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ORIGINAL ARTICLE

Synthesis and characterization of Silver nanoparticles from *Cinnamomum tamala* leaf extract and its antibacterial potential

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Abstract

The novel approach has been carried for the green synthesis of silver nanoparticles using leaf extract of *Cinnamomum tamala* and silver nitrate solution. The optimal condition for synthesizing Ag-NPs was obtained by varying the leaf extract concentration, temperature, $AgNO_3$ concentration, effect of ratio of leaf extract to $AgNO_3$ solution, pH and reaction time. The formation of silver nanoparticles was confirmed by UV–Vis spectrophotometer. Fourier transform infrared spectroscopy (FTIR) was used to key out the specific functional groups responsible for the reduction of silver nitrate to form silver nanoparticles and the capping agents present in the leaf extract. Scanning electron microscopy (SEM) represents the morphological characterization of synthesized nanomaterials. Transmission electron microscopy (TEM) analysis revealed that the particles were crystalline, spherical and irregular in shape and the size were 16 nm and 9 nm at 25 °C and 60 °C, respectively. Electron X-ray diffraction (XRD) analysis is used to determine the phase distribution, crystallinity and purity of the synthesized nanoparticles. The synthesized nanoparticles are found to be highly effective against some pathogenic bacteria species, thus signification of the present study is in production of various pharmaceutical and bioactive products.

Keywords: *Antibacterial Activity; Cinnamomum Tamala; Green Synthesis; Silver Nanoparticles; Spectral Analysis.*

How to cite this article

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INTRODUCTION

The field of nanotechnology is one of the vital areas for current material science researcher. Nowadays, interest of nanobiotechnology is the development of environmentally benign technology for the synthesis of metal/metal oxide nanoparticles with miraculous and boundless applications in the fields of agriculture, cosmetics, defense, environmental safety, food, health and pharmaceutical [1].

Nanoparticles can be broadly classified into two groups: Organic nanoparticles and Inorganic nanoparticles. Organic nanoparticles are carbon nanoparticles (fullerenes) and inorganic nanoparticles are magnetic nanoparticles, noble nanoparticles (gold and silver), and semiconductor nanoparticles (titanium oxide and zinc oxide). Researchers have paid special attention to the synthesis of inorganic nanoparticles for their superior material properties with versatile functions, which can be easily used for chemical imaging drug agents and drug due to nano size feature. They can be easily used for chemical imaging drugs agents and drug due to nano size feature. They can be used as multipurpose function agents for cellular delivery as they are widely available, have rich functionality and good biocompatibility. These are also good carriers of targeted drug delivery and controlled drug release [2]. It has been reported that since ancient times silver metal is known to have antimicrobial activities [3] and silver nanoparticles

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(Ag-NPs) are of particular interest due to their peculiar properties and wide applications. Silver nanoparticles are used to treat infections in open wounds, chronic ulcers [4] and in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances [5], catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine [6-8], treatment of brucellosis [9], anti-inflammatory [10], mosquito larvicidal [11], etc.

Nanoparticles can be obtained by using conventional or unconventional methods, using two different approaches: "top-down" and "bottom-up". The first approach is the "top-down" method which calls for breaking down of solid materials into small pieces by applying external force. In this approach, many physical, chemical and thermal techniques are used to provide the necessary energy for nanoparticle formation. The second approach, known as "bottom-up", is based on gathering and combining gas or liquid atoms or molecules. These two approaches have advantages and disadvantages relative to each other. In the top-down approach, which is costlier to implement, it is impossible to obtain perfect surfaces and edges due to cavities and roughness that can occur in nanoparticles; whereas excellent nanoparticle synthesis results can be obtained by bottom-up approach. In addition, with the bottom-up approach, no waste materials that need to be removed are formed, and nanoparticles having smaller size can be obtained thanks to the better control of sizes of the nanoparticles [12].

In recent years green chemistry and biosynthetic methods have become more attractive ways to obtain Ag-NPs. These unconventional methods use either biological microorganisms (e.g.: bacteria, fungi, marine algae, yeasts) or different alcoholic or aqueous plant extracts. Green synthesis has multiple advantages over classical routes: it is cost effective, eco-friendly and does not require high pressure, energy, temperature or the use of toxic chemical reagents [13]. Plant-mediated synthesis of Ag-NPs is more advantageous compared to the methods that use microorganisms especially because they can be easily improved, are less biohazardous and do not involve the elaborate stage of growing cell cultures.

