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FACILE GREEN SYNTHESIS OF *Cinnamomum tamala* EXTRACT CAPPED SILVER NANOPARTICLES AND ITS BIOLOGICAL APPLICATIONS

Abstract: The plant mediated biogenic synthesis of nanoparticles is of magnificent concern due to its eco-benign and single pot nature. Here, *Cinnamomum tamala* (*C. tamala*) aqueous leaf extract was utilised for the silver nanoparticles' (Ag NPs) synthesis. The phytoconstituents in the leaf extract were analysed by standard methods. These metabolites, especially carbohydrate polymers reduce Ag ions to Ag NPs accompanied by a reddish-brown coloration of the reaction mixture. The visual observation of intense brown colour is the first indication of the formation of Ag NPs. Various spectro-analytical techniques further characterise the Ag NPs. The green synthesised spherical Ag NPs were crystalline with an average size of 38 nm. The Ag NPs were scrutinised for antioxidant, antimicrobial and cytotoxic activity and obtained good results. The free radical scavenging was studied by 2, 2-Diphenyl-l-picrylhydrazyl (DPPH) assay. The antibacterial activity of Ag NPs was assessed against human pathogens, and it shown to have good antibacterial potency against a wide spectrum of bacteria. The cytotoxic activity against HEK-293T (human embryonic kidney) cell line was evaluated by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay. These potent biological activities enable *C. tamala* capped Ag NPs to be suitable candidates for the future applications in various fields, predominantly clinical and biomedical.

Keywords: green synthesis, silver nanoparticles, antioxidant, antibacterial, cytotoxicity

Introduction

The noble metal nanoparticles have been explored in various sectors by the virtue of its interesting properties and has received widespread acceptance in the field of

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bionanotechnology [1, 2] Ag NPs received special interest among the metal NPs due to its characteristic features such as enhanced stability, catalysis, electrical conductivity, sensing capability, antimicrobial etc. [3-6]. Although numerous synthesis strategies were available for Ag NPs synthesis, chemical reduction method was commonly employed due to rapid process of obtaining uniform nanoparticles [7]. The toxic chemicals associated with the nanoparticles during synthesis limits its biomedical and clinical applications. Thus, there is an urgent need for newer methods, which can dominate the conventional methods for the synthesis of nontoxic nanoparticles. Nowadays, the increased awareness of hazardless procedures has given more acceptance for the bio-inspired synthesis [8-11]. The metal nanoparticle synthesis using plant biomass is beneficial over other methods since it is non-toxic, economically viable and environmentally friendly [12-15]. The extracts prepared from plant parts is rich of phytoconstituents such as alkaloids, polyphenols, terpenoids, flavanoids, glycosides etc. accountable for the reduction and stabilisation of NPs during the biosynthetic pathway [16-18]. This gives rise to exquisite bactericidal activity for the synthesised Ag NPs against a broad spectrum of pathogens [19]. Ag NPs capped with the biomolecules gives better biological properties compared to the nanoparticles synthesised through conventional methods.

Currently, nanoparticles synthesised with medicinal plants have acquired specific attention. The plant metabolites in these templates have a particular contribution in the formation of less toxic nanoparticles [11, 17, 20, 21]. In this study, leaf extract of *Cinnamomum tamala* (*C. tamala*, commonly known as Indian bay leaf - Lauraceae family) was utilised for Ag NPs synthesis. This plant is commonly available in India and these leaves were recommended for several ailments in traditional medicinal systems such as heart troubles, scabies, bad taste, piles, etc. due to its heating and alexiteric properties [22-24]. The leaves have been used medicinally for the treatment of diarrhea, colic trouble, rheumatism, nausea, and vomiting [25-27]. By the virtue of the active phytochemical constituents, *C. tamala* leaf extract exhibits eloquent biological properties. As per the earlier reports on chemical constitution of *C. tamala* essential oil, it contains monoterpenoids including eugenol, phellandrene, linalool, traces of α-pinene, p-cymene, ß-pinene, camphene, myrcene, limonene and methyl ether of eugenol [28, 29]. The terpenoids, proteins, alkaloids, polyphenols, phenolic acids, sugars and other secondary metabolites are crucial during the synthesis process [30]. Carbohydrates and flavonoids take part in the reduction of noble metal nanoparticles. Polymeric moieties with different functionalities, mainly polyphenols have decisive role in the stabilisation of synthesised nanostructures [31].

