

RESEARCH ARTICLE

Fabrication and Characterization of Biosynthesized Silver Nanoparticles using *Cymbopogon citratus* and Evaluation of its Antioxidant, Free Radicals and Reducing Power Activity

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ABSTRACT

The study aims at synthesizing silver nanoparticles using leaf extract of *Cymbopogon citratus* along with the evaluation of its antioxidant, free radicals scavenging, and reducing power properties. Biosynthesized silver nanoparticles were characterized X-Ray diffractometry, Scanning Electron Microscopy, Transmission Electron Microscopy, Fourier Transform Infrared spectroscopy and Energy Dispersive X-ray spectroscopy. The antioxidant, free radicals and reducing power activity were determined by 2, 2-diphenyl-1-picrylhydrazyl, hydrogen peroxide scavenging, hydroxyl radicals scavenging, superoxide scavenging and reducing power activity methods. The silver nanoparticles were synthesized by *Cymbopogon citratus* extract that was confirmed by visible color changes of solution and spectral analysis. The biosynthesized silver nanoparticles having a surface plasmon resonance band centered at 450 nm were characterized using different techniques. The data obtained from SEM and TEM revealed the formation of spherical shape nanoparticles with size ranging from 5-35 nm in diameter while XRD suggested highly crystalline nanoparticles having Bragg's peak at (111), (200) and (220) plane. FTIR confirmed the presence of various function groups in the extract and on the surface silver nanoparticles. The biosynthesized silver nanoparticles had greater antioxidant, free radicals scavenging and reducing power activity than *Cymbopogon citratus* extract while lesser activity than vitamin C.

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INTRODUCTION

Nanotechnology is a branch of science that deals the study of matter and systems in nanoscale [1-100nm] (Mody et al., 2010). This nanoscale bestows diversified physico-chemical properties to the nanoparticles [1]. Various types of nanoparticles have been synthesized such as silver, copper, gold

and zinc etc. but nowadays silver nanoparticles have gained more importance because of their unique properties like antimicrobial activity [2] and drug resistance against commonly used antibiotics [3]. The silver nanoparticles (AgNPs) can be synthesized by physical, chemical and biological methods [4][5][6] (Panigrahi et al., 2004). The physical and chemical methods are used in the synthesis of metallic

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nanoparticles are not very suitable due to high cost, low yield, more time consumption and utilization of toxic chemicals [7][8]. Similarly, pathogenicity issue is involved with bacteria and fungi, and therefore, green synthesis of nanoparticles using plants have gained much attention among the research community [9][10]. The other advantages of green synthesis of metallic nanoparticles include its cost-effectiveness, easy availability, eco-sustainability, easy handling and relief from maintaining septic cultures. Besides, various medicinal properties of a plant or its parts can also be naturally incorporated in the nanoparticles (Parveen et al., 2016). Silver nanoparticles synthesized using green synthesis is more advantageous as silver functions as an important biocidal agent against a wide range of microorganisms. It acts by disrupting their unicellular membrane leading to disruption in the activities of various enzymes[11]. Various reports have been depicted the synthesis of silver nanoparticles using plants such as *Lantana camara* [12], *Ocimum sanctum* [13], *Chrysanthemum morifolium* Ramat [14](Hu et al., 2013), Banana, Neem and *O. tenuiflorum* [15], Coffee and Tea [16], *Withania somnifera* [17], etc.. *Cymbopogon citratus* (Family- Poaceae) commonly known as lemongrass is a perennial grass plant widely distributed worldwide. Its aqueous extract is commonly used as an aromatic drink while the whole plant is well incorporated into traditional food for its lemon flavor and enjoys wide application in folk medicine. Traditionally, tea made from lemongrass leaves has been widely utilized for its antiseptic, antipyretic, antidiarrheal, carminative and, anti-inflammatory effects. Lemongrass leaves contains several important bioactive compounds like myrcene, citral, geranial, geraniol, etc. [18]. In this study, the leaf extract of *Cymbopogon citratus* was used for the biogenic synthesis of silver nanoparticles and its antioxidant, free radicals scavenging and reducing power potential.

MATERIALS

Silvernitrate, 2, 2-Diphenyl-1-Picrylhydrazyl, hydrogen peroxide, deoxyribose, trichloroacetic acid, NADH, NBT and PMS were purchased from Sigma Aldrich Chemicals.

METHODS

Preparation of *Cymbopogon citratus* extract

Lemongrass leaves were obtained from the Department of Horticulture (25.2648° N, 82.9913°

E), Banaras Hindu University (BHU), Varanasi and authenticated by Prof. N. K. Dubey, Department of Botany, BHU. The voucher specimen number to it was designated as Poa.2018/1. The leaves were dried in a digital oven (37°C) for 7 days and were then ground with the help of the grinder. Then 4 gram lemongrass leaf powder was mixed in 100 ml deionized water and boiled for 5-7 minutes and then cooled. This solution was centrifuged (3000 rpm, 15 minutes) and filtered using Whatman no.1 filter paper. Then, supernatant was collected and stored at 4 °C for further experimental use (Keshari et al., 2018).