Cinnamomum tamala (Family- Lauraceae) is a medium sized evergreen tree mainly grows in tropical and subtropical Himalayas, Uttar Pradesh, Eastern Bengal, Burma, Khasi and Jaintia Hills of India [14]. It is commonly used for flavorings food and widely used in pharmaceutical preparation because of its hypoglycemic, stimulant and carminative properties [15]. The leaves of this tree are used as spice having clove like taste and pepper like odor.

In the present work, an attempt has been made to synthesize silver nanoparticles using aqueous leaf extract of *C. tamala*. The characterization was done using several spectral analyses. The synthesized silver nanoparticles were also evaluated for their antibacterial activity.

MATERIALS AND METHODS

Plant material and chemical

C. tamala leaves were collected from the local market in Kushtia, Bangladesh. Silver nitrate was purchased from commercial sources (Merck, Germany). All glassware was sterilized with nitric acid and further rinsed with distilled water and then deionized water and dried in oven before use.

Preparation of leaf extract

Primarily the leaves were thoroughly washed with distilled water to remove dirt particles and then sun dried to remove residual moisture. The dried leaves were cut into small pieces. 2 g of powdered leaves in 100 ml deionized water boiled for 15 min at 80 ºC. The aqueous plant extract was filtered through Whatman no.1 filter paper. Then the filtered extract was stored in 4 ºC for further use in synthesis of silver nanoparticles.

Synthesis of Silver Nanoparticles

An aqueous solution of 80 mL 0.01M $AgNO_3$ was added to 20 mL *C. tamala* leaf extract and the mixture was stirred with a magnetic stirrer at room temperature. The color of the solution changed from colorless to light yellow and then to brown color. Silver nanoparticles formation was primarily identified by color change. The separation of silver nanoparticles from the dispersion was carried out by centrifugation after that Ag-NPs were washed four times with distilled water and acetone to remove water soluble impurities and then nanoparticles were lyophilized and stored in dry bottles for further analysis.

Standardization

For efficient synthesis of silver nanoparticles, effect of concentration of leaf extract, effect of reaction time, effect of temperature, effect of pH, effect of ratio of plant extract to $AgNO_3$ solution, and effect of concentration of $AgNO_3$ on Ag-NPs formation were investigated and the optimum conditions for the reaction were selected.

Characterization of silver nanoparticles

Characterization of silver nanoparticles was carried out by using visual observation and various techniques, in visual observation, change in color of the solution was observed by naked eye. For identification of Ag-NPs in solution absorbance value was determined using Shimadzu UV-visible 2900 spectrometer in the wavelength (λ) range 300-600 nm. Characterization of the surface chemistry of Ag-NPs and biomolecules in *C. tamala* solution were done by using a Fourier transform infrared spectroscopy (Shimadzu FTIR spectrophotometer, FTIR 8400). The FTIR spectra were recorded from 4000-600 cm^{-1}. The particle size and surface morphology was confirmed using Transmission electron microscopy (TEM), operated at an accelerated voltage of 100 kV. The shape, size and surface of the synthesized Ag-NPs were analyzed using scanning electron microscopy (SEM) with high-resolution images and selected area, electron X-ray diffraction (XRD) was used to determine the phase distribution, crystallinity and purity of the synthesized nanoparticles. The Debye–Scherer's equation was used for calculating the mean size of silver nanoparticles by using the following equation:

$$D = 0.94\lambda/B\cos\theta.$$

Photochemical screening

The qualitative phytochemical analysis of *C. tamala* extract was performed to determine the presence of alkaloids (Dragendorff's), flavonoids (alkaline reagent), phenolics (lead acetate, alkaline reagent test), triterpenes (Liberman test), saponins (foam test), tannins (Few $FeCl_3$) and carbohydrates (Molish test) [16-18].

Antibacterial activity assay

The antibacterial test was carried out by agar disc diffusion method [19] using 100 µl of standardized inoculums suspension containing 10^7 CFU/mL of bacteria. Two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus)* and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)* bacteria were used in this study. The strains were obtained from the Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh. Each tested sample (30µL/disc) was applied on the filter paper discs (6 mm diameter) and placed on the inoculated LB agar. Standard reference antibiotics, tetracycline (Sigma-Aldrich Co., St. Louis, MO, USA), was used as positive controls for the tested bacteria. The plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria.