A reliable, eco-friendly method was designed for the synthesis of Ag NPs using silver nitrate ($AgNO_3$) solution and *C. tamala* aqueous extract. The Ag NPs were characterised by UV-Vis spectrophotometry, FTIR, XRD, FESEM, EDX and HR-TEM. After phytochemical screening of *C. tamala* leaf extract, aqueous extract was utilised for Ag NPs synthesis. The biological activities such as free radical scavenging, antibacterial activity and cytotoxic effects were evaluated for further applications.

Materials and methods

Reagents

Silver nitrate ($AgNO_3$), Kanamycin and petroleum ether were obtained from Sigma-Aldrich. The cell lines HEK-293T was procured from NCCS (National Centre for

Cell Science), Pune. Bacterial strains were acquired from CSIR-IMTECH (Institute of Microbial Technology), Chandigarh. The Cell Proliferation Kit II (XTT) - 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium5-carboxanilide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), fetal bovine serum (FBS), phenazine methosulphate (PMS), ascorbic acid, sterile discs, streptomycin, ampicillin, alpha naphthol, Anthrone reagent, ninhydrin, trichloro acetic acid, millions reagent, sodium citrate tribasic, ferric chloride, sodium phosphate monobasic, sodium phosphate di basic, methanol, Luria Bertani Agar (LBA), Luria Bertani Broth (LBA) and potassium ferricyanide were purchased from Hi-Media. Chloroform, benzene and Fehling's A and B, acetic acid, sulfuric acid, ethanol, hydrochloric acid and acetone were obtained from Merck life science Pvt. Ltd. Sodamide and glacial acetic acid were obtained from Loba chemie Pvt. Ltd. Pottassium iodide, iodine and phenolphthalein were obtained from Sisco Research Laboratories Pvt. Ltd. The leaves of *C. tamala* were gathered from Payyannur, located in Kannur district, Kerala. Millipore water was used throughout the experiment.

Instrumentation techniques

The morphology and elemental composition of synthesised Ag NPs was analysed by field emission scanning electron microscopy (FESEM - Carl Zeiss, Germany) equipped with Energy dispersive X-ray spectroscopy (EDAX - Oxford Instruments, England). The particle size distribution was obtained from HR-TEM analysis with transmission electron microscopy (TEM) (Jeol/JEM 2100). An ultraviolet (UV)-visible spectrophotometer (Perkin Elmer Lambda 35) was adopted to scan in the wavelength region of 200 nm - 700 nm for the determination of its optical properties. The crystallinity and purity confirmations were done with the X-Ray Diffraction (XRD) patterns seized from a diffractometer (Rigaku Miniflex 600, Tokyo, Japan) for the diffraction angle (2θ) between 20° and 90°. The identification of surface functionalities in the NPs were obtained from the Fourier transform infrared (FTIR) spectrum, recorded by a spectrometer (Perkin-Elmer FTIR Spectrum Two) in between 4000 cm^{-1} and 400 cm^{-1}.

Preparation of *C. tamala* extract

Freshly collected *C. tamala* leaves were shade dried for 20 days - 30 days and powdered well. Then leaf extract was prepared by using soxhlet extraction method. Different solvents such as petroleum ether, benzene, chloroform, ethanol and water were used for extraction. 10 g leaf powder was packed well and extracted using 250 mL solvent for 2 h. The extract was stored in 4 ^{o}C and used as a stock solution for further experiments.

Evaluation of phytochemicals

Leaf extracts were prepared with different solvents of varying polarity and subjected to qualitative analysis of phytochemical constituents. Extracts were analysed for alkaloids, glycosides, carbohydrates, reducing sugar, steroids, proteins and amino acids, saponins, terpenoids, flavonoids, fixed oils and fat, gums and mucilages, phenol and tannins by using standard methods [32]. Tests were performed to check whether the constituents were present in these extracts (Table S1 in supplementary data).

Synthesis of Ag NPs

30 mL of *C. tamala* leaf extract was added to 100 mL of $AgNO_3$ solution (1 mM) in a 250 mL Erlenmeyer flask. The reaction mixture was stirred vigorously (700 rpm) on a magnetic stirrer at 80 °C for 30 min. The resultant solution was centrifuged, and the collected blackish powder washed thoroughly with millipore water to remove the adhered impurities. The final product was dried at 80 °C.

The Ag NPs synthesis is optimised, by evaluating influencing variables such as of silver nitrate concentration, extract concentration (volume), reaction temperature and incubation time.