Qualitative Phytochemical Analysis

The *Cymbopogon citratus* extract was tested for the presence of phytochemicals like proteins, carbohydrates, starch, phenols/tannins, flavonoids, saponins, glycosides, steroids, terpenoids, quinones, phlobatannins and, alkaloids by following standard methods. (Keshari et al., 2018)

Synthesis of silver nanoparticles

A silver nanoparticle (AgNPs) was synthesized by using *C. citratus* extract. For AgNPs synthesis, 10 ml *C. citratus* extract were added separately with 90 ml aqueous solution of AgNO₃ (1mM) and kept at room temperature [19]. Within a short period of time, the colour changed from pale yellow to brown indicating the formation of AgNPs (Keshari et al., 2018). The reduction of pure AgNO₃ into AgNPs was monitored by analysing the UV-visible spectrum obtained by using UV-Vis spectrophotometer (Perkin Elmer, lambda 35, Germany) in the range of 350-750 nm at different time intervals up to 5 hours and absorption was taken after 1, 2 and 5 hours.

Characterization of silver nanoparticles

XRD graphs were obtained by Rigaku MiniFlex 600 using Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) at 2 θ angle, voltage, 40kV and current of 20 mA. TEM observations were performed on JEOL JEM 200 CX, a drop of AgNPs was placed on the grid and dried under a lamp. SEM (NOVA NANOSEM 450) analysis was performed at an accelerating voltage of 5-10KeV. SEM sample was prepared by dropping AgNPs on the silicon wafer and dried in the oven [20] (maleki et al 2019). FTIR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer) was performed for the analysis of chemical composition of AgNPs. The solutions

were dried at 75° C and the dried powder was characterized in the range 4000–400 cm⁻¹ using KBr (Keshari et al., 2018).

Analysis of Antioxidant Activity

2, 2-Diphenyl-1-Picrylhydrazyl Method

1000µl DPPH solution (1mM in methanol) was added with 1000µl (10-100 mg/ml) silver nanoparticles and wait for 30 minutes at room temperature in the dark room. After the incubation absorbance was recorded at 517nm using UV-Vis spectrophotometer (Systronics, AU-2701). Similar procedure was followed for the preparation of *C. citratus* extract and vitamin C. Methanol solution was used as blank while DPPH solution was used as control. The % of Antioxidant activity was calculated as:

$$\text{Percentage of Antioxidant Activity} = \frac{Aa - Ab}{Aa} \times 100$$

Aa- denotes absorbance of control; Ab-denotes absorbance of sample [21].

Analysis of Hydrogen Peroxide Scavenging Activity

600 µl, 2 mM hydrogen peroxide solution (phosphate buffer {50mM, pH. 7.4}) was mixed with 100µl silver nanoparticles (25-250 mg/ml; in phosphate buffer {50mM pH. 7.4}) and 300µl phosphate buffer (50mM, pH. 7.4). Then solution was vortexed properly and absorbance was measured at 230 nm using UV-Vis spectrophotometer (Systronics, AU-2701) after 10 minutes of incubation. Same procedure was followed for the preparation of *C. citratus* extract and vitamin C. Then % of hydrogen peroxide scavenging activity (HPSA) was calculated as:

$$\% \text{ of HPSA} = \frac{Bc - Bs}{Bc} \times 100$$

Bc- Represents the absorbance of control; Bs- represents the absorbance of sample [22].

Determination of Hydroxyl Radical Scavenging Activity

450µl sodium phosphate buffer (200mM, pH, 7.0), 525µl deionized water, 150µl FeSO₄EDTA (10 mM), 150µl deoxyribose (10mM), and 150µl H₂O₂ (10mM) were mixed with 75µl silver nanoparticles (25-250 mg/ml in methanol) in a test tube. Then test tube was kept in the incubator at 37 °C for 4 hours. 750µl TBA (1% in 50 mM NaOH) and 750µl trichloroacetic acid (2.8%) was mixed to stopped the reaction. Further, this tube was placed in the

boiling waterbath for 10 minutes and cooled. Then absorbance was recorded at 520 nm using Uv-vis spectrophotometer. Same procedure was followed for the preparation of *C. citratus* extract and vitamin C. The % of hydroxyl radical scavenging activity (HRSA) was calculated as:

$$\text{Percentage of HRSA} = \frac{Cc - Cs}{Cc} \times 100$$

Cc- Absorbance of control; Cs= Absorbance of sample [23].