RESULTS AND DISCUSSION

Visual Observations

Silver nanoparticles formation was primarily identified by color change. Color changes of the solutions are due to some chemical compound such as alkaloids, flavonoids, saponins, steroids, and color present in plant extract acts as a reducing agent that reduced silver ions (Ag^+) to a silver atom (Ag^0). Visually *C. tamala* leaf extract was treated with 0.01M silver nitrate aqueous solution showed a color change from colorless to light yellow within 60 min (Fig. 1b) then changes

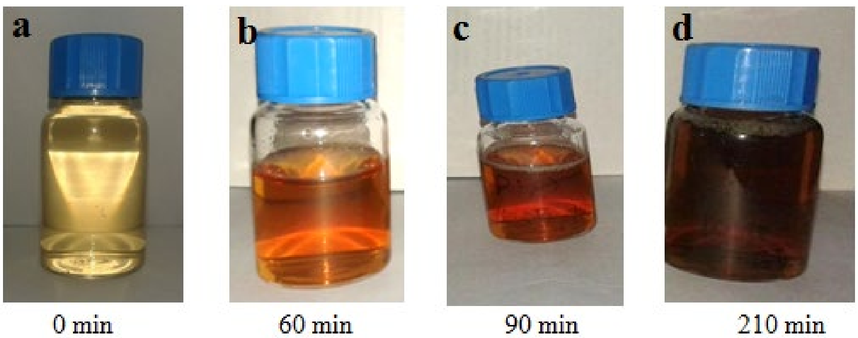

Fig. 1. Color change at different time interval of *C. tamala* leaf extract solution with 0.01M $AgNO_3$ solution, (a) 0 min, (b) 60 min, (c) 90 min, (d) 210 min.

of the solution from light yellow to brown (Fig. 1c) were observed and finally it was turned to dark brown after 210 min at 25 °C (Fig. 1d). Thereafter no further color of the solution was changed. This brown color of silver nanoparticles arises due to the surface Plasmon resonance in the aqueous solution [20]. Vijayaraghavan *et al*. [21] and Rajoriya [22] also reported similar changes in the color during nanoparticles formation.

Standardization

Optimization of some parameters was essential for the efficient formation of silver nanoparticles. There was a difference in the formation of Ag-NPs by increasing the concentration of leaf extract (Fig. 2). On increasing concentration of extract there is increase in intensity of absorption. The highest absorption peak was observed when using 80 g/L *C. tamala* extract. The synthesis of nanoparticles also increased by increasing the reaction time from 30 min to 210 min as shown in Fig. 3. Maximum production of nanoparticles was confirmed by maximum absorption which occurs in the UV-Vis spectra. The spectra show no peak at the time of 30 min. With the increase in reaction time, UV-Vis spectra show sharp narrow peak after 210 min which indicates the maximum formation of nanoparticles. This single and strong band indicates that the particles are isotropic in shape and uniform size [20]. The absorption spectra of Ag-NPs at different temperatures were investigated in the range of 20 °C to 80 °C. The

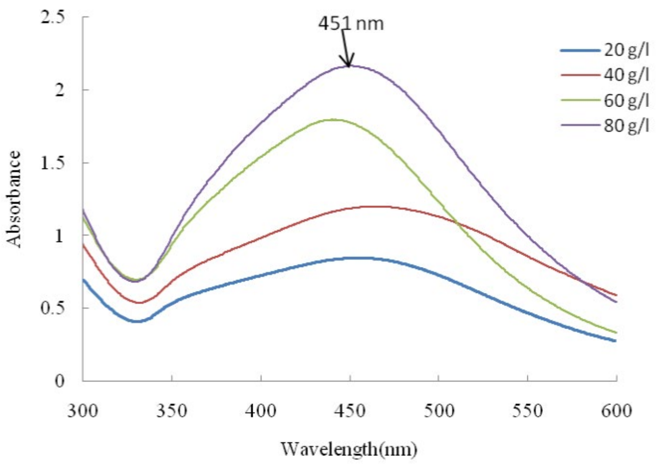

Fig. 2. Effect of concentration of leaf extract.