Evaluation of antioxidant activity by DPPH free radical scavenging method

0.1 mM DPPH solution in methanol was prepared. 2 mL DPPH solution was mixed with 1 mL of different concentrations (10, 400) µg/mL of Ag NPs. DPPH without Ag NPs was used as control [33]. This reaction mixture was incubated at room temperature for 30 min in a vortex shaker. The UV-Vis spectrophotometer monitored absorbance of the solution at 517 nm. The radical scavenging activity was compared with the standard reference, L-ascorbic acid. The higher antioxidant potential of the reaction mixture was indicated by the lowering of absorbance value. The free radical scavenging activity, RSA [%] - is obtained from equation:

$$\mathrm{RSA} = (A_c - A_s) \cdot 100 \quad (1)$$

where A_c denotes the absorbance of control, absorbance of DPPH without sample and A_s is the absorbance of sample. Antioxidant activity of *C. tamala* aqueous leaf extract was also determined by the same method.

The effective concentration 50 % (EC_{50}), is the amount of substance for the 50 % scavenging of DPPH was calculated. It is the amount of antioxidant to reduce the concentration of DPPH radical to 50 %.

Antibacterial activity of Ag NPs

The antimicrobial activity of green synthesised Ag NPs against human pathogens was evaluated by the agar disc diffusion method [34]. Strains of human pathogenic bacteria such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*) and *Staphylococcus aureus* (*S. aureus*) were used. The subculturing of bacteria was carried out using Luria Bertani (LB Broth) medium and were kept for incubation at 37 °C for 24 hrs. The fresh bacterial cultures were evenly strewed on the Luria Bertani agar plates for bacterial cultivation. Sterile discs were placed on it and added 20 µL of plant extract and colloidal Ag NPs. The Petri plates were incubated again at 37 °C for 24 hrs.

Evaluation of cytotoxicity of Ag NPs using XTT assay

The DMEM medium complimented with 10 % (v/v) heat inactivated FBS and 1 % (v/v) Kanamycin was used as the culture medium for HEK-293T cell lines. And the cells were kept in 5 % CO_2 humidified incubator at 37 °C. Seed the well-grown cells into microplates (96 wells) at a concentration of 10×10^3 cells/well for the experiments.

XTT assay is a tool for measuring cellular metabolic activity to indicate cell viability and cytotoxicity. It is a colorimetric assay, based on the reduction of yellow tetrazolium salt

(XTT) to an orange formazan dye by the metabolically active cells. Seed the cells in a microplate (96 wells) and incubate the cell cultures overnight at 37 °C and 5 % CO_2. After incubation, the morphologies of cells were observed in an inverted microscope before exposure to Ag NPs. The supernatants were removed and then added different concentrations of Ag NPs solution to the wells. Incubate the treated cells at 37 °C and 5 % CO_2 for 24 hours. After the subsequent addition of 10 μL XTT solution (5 mg/mL), the plates were again kept in incubator for 4 h. A multi-plate reader was used to record the absorbance of the mixture at two wavelengths (570 nm and 630 nm). The blank wells with growth media alone were used to correct the absorbance values obtained for Ag NPs. Independently triplicate experiments were carried out.

The percentage of cell viability, XTT [%] was calculated using equation:

$$XTT = [(A_t - A_b) / (A_c - A_b)] \quad (2)$$

where: A_t - absorbance of the test solution, A_b - absorbance of the blank.

Results and discussion

Qualitative phytochemical analysis

The qualitative determination of phytochemicals present in of *C. tamala* leaf extract was executed and tabulated below (Table 1). It disclosed the existence of a number of metabolites such as alkaloids, flavonoids, terpenoids etc. in the extract. The *C. tamala* leaf extract is enriched with bioactive compounds. The ethanolic and aqueous extracts were shown to have glycosides, terpenoids, phenol and tannins in common. In addition to this, carbohydrates, flavonoids and reducing sugar were also present in the aqueous extract. Saponins were present in benzene and ethanolic extracts. Chloroform and petroleum ether fractions contain fixed oil and fat. The study provides evidence for medicinally critical bioactive components in the aqueous extract, compared to others and can be used for further studies. These biomolecules account for the reduction and stabilisation of metal ions. The biopolymers like carbohydrates and flavonoids as reductants provide stability to the synthesised Ag NPs. The polyphenols as a capping agent and stabilising agent impart extra stability to the synthesised Ag NPs [15, 31, 35]. The details of phytochemical screening tests were presented in Table S1 (Supplementary data).