Analysis of Superoxide Radical Scavenging Activity

1000µl NBT (50 mM), 1000µl PMS (10 mM), 1000µl Tris HCl buffer (16mM, pH. 8), 1000µl NADH(78mM) and 200µl silver nanoparticles (100-500 mg/ml; methanol) were mixed in eppendorff tube. Then this tube was placed in the incubator at 25 °C for 5 minutes and absorbance was recorded at 560nm using UV-Vis spectrophotometer. This procedure was followed for the preparation of *C. citratus* extract and vitamin C. The % of superoxide scavenging activity (SCA) was calculated as:

$$\text{Percentage of SCA} = \frac{Dc - Ds}{Dc} \times 100$$

Dc- Absorbance of control; Ds- Absorption of sample [22].

Analysis of Reducing Power Activity

2500µl K₃Fe (CN)₆ (1%), 2500µl phosphate buffer (0.2M, pH; 6.6) were mixed with 1000µl silver nanoparticles (100-500 µg/ml) in a eppendorff tube. This tube was placed in the incubator at 50°C for 20 minutes. Then 2.5ml trichloroacetic acid solution (10%) was mixed and centrifuged at 3000rpm for 10 minutes. Then 2500 µl of this solution was mixed with 2500 µl distilled water and 500 µl FeCl₃. Then absorbance was recorded at 700 nm using Uv-vis spectrophotometer. Same procedure was followed for the preparation of *C. citratus* extract. The reducing power activity was calculated as vitamin C equivalent per 100 gm of dry sample (Keshari et al. 2018).

RESULTS

Phytochemical Analysis

The qualitative test was performed for the determination of presence of phytochemicals in the *C. citratus* extract. In the *C. citratus* extract phenols, tannins, carbohydrates, proteins, saponins, flavonoids, glycosides and terpenoids were present but starch, steroids, quinones and phlobatannins were absent (Table 1).

Table 1: The table 1 shows the phytochemical analysis of leaf extract of *C. citratus*

| Phytochemical Analysis | Observation | Result |
|------------------------|---|----------|
| Protein | Violet color appeared | Positive |
| Carbohydrate | Violet ring appeared at the interphase | Positive |
| Phenols | Black color appeared | Positive |
| Flavonoids | Intense yellow color turned colorless upon addition of dil. HCl | Positive |
| Saponins | Stable foam formed | Positive |
| Glycosides | Brown ring appeared at the interphase | Positive |
| Iodine | Blue/violet color did not appear | Positive |
| Steroids | Green color did not appear | Positive |
| Terpenoids | Reddish brown color appeared | Positive |
| Quinones | Blue/green color did not appear | Negative |
| Phlobatannins | Red precipitate did not form | Negative |
| Alkaloids | Yellow color precipitate did not form | Negative |

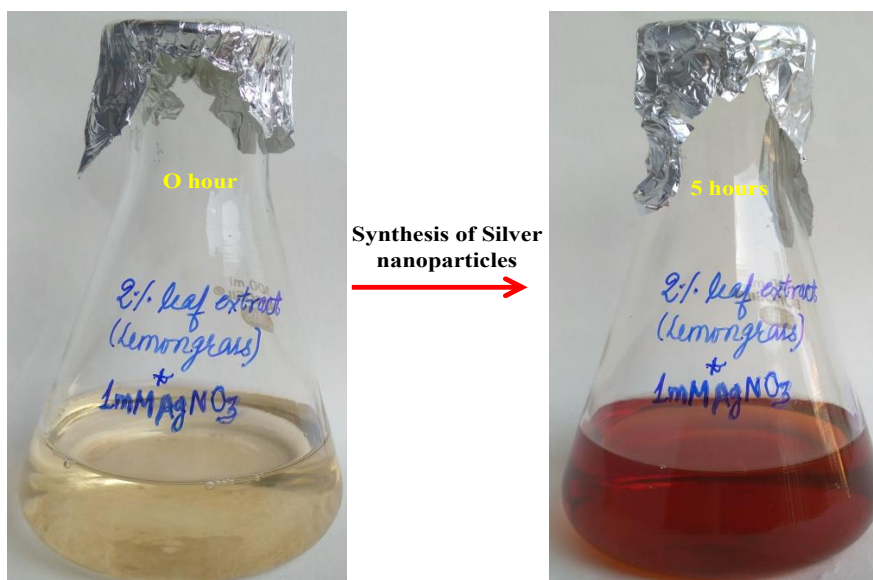


Fig. 1: The image represents the color changes of solution before (0 hour) and after (5 hours) the synthesis of silver nanoparticles

Synthesis of Silver nanoparticles

The results confirmed that AgNO_3 was reduced into silver nanoparticles (CCAgNPs) that was observed visually by the development of reddish brown color. The *C. citratus* extract synthesized the CCAgNPs after 5 hours of reaction (Fig. 1). The UV-Vis spectrophotometer was recorded in different time interval and wavelength of the solution at 450nm was obtained. The presence of peaks at 450nm confirmed the formation of silver nanoparticles. The synthesis of CCAgNPs was started after 1 hour of reaction and completed after 5 hours (Fig. 2).