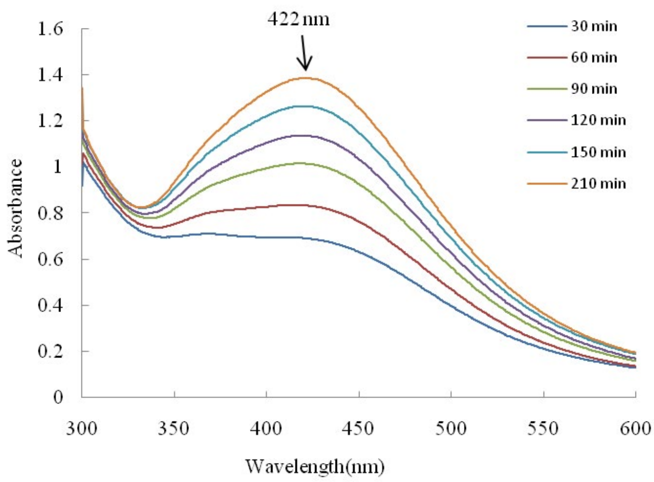

Fig. 3. Effect of reaction time.

increase in temperature increased the rate of formation of Ag-NPs from silver ions, retarding the secondary reduction process. Our results are in good agreement with Song *et al*. [23] and Kaviya *et al*. [24]. The peak absorption wavelength shifted toward blue from 441 to 426 nm, as temperature varies from 20 °C to 80 °C (Fig. 4). The shift in the band maximum is due to localization of surface Plasmon resonance of the Ag-NPs. This indicates that the size of the synthesized nanoparticles decreases with increasing temperature. At high temperature, the kinetic energy of the molecules increases and silver ions gets consumed faster, thus leaving less possibility for particle size growth. Change in pH affects the shape and size of the particles, as pH has the ability to alter the charge of biomolecules, which might affect their capping as well as stabilizing abilities. At low pH (pH 2), no silver nanoparticles are formed [25]. As the pH increases from 2 to 9 the absorption intensity increases but at pH 11 the absorbance again decreases (Fig. 5). This indicates that pH 9 is the most favorable pH for the synthesis of Ag-NPs using *C. tamala* leaf extract. Rajoriya [22] also reported that Ag-NPs show maximum stability at the pH 9. The concentration of $AgNO_3$ also affects the formation of Ag-NPs. If increase concentration of $AgNO_3$ solution from 0.001M to

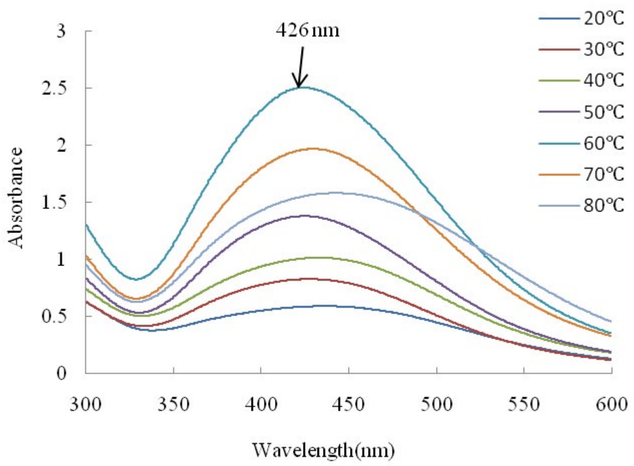

Fig. 4. Effect of temperature.

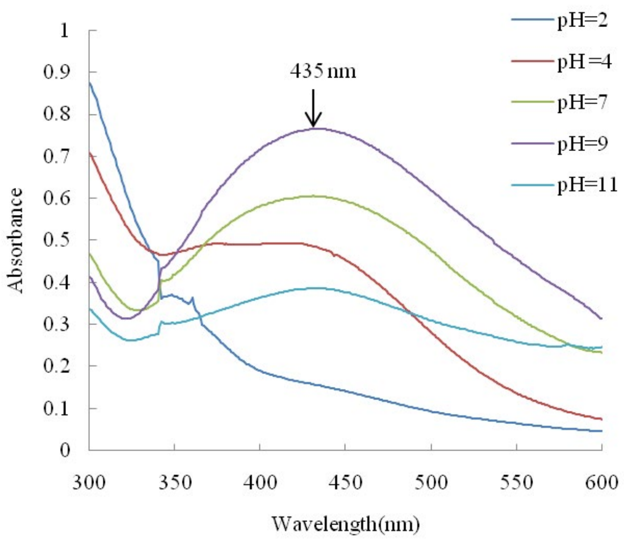

Fig. 5. Effect of pH.