Table 1

The phytochemical analysis of aqueous, ethanol, chloroform, benzene and ether extracts of *C. tamala* leaves

Phytochemicals	Petroleum ether	Benzene	Chloroform	Ethanol	Water
Alkaloids				+	
Glycosides				+	+
Carbohydrates					+
Reducing sugar					+
Steroids					
Proteins and amino acids					
Saponins		+		+	
Terpenoids				+	+
Flavonoids					+
Fixed oils and fat	+		+		
Gums and mucilage					
Phenol				+	+
Tannins				+	+

where [+] indicates presence of the constituent

Characterisation of synthesised Ag NPs

UV-Visible Spectroscopy

Initially, the reaction mixture was colourless, and turned reddish-brown, which signifies the formation of Ag NPs in solution (Fig. 1). The colour transition is an implication of the reduction of Ag^{+} to Ag^{0}. In general, Ag NPs are shown to have a characteristic absorption in the range of 400 nm - 460 nm. The surface plasmon resonance (SPR including quantum size effect) of metal NPs leads to the formation of an absorption band. The biofabricated Ag NPs show a strong absorption band at 430 nm, typical absorption for Ag NPs.

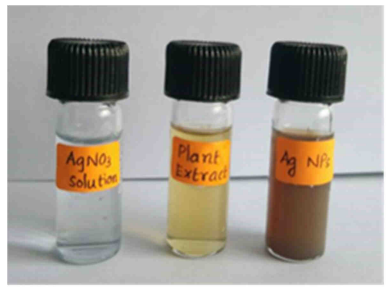


Fig. 1. Formation of Ag NPs in solution

The various physico-chemical conditions were optimised to scale up the synthesis of Ag NPs. The concentration of silver nitrate solution, amount of *C. tamala* extract, reaction temperature and incubation time are the parameters altered to optimise the synthesis of Ag NPs. For optimum amount of *C. tamala* extract, 1 mL to 5 mL of plant extract was added to 10 mL silver nitrate solution of a particular concentration. The concentration of silver nitrate solution was standardised by taking different concentrations (0.2, 0.4, 0.6, 0.8, 1.0) mM. In order to check the effect of incubation temperature, the reaction is carried out at various temperatures such as room temperature (40, 50, 60, 70 and 80) °C. To study the influence of incubation time on Ag NPs synthesis, the reaction was monitored at regular intervals (15, 30, 45, 60, 75, 90, 105, 120) min (Fig. 2a-d).

By analysing the spectra, on increasing concentration of silver nitrate solution, the absorbance of the resultant solution also increases (Fig. 2a). It may attribute to the increase in Ag NPs concentration in the solution, and is also evidenced from the intensity of the reddish brown colour of the reaction mixture. The Ag NPs synthesis is optimum at 1.0 mM silver nitrate. From the investigation of the effect of the amount of plant extract, it is clear that the peak broadens on the increase in amount of extract (Fig. 2b). The broadening of the peak may be due to the slow reduction process that occurred [36]. The peak obtained for 3 mL had higher absorbance, indicating immense Ag NPs formation and thus setting the optimum value for further study. As for the reaction temperature relation to Ag NPs synthesis, the synthesis process occurs at a higher temperature (Fig. 2c). The intensity enhancement occurs at elevated temperature due to a faster reaction rate, as the conversion of Ag NPs occurs at a higher rate, leaving less possibility of particle size growth [37]. The effect of incubation time on Ag NPs synthesis was investigated by monitoring the reaction at regular intervals. At 30 min, Ag NPs formation started, indicated by an intense

peak, which is characteristic SPR band of Ag NPs (Fig. 2d). The intensity of the peak increases with the progress of Ag NPs formation. The time at which a narrow peak was obtained with intense colour can be taken as the optimum time of the reaction. The Ag NPs, synthesised by reducing 1 mM of $AgNO_3$ (100 mL) with 30 mL of plant extract for 30 min of heating at 80 ºC temperature showed strong absorption at 430 nm (Fig. 3).