X-Ray Diffraction

The XRD technique was confirmed the phase identification of CCAgNPs. The result indicated the formation of crystalline CCAgNPs that was confirmed by the peaks at $2\theta^\circ$ values of 38.06, 44.23, and 67.43 representing the planes 111, 200 and 220 respectively (Fig. 3).

Transmission Electron Microscopy

TEM was used for the analysis of morphology of CCAgNPs. The TEM results confirmed the spherical shape and variable size of CCAgNPs (Fig. 4A). The diffraction pattern confirmed the formation

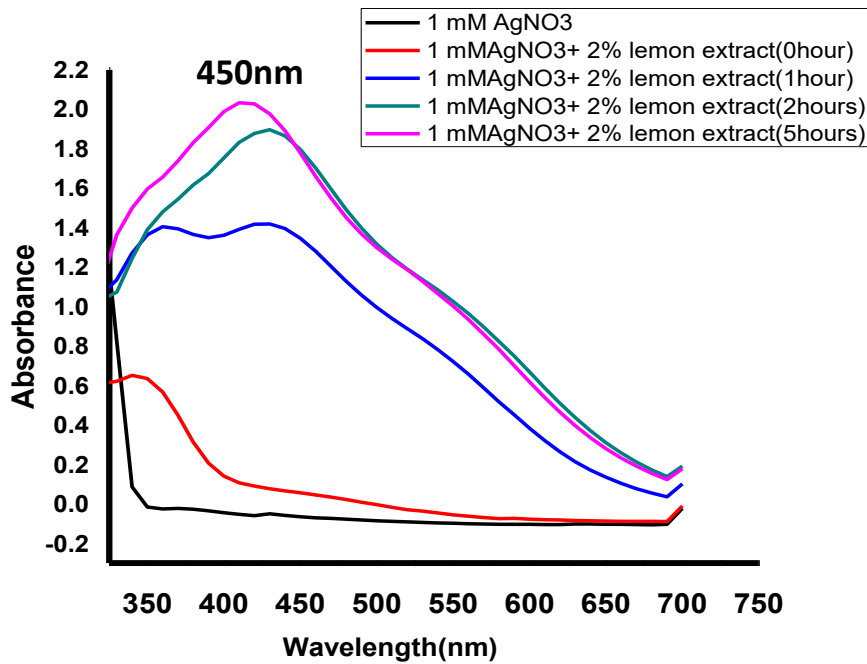


Fig. 2: The image shows the synthesis of silver nanoparticles in time dependent manner

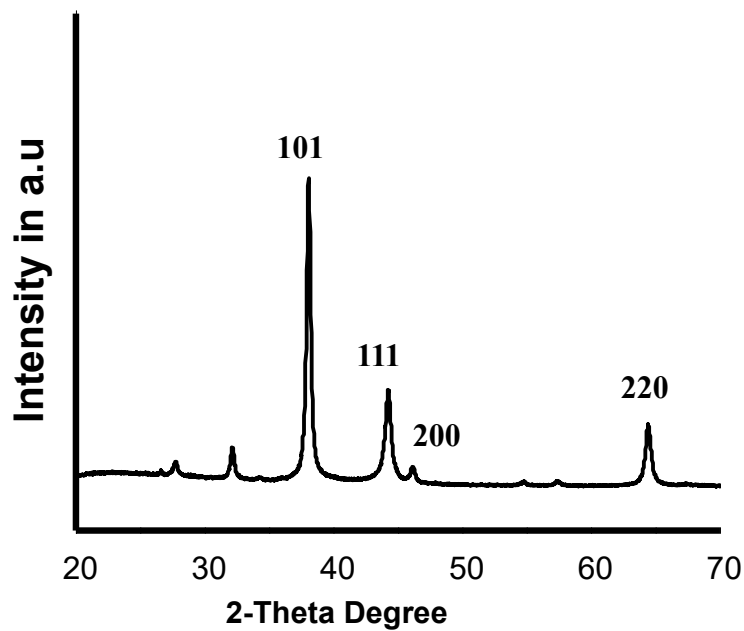


Fig. 3: The image represents the crystalline silver nanoparticles

of polycrystalline CCAgNPs (Fig. 4B). The size of CCAgNPs was ranges from 5-35nm while 20 nm size was maximum in number (Fig. 4C). The EDAX results confirmed that CCAgNPs was elemental (Fig. 4C).

Scanning Electron Microscopy

SEM was used for the determination of size of CCAgNPs. The results confirmed the formation of variable size of CCAgNPs (Fig. 5).