0.01M the intensity increases with concentration and wavelength changed from 415 nm to 428 nm. Maximum absorbance observed in 0.01M salt solution at wavelength 428 nm (Fig. 6). Fig. 7 shows the ratios of *C. tamala* leaf extract and silver nitrate in the ranges of 1 : 1, 1 : 2, 1 : 3, 1 : 4, 1 : 5 and 2 : 1 were utilized in order to find out the optimum composition for the preparation of SNPs. The maximum silver nanoparticle synthesis occurred in 1 : 1 ratio, which was confirmed by the formation of highest peak in spectroscopy.

Characterization

UV-Visible absorption spectrum

The optical properties of silver nanoparticles were studied by absorption spectroscopy. The structural change of the particles can be easily examined by the UV-Vis absorption spectrum, which can help us to know the complex formation. It is the primary method to indicate the bioreduction of silver from aqueous silver nitrate solution to silver nanoparticles. Surface Plasmon resonance bands play a vital role in size, shape, morphology [26]. Fig. 8 shows the UV–visible spectra of the nanoparticles obtained by *C. tamala* leaf extract. The particles synthesized gave a Plasmon resonance band at 424 nm. UV-Vis spectroscopy almost used to detect the presence of Ag-NPs through green syntheses [27, 28]**.** In particular, absorbance in the range of 400 nm to 450 nm has been used as an indicator to confirm the reduction of Ag^+ to metallic Ag^0 [27, 29]**.**

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy measurements are carried out to identify the

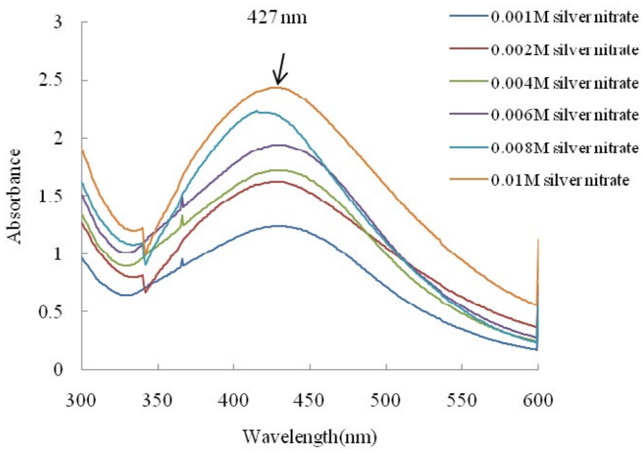

Fig. 6. Effect of concentration of $AgNO_3$.

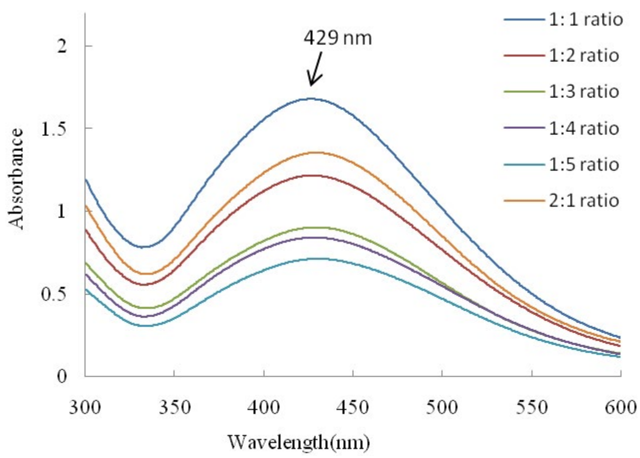

Fig. 7. Effect of ratio of leaf extract and $AgNO_3$ solution.

possible biomolecules responsible for reduction, capping and efficient stabilization of silver nanoparticles [30]. There were many functional groups present which may have been responsible for the bio-reduction of Ag^+ ions. The band intensities in different regions of the spectrum for plant extract and silver nanoparticles were analyzed and are shown in Fig. 9. FTIR spectrum of *C. tamala* leaf extracts shows different major peak positions at 3458, 2098, 1638 and 739 cm^{-1} and FTIR spectrum of *C. tamala* nanoparticles shows different major peak positions at 3457, 2093, 1635, 1384, and 733 cm^{-1}. The spectra with some marginal shifts in peak position clearly indicate the presence of the residual plant extract in the sample as a capping agent to the silver nanoparticles. The

