supernatant was collected and was re-centrifuged at 13,000 rpm for 30~45 minutes. The silver nanoparticles pellet was suspended in sterile distilled water and it was used for further applications.

Table 1 List of microorganisms used with respective Antibiotics/Antifungals.

Microorganisms	Antibiotics/Antifungals
<i>Staphylococcus aureus</i> (MDR)	Gentamicin (20 µg/ml), Tetracycline (20 µg/ml)
<i>Escherichia coli</i> (MDR)	Gentamicin (10 µg/ml), Cefoperazone (50 µg/ml)
<i>Proteus mirabilis</i> (MDR)	Streptomycin (50 µg/ml), Piperacillin (100 µg/ml)
<i>Klebsiella pneumoniae</i>	Gentamicin (10 µg/ml), Ampicillin (10 µg/ml)
<i>Salmonella typhi</i>	Gentamicin (10 µg/ml), Chloramphenicol (20 µg/ml)
<i>Candida albicans</i>	Amphotericin B (20 µg/ml), Fluconazole (10 µg/ml)
<i>Aspergillus niger</i>	Amphotericin B (20 µg/ml), Fluconazole (10 µg/ml)

Table 2 Abbreviations.

I	10% of concentrated Silver Nanoparticles
II	25% of concentrated Silver Nanoparticles
III	50% of concentrated Silver Nanoparticles
IV	75% of concentrated Silver Nanoparticles
I+Antibiotics	15 µl, 10% of concentrated respective Silver Nanoparticles+15 µl of respective concentration of antibiotic
II+Antibiotics	15 µl, 25% of concentrated respective Silver Nanoparticles+15 µl of respective concentration of antibiotic
III+Antibiotics	15 µl, 50% of concentrated respective Silver Nanoparticles+15 µl of respective concentration of antibiotic
IV+Antibiotics	15 µl, 10% of concentrated respective Silver Nanoparticles+15 µl of respective concentration of antibiotic

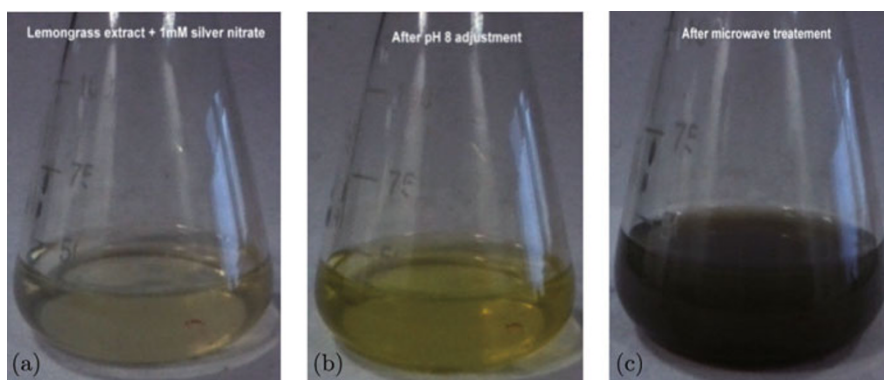


Fig. 1 (a) Initial reaction mixture containing extract of *Cymbopogon citratus* leaves and 1 mM silver nitrate in 1:4 ratio; (b) Color change of reaction mixture after adjusting pH 8.0; (c) Color change of reaction mixture after microwave irradiation.

UV-visible spectrophotometric analysis

The synthesis of silver nanoparticles by reduction of aqueous metal ions during exposure of *Cymbopogon citratus* leaves extract can be easily monitored by using UV-visible spectrophotometry. Figure 2 illustrates the absorbance spectra of reaction mixture containing aqueous solution of 1 mM silver nitrate and extract of *Cymbopogon citratus* leaves after microwave irradiation. Reaction mixture showed an absorbance peak at around 430 nm, which is characteristic of silver nanoparticles, due to its surface plasmon resonance absorption band [18].