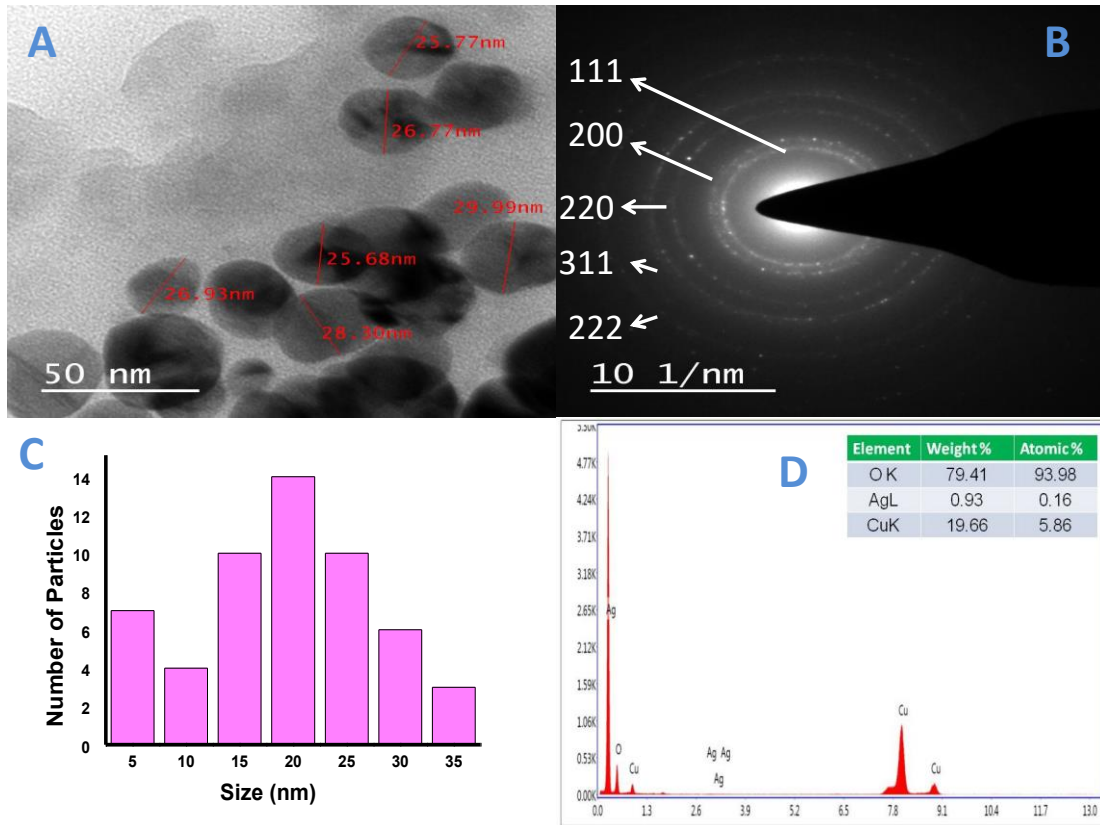


Fig. 4: The images represent the silver nanoparticles are spherical (A), polycrystalline (B), variable in size (C) and elemental (D)