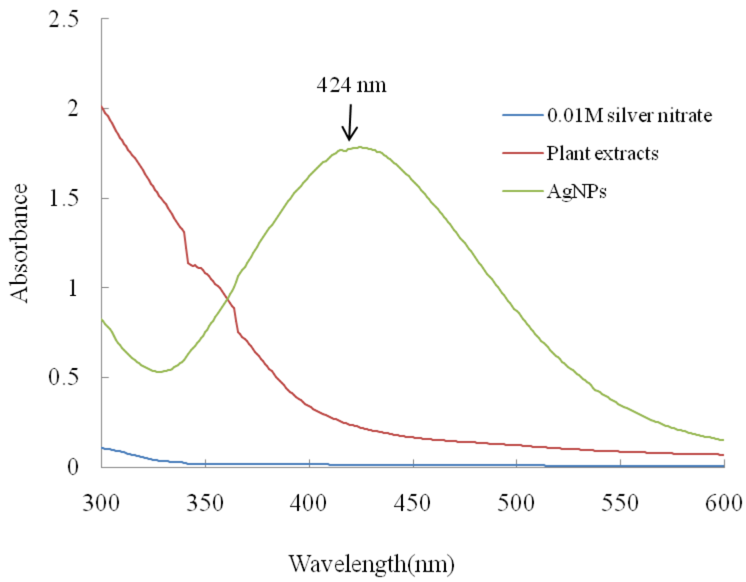

Fig. 8. UV- visible spectra of 0.01M $AgNO_3$ solution, *c. tamala* leaf extract and AgNPs.

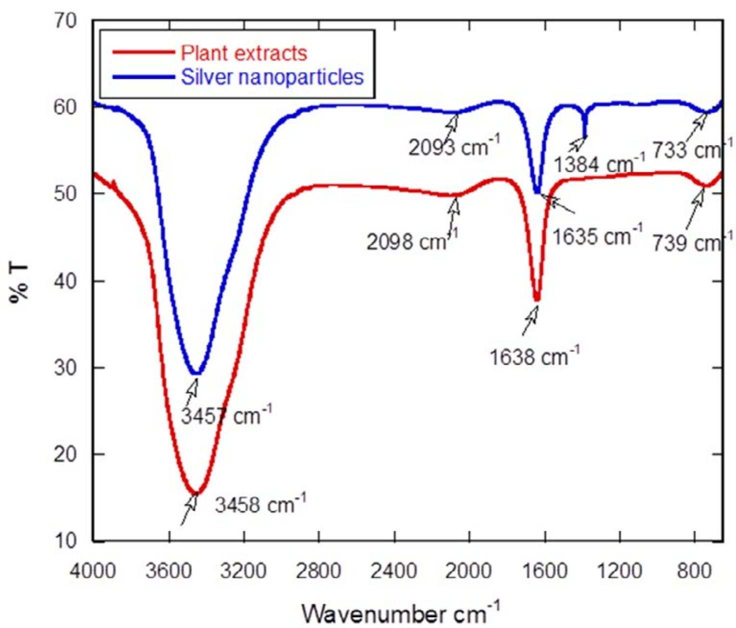

Fig. 9. FTIR spectra of *C. tamala* silver nanoparticles.

broad and intense peak at 3457 cm^{-1} corresponds to OH stretching vibrations of phenol/carboxylic group present in extract, a peak at 2093 cm^{-1} can be assigned to alkyne group present in phyto-constituents of extract. The peak located at 1635 cm^{-1} could be assigned to C=O stretching or amide bending [31]. The peak at 1384 cm^{-1} assigned to nitro N-O bending [32] and 733 cm^{-1} assigned to C–H alkenes stretch. The observed peaks are mainly attributed due to presence of some secondary metabolites like flavonoids, triterpenes, tannins, steroids and saponins excessively present in plants extract (Table 1) as also suggested by other researchers [33, 34].

Scanning Electron Microscopy Analysis (SEM)

SEM images are used to study the morphology of silver nanoparticles. It has been observed that the size differences, size distribution, and capacity for aggregation depends on experimental conditions, stabilities etc. [35]. Fig. 10a and 10b shows the SEM image of the synthesized silver nanoparticles sample at 25 °C and 60 °C, respectively. SEM analysis shows that the nanoparticle formed was spherical in shape with average size 27 nm (15nm - 43nm) at 25 °C and 19 nm (9 nm - 35 nm) at 60 °C.