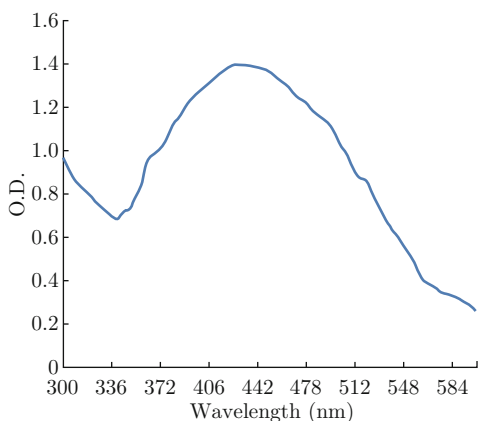


Fig. 2 UV-visible spectrophotometer analysis of silver nanoparticles synthesized using extract of fresh *Cymbopogon citratus* (Lemongrass) leaves.

NTA measurements

NTA measurements revealed that the mean size of synthesized silver nanoparticles was found to be 32 nm (Fig. 3) with concentration of 6.3×10^{10} particles/ml. The brownian motion of silver nanoparticles was recorded as video clip. No aggregations or debris were detected on NTA measurements.

TEM and EDX analysis

TEM analysis revealed that the silver nanoparticles are prominently spherical (Fig. 4). The TEM image at

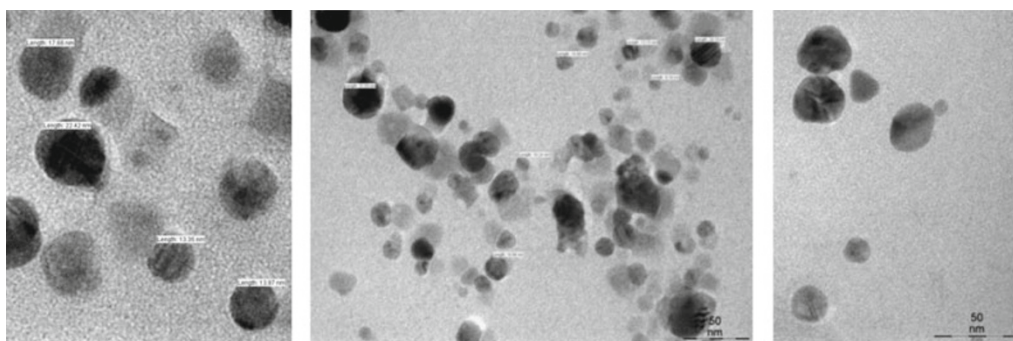


Fig. 4 TEM micrograph of silver nanoparticles synthesized using extract of Lemongrass fresh leaves.

high resolution also revealed that silver nanoparticles are not in physical contact but are separated by uniform distance. The capping of silver nanoparticles was also observed under TEM micrograph. This capping might be because of presence of bio-organic compounds present in extract [13]. The EDX analysis revealed that the silver is present in the solution (Fig. 5). The silver content in the particles was found to be 69.81%.

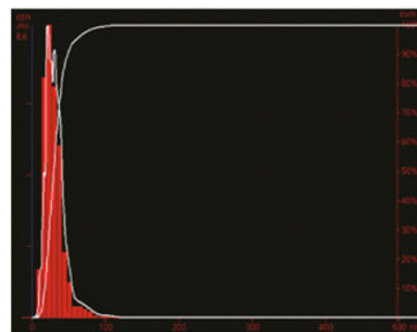


Fig. 3 Frequency size distribution graph of silver nanoparticles synthesized using extract of fresh *Cymbopogon citratus* (Lemongrass) leaves; X axis: particles size (nm), Y axis: concentration/ml $\times 10^6$.

Antimicrobial studies

The antibacterial activity of silver nanoparticles was checked against multiple drug resistant hospital isolates of *E.coli*, *S.aureus*, *P.mirabilis* and hospital isolates of *S.typhi*, *K.pneumoniae*. The antifungal activity was checked against *C.albicans* (hospital isolate) and *A.niger* (NCIM 616). Two antibiotics were served as a control for each microorganism (Table 1, 3). Silver nanoparticles showed clear zone of inhibition against all tested microorganisms. Zone of inhibition was found to be in the range of 13~16 mm for various tested bacteria and 15~18 mm for tested fungi (Fig. 6, Fig. 7). The synergistic effect of silver nanoparticles along with antibiotics was also checked on various multiple drug resistance pathogenic bacteria. The synergistic effect was found to be more prominent than effect of antibiotics alone.