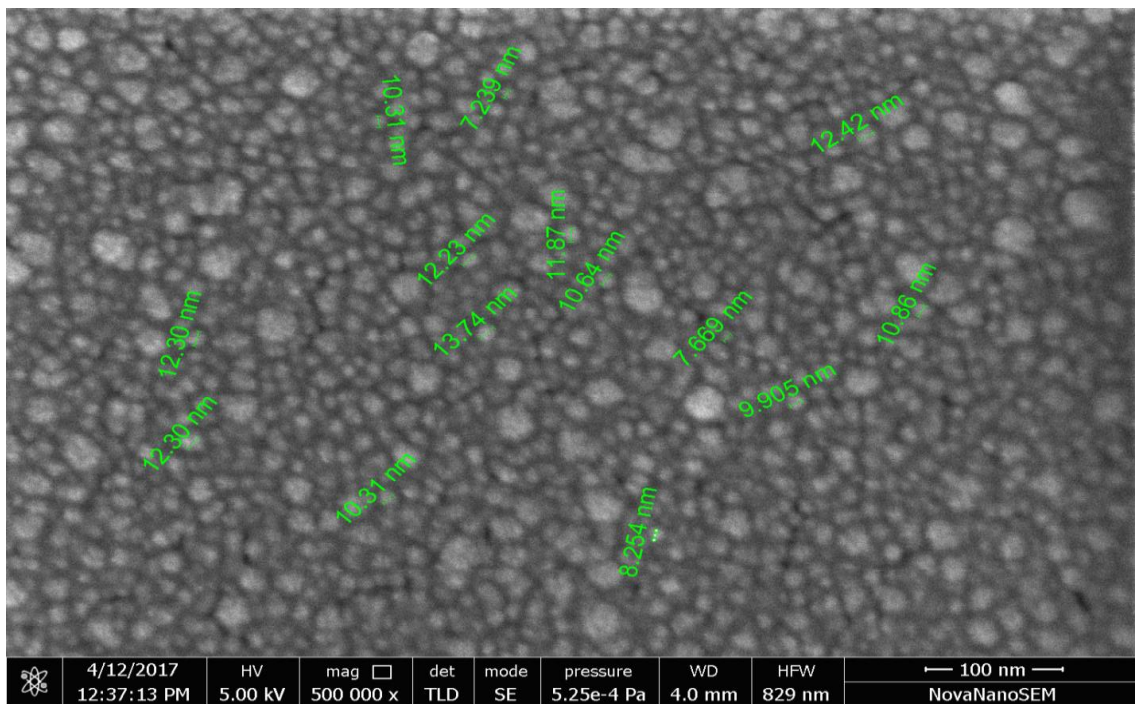


Fig. 5: The image shows the variable size of silver nanoparticles

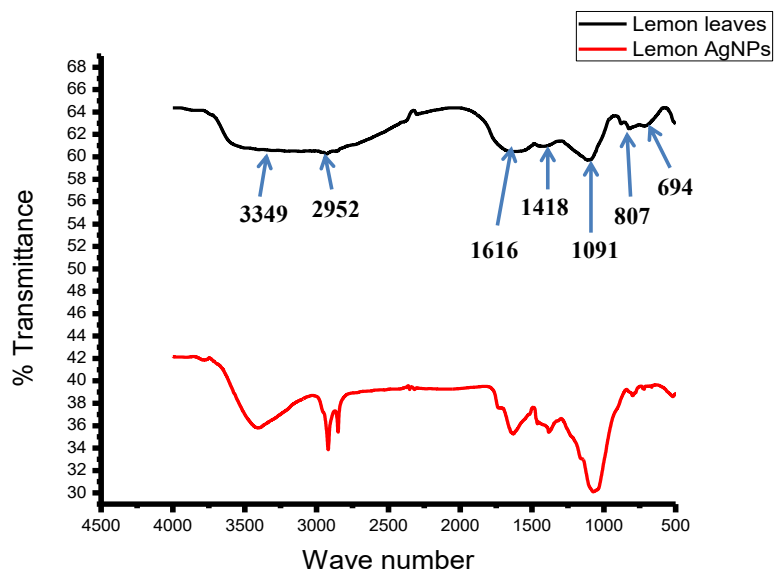


Fig. 6: The image shows the functional groups present in the lemon extract (black line) and on the surface of silver nanoparticles (Red line)

Fourier Transform Infrared

The FTIR was performed for the determination of different phytochemicals in the extract and on the surface of CCAgNPs. The results confirmed that different absorption peaks in the extract and CCAgNPs was present. The absorption band at 3349 cm^{-1} corresponds to O-H stretching for carboxylic acid, 2952 cm^{-1} corresponds to C-H stretching for alkane, 1616 cm^{-1} indicated the N-H for primary amines, 1418 cm^{-1} represented C-C stretch for aromatics, 1091 cm^{-1} represented the C-O stretch for the alcohols, carboxylic acids, esters and ethers, 807 cm^{-1} determined N-H, strong C-H and medium C-Cl for primary and secondary amine, aromatics and alkyl halides respectively and 694 cm^{-1} represented the strong and broad $-\text{C}\equiv\text{C}-\text{H}; \text{C}-\text{H}$ bend for alkynes (Fig. 6). These results were compared and confirmed by Keshari et al 2020 and Adabi et al 2010 (Keshari et al. 2020)[25].

Analysis of Antioxidant Activity

2, 2-Diphenyl-1-picrylhydrazyl method

The antioxidant activity of AgNPs, *Cymbopogon citratus* and vitamin C were determined by DPPH method. The results confirmed that vitamin C has greater antioxidant activity than AgNPs and *C. citratus* extract. 200 $\mu\text{g}/\text{ml}$ vitamin C, AgNPs and *C. citratus* extract have 53%, 51 % and 48 % antioxidant activity respectively (Fig. 7).

Analysis of Free Radicals Scavenging Activity

Determination of Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of vitamin C, AgNPs and *C. citratus* extract were determined by hydrogen peroxide scavenging method. The results confirmed that vitamin C, has greater hydrogen peroxide scavenging activity than AgNPs and *C. citratus* extract. 200 $\mu\text{g}/\text{ml}$ vitamin C, AgNPs and *C. citratus* extract have 90 %, 29% and 11% hydrogen peroxide scavenging activity respectively (Fig. 8).

Analysis of Hydroxyl Radicals Scavenging Activity

The hydroxyl radicals scavenging activity of vitamin C, AgNPs and *C. citratus* extract were determined by hydroxyl radicals scavenging method. The results confirmed that vitamin C has greater hydroxyl radicals scavenging activity than AgNPs and *C. citratus* extract. 200 $\mu\text{g}/\text{ml}$ vitamin C, AgNPs and *C. citratus* extract have 42%, 36% and 29% hydroxyl radicals scavenging activity respectively (Fig. 9).