Transmission Electron Microscopy Analysis (TEM)

The TEM image of the Ag-NPs is depicted in Fig. 11. The average size of the nanoparticles was found to be 16 nm at 25 °C and the shape was almost spherical, hexagonal and irregular with a wide size distribution (Fig. 11a). On the other hand, at 60°C the average particle size was found 9 nm as shown in Fig. 11b. It was revealed that increasing the reaction temperature leads to Ag-NPs with narrow size distribution. The images also show the existence of nanocrystalline structure in the particles.

X-ray diffraction

XRD is a valuable characterization tool to prove the formation of Ag-NPs, determine the crystal structure and calculate the crystalline nanoparticle size [36]. Fig. 12a shows the silver nanoparticles synthesis at 25 °C having three intense peaks were 38.2, 44.48 and 64.66 corresponding to the planes of (111), (200) and (220), respectively which were in good agreement with reference to the unit cell of face-centered-cubic (FCC) structure of metallic silver (Joint Committee for Powder Diffraction

Table 1. Phytochemical test of cinnamomum tamala leaf extract.

Phytochemicals	Result
Flavonoids	+
Carbohydrates	+
Triterpenes	+
Tannins	+
Alkaloids	-
Steroids	+
Saponins	+

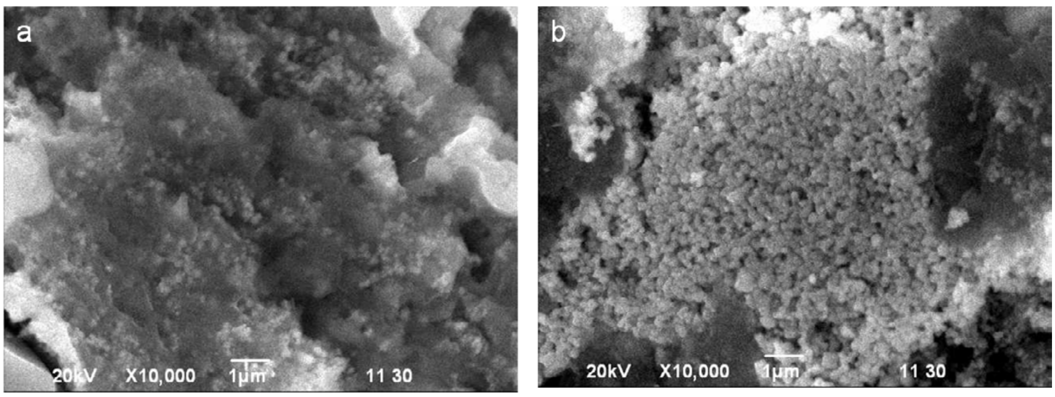

Fig. 10. SEM images of the AgNPs (a) at 25°C temperature; (b) at 60°C temperature.

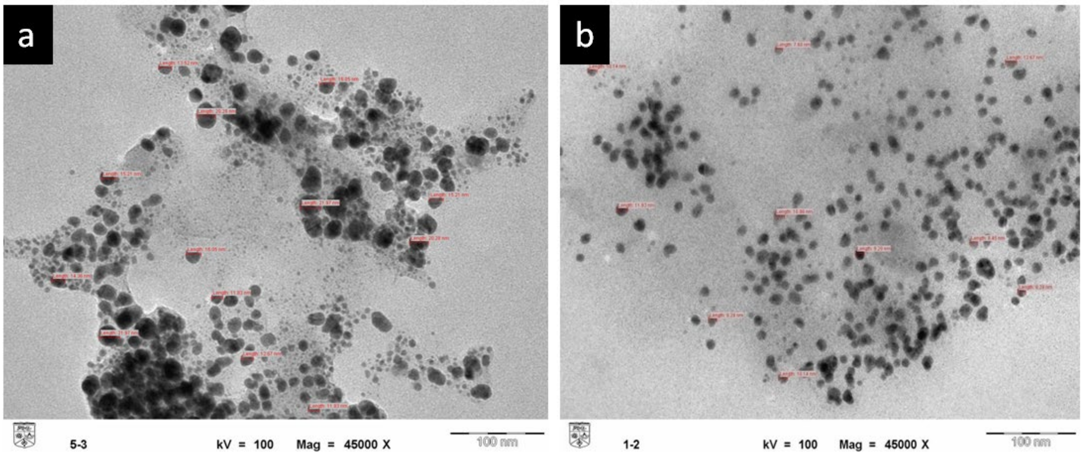

Fig. 11. TEM images of the AgNPs (a) at 25°C temperature; (b) at 60°C temperature.