Table 3 Zone of inhibition of Antibiotics/Antifungals and control used with respective microorganisms.

Microorganism	Antibiotics/Antifungals used and respective zone of inhibition in mm	Zone of inhibition of distilled water in mm	Zone of inhibition for 1 mM silver nitrate in mm
<i>E.coli</i>	Cefoperazone- 0	Gentamycin- 0	10
<i>S.aureus</i>	Tetracycline- 0	Gentamycin- 0	10
<i>P.mirabilis</i>	Streptomycin- 12	Piperacillin- 0	10
<i>K.pneumoniae</i>	Gentamicin- 0	Ampicillin- 0	10
<i>S.typhi</i>	Gentamicin- 0	Piperacillin- 0	10
<i>C.albicans</i>	Amphotericin-B- 0	Fluconazole- 20	0
<i>A.niger</i>	Amphotericin-B- 0	Fluconazole- 0	0

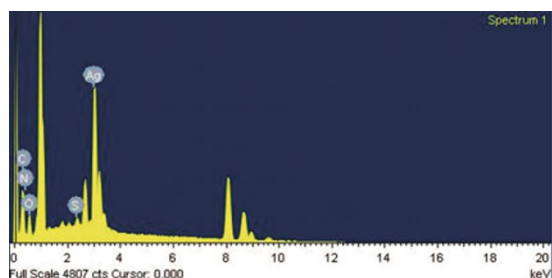


Fig. 5 EDX spectra of silver nanoparticles solution.

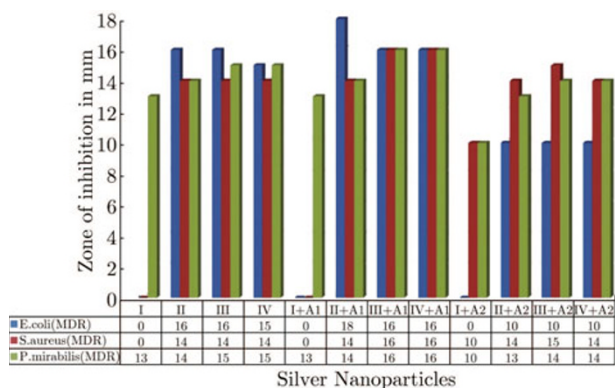


Fig. 6 Effect of silver nanoparticles synthesized using *Cymbopogon citratus* (Lemongrass) leaves extract on various microorganisms.

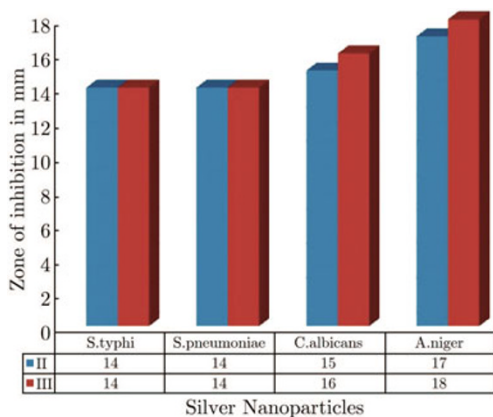


Fig. 7 Effect of silver nanoparticles synthesized using extract of *Cymbopogon citratus* (Lemongrass) leaves on bacteria and fungi.

Conclusion

The silver nanoparticles were green synthesized using leaf extract of *Cymbopogon citratus*. The method represents an example of clean, nontoxic and ecofriendly method for obtaining silver nanoparticles. Further, the above nanoparticles revealed to possess antibacterial activity against multiple drug resistant hospital isolates of *E.coli*, *S.aureus*, *P.mirabilis* and hospital isolates of *S.typhi*, *K.pneumoniae*, as well as antifungal activity against *C.albicans* (hospital isolate) and *A.niger* (NCIM 616). Synergistic effect of silver nanoparticles and antibiotics was found to be effective against multiple drug resistant bacteria. The present study emphasizes the use of plant materials for the synthesis of silver nanoparticles with antibacterial and antifungal effect.

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