Analysis of Superoxide Scavenging Activity

The superoxide scavenging activity of vitamin C, AgNPs and *C. citratus* extract were determined by superoxide scavenging method. The results confirmed that vitamin C, AgNPs and *C. citratus*

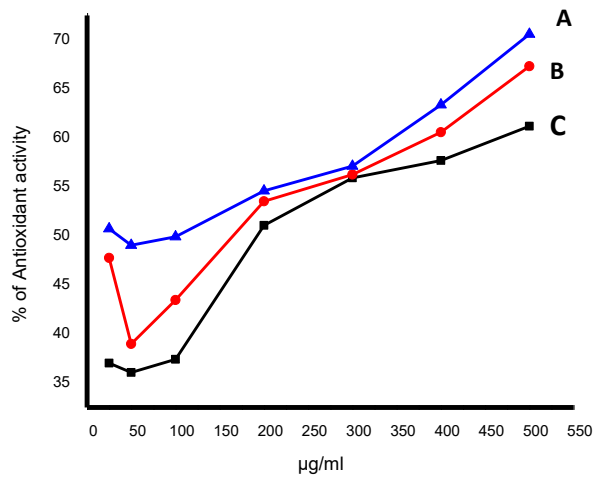


Fig. 7: The graph shows the antioxidant property of vitamin C (A), silver nanoparticles (B) and *Cymbopogon citratus* extract (C)

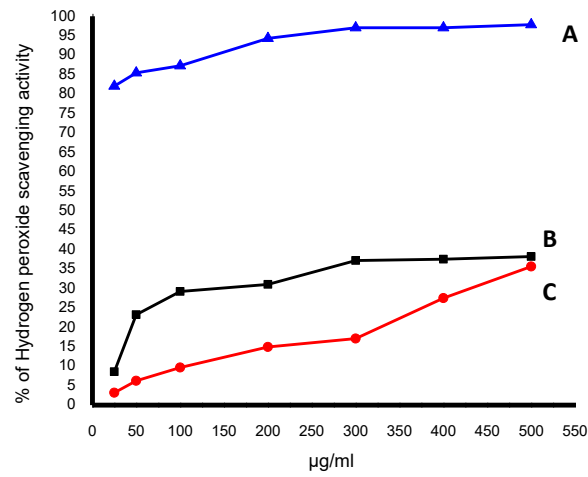


Fig. 8: The graph shows the hydrogen peroxide scavenging activity of vitamin C (A), silver nanoparticles (B) and *Cymbopogon citratus* extract (C)

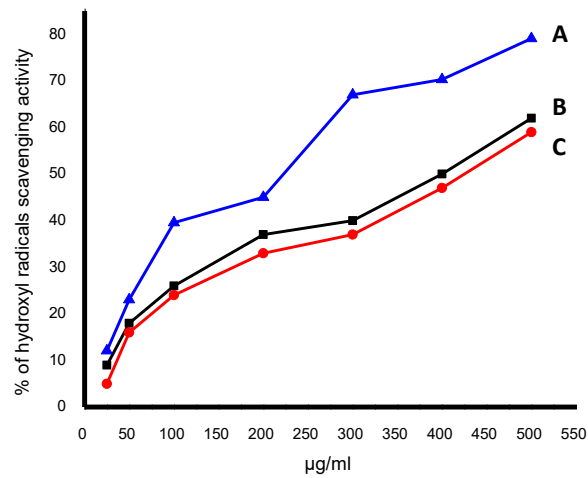


Fig. 9: The graph shows the hydroxyl radicals scavenging activity of vitamin C (A), silver nanoparticles (B) and *Cymbopogon citratus* extract (C)

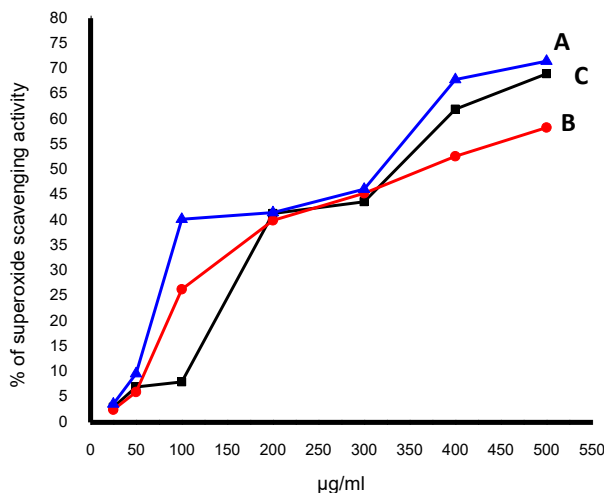


Fig. 10: The graph shows the superoxide scavenging activity of vitamin C (A), silver nanoparticles (B) *Cymbopogon citratus* (C)