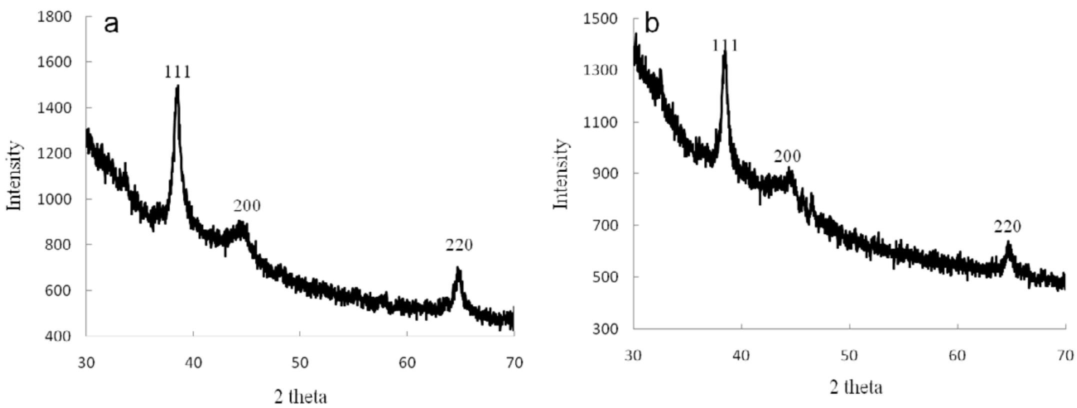

Fig. 12. XRD of the AgNPs (a) at 25°C temperature; (b) at 60°C temperature.

Table 2. Antibacterial activity of tested samples.

Name of bacteria	Zones of inhibition (mm)				
	$AgNO_3$	Plant extract	Standard antibiotic (Tetracyclin)	AgNPs	
				25°C temperature	60°C temperature
Bacillus subtilis	-	7	8	9	10
S. aureus	-	7	8	8	10
Escherichia coli	-	7	7	12	13
P. aeruginosa	-	7	8	10	12

Standards, JCPDS File No. 01089- 3722). Fig. 12b shows the silver nanoparticles synthesis at 60°C display three intense peak at 38.58, 44.32 and 64.88 corresponding to the planes of (111), (200) and (220), respectively. Also, the XRD result was in agreement with the other earlier researches [37, 38].

Antibacterial activity

Silver nanoparticles have been using in many industries such as the health, pharmaceuticals, water treatment, paint, food storage because of its antibacterial properties [39]. In the present study, the antibacterial activity of bio synthesized Ag-NPs was tested against some bacteria. It is apparent that the Ag-NPs showed inhibition zone against tested bacteria (Table 2). The power of Ag-NPs against human pathogen was depended on the size and dose. The synthesized Ag-NPs were found to have a higher inhibitory action at higher dose and smaller size to tested bacteria. The larger sizes of nanoparticles have less activity than smaller size nanoparticles due to small surface area. One of the possible modes of action of the plant mediated Ag-NPs might be to attach to the cell surface and disrupt the cell membrane and interact. After penetration, Ag-NPs released silver ion. These ions interacted with DNA, proteins, and sulfur containing cell constituents, therefore, the organisms were prevented [40].

CONCLUSIONS

The green synthesis of silver nanoparticles by using leaf extract of *C. tamala* is very simple and eco-friendly method. The separation of this nanoparticle was performed by centrifugation while the identification was by UV-Vis spectra, XRD, FT-IR, SEM and TEM methods. Reduction of the Ag^+ to Ag^0 during exposure to the *C. tamala* leaf extract was followed by color change of the solution from colorless, yellow to dark brown. Optimization of some parameters was also done for the efficient formation of silver nanoparticles. It is observed that surface Plasmon resonance peaks of the maximum absorbance of silver-nanoparticles occur at 424 nm, indicating that Ag-NPs were produced. The Ag-NPs obtained were subjected to biological evaluation and tested against Gram-positive and Gram-negative bacterial strains. It was evident that the Ag-NPs displayed moderate to very good bactericidal properties; however, it was also observed that a higher inhibitory action at higher dose and smaller size to tested bacteria. Future studies will probably focus on obtaining nanoparticles with antimicrobial effects at its maximum level and toxicity at minimum. Because of this reason, synthesizing metallic nanoparticles, especially by non-toxic green synthesis method, which is used in many application fields such as cancer treatment, drug transport, biosensor construction is of great importance today.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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