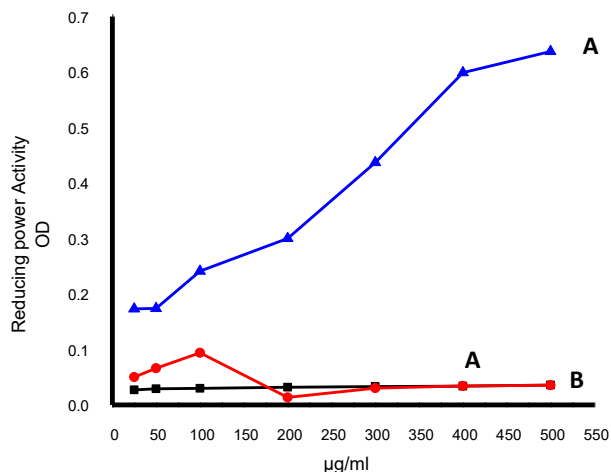


Fig. 11: The graph shows the reducing power activity of vitamin C (A), silver nanoparticles (B) *Cymbopogon citratus* extract (C)

extract have approximately similar superoxide scavenging activity. 200 µg/ml vitamin C, AgNPs and *C. citratus* extract have nearly 40% superoxide scavenging activity (Fig. 10).

Analysis of Reducing Power Assay

The reducing nature of vitamin, AgNPs and *C. citratus* were determined by reducing power activity method. The results confirmed that vitamin C has greater reducing power activity than AgNPs and *C. citratus* extract. 200 µg/ml vitamin C, AgNPs and *C. citratus* extract have optical density 0.3, 0.02 and 0.03 respectively. As we know that higher the optical density greater will be the reducing power activity (Fig. 11).

DISCUSSION

The synthesis of silver nanoparticles was confirmed by the development of reddish-brown color (Fig. 1) and subsequently confirmed by the UV-Vis spectrophotometer. The maximum absorbance of AgNPs was obtained at 450nm (Fig. 2). Similar changes in color have been observed in previous studies also [26] (Farghaly and Nafady, 2015). The XRD result confirmed the formation of crystalline AgNPs that was confirmed by the peaks obtained at $2\theta^\circ$ values of 38.06, 44.23, and 67.43 representing the planes (111), (200) and (220), respectively (Fig. 3). Exact result was obtained from the structural analysis of XRD of AgNPs [19]. TEM results indicated that the green

synthesized AgNPs had spherical shape (Fig. 4A). The diffraction pattern confirmed the synthesis of metallic nanoparticles (Fig. 4B). The size of biosynthesized silver nanoparticles was ranges from 5-35 nm (Fig. 4C). The result of EDX determined the major constituents were elemental silver with small amounts of copper signals. The weak signals originated due to capped biomolecules and copper grid used as supporting filaments (copper signals) (Fig. 4D). SEM results indicated the formation of variable size of AgNPs (Fig. 5). Similar size ranges of AgNPs have been reported (Farghaly and Nafady, 2015)[27][28]. FT-IR analysis of the synthesized AgNPs clearly revealed the absorption peak locations on 2917, 1656, 1540, 1255, 1028, 815, 696 cm^{-1} . The band at 2917 indicated the formation of O-H stretching that corresponds to carboxylic acid, 1656 stretching for -C=C- corresponding to alkenes and 1540 stretching for N-O corresponds to nitro compounds (Fig. 6). These observations confirmed the presence of bioactive molecules such as phenols, tannins, steroids and, alkaloids in the extract. Ethylene groups (=C-H , C=C) detected by FT-IR have been reported, and these groups are capable of acting as reducing or capping agents [29]. The results of DPPH confirmed that the extract, AgNPs and vitamin C have antioxidant properties. The AgNPs have greater antioxidant property as compared to the extract but both have lesser antioxidant property in comparison with vitamin C (Fig.7). Hydrogen peroxide scavenging results confirmed that vitamin C has greater hydrogen peroxide scavenging as compared to the extract and AgNPs (Fig. 8). The results of hydroxyl radicals scavenging confirmed that vitamin-C has greater hydroxyl radicals scavenging activity than AgNPs and extract (Fig. 9). The results of superoxide scavenging activity proved that the AgNPs have lesser superoxide scavenging activity as than vitamin-C and greater superoxide scavenging activity than extract (Fig. 10). The AgNPs have greater reducing power activity than extract and lesser activity than vitamin C (Fig. 11).

CONCLUSION

In the present study, we are reported the synthesis of AgNPs from *Cymbopogon citratus* leaf extract. In this method, naturally occurring bioactive compounds (phenol and flavonoids) are acting as reducing and proteins act as capping and stabilizing agents. The nanoparticles synthesized were in the range of 5-35 nm and were

spherical shape. Most importantly, the AgNPs demonstrated strong antioxidant, free radicals scavenging and reducing power activity against the standard vitamin C which was confirmed by DPPH, hydrogen peroxide, hydroxyl radicals and superoxide scavenging activity. These green synthesized AgNPs may act as a drug for the prevention of oxidative stress and related diseases but further studies are needed for more exploration regarding this subject.

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CONFLICT OF INTEREST

All the authors were declared that they have no conflict of interest.

SUBMISSION DECLARATION

The present work has not been published previously.

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