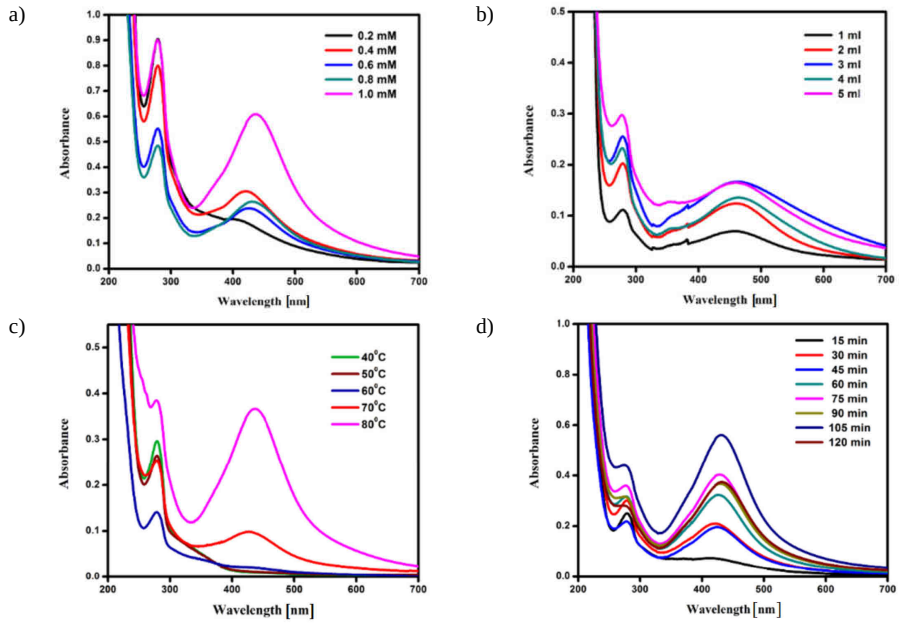


Fig. 2. The UV-Vis spectrum of Ag NPs synthesis at varying: a) concentration of $AgNO_3$ solution, b) amount of *C. tamala* extract added to 10 mL $AgNO_3$ solution, c) temperature of reaction, d) incubation periods

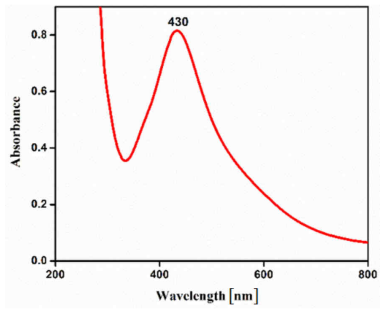


Fig. 3. UV-Vis spectra of Ag NPs

FTIR Spectroscopy

The FTIR spectroscopy explicated the presence of various functionalities present in the leaf extract. These biomolecules are responsible for the reduction of Ag^+ to Ag^0 and its

subsequent stabilisation. The *C. tamala* extract's absorption spectrum has four prominent peaks located at (3293, 2924, 1603 and 1021) cm^{-1} as reported in our previous work (Fig. 4) [38]. And it arises from OH stretching frequency, stretching vibrations of C-H, C=C and C-O respectively. However, the synthesised Ag NPs shows peak shifts with intense absorption bands at 3353 cm^{-1} and 1638 cm^{-1}. The broad band present in 3353 cm^{-1} correlated to –OH groups of flavonoids (phenolic compounds), tannins and glucose [39]. The characteristic band at 1638 cm^{-1} attributed to the carbonyl stretching vibration of flavonoids. The shift in band position in *C. tamala* extract and the Ag NPs clearly indicate that the biomolecules are involved in the reduction reaction with silver nitrate for Ag NPs formation. From the FTIR spectra of Ag NPs, it was clear that the formed nanoparticles were capped with the biomolecules, which stabilises them by preventing further agglomeration (this can be achieved by multiple supramolecular interactions [40]). It was mainly contributed by flavonoids, phenolics and tannins. Such hybrid inorganic-organic nanoparticles have multiple applications of interest [41].

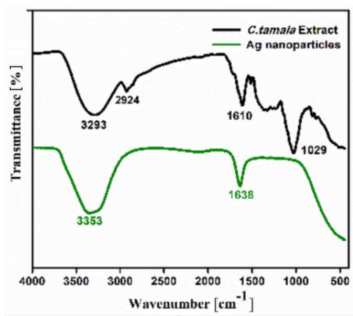


Fig. 4. FTIR spectra recorded for *C. tamala* leaf extract and the synthesised Ag NPs

XRD Analysis

The synthesised Ag NPs were characterised by powder XRD (PXRD) to determine crystalline nature and purity. X-ray diffraction pattern for Ag NPs shows five diffraction peaks (in degrees) at 2θ values: 38.31°, 44.27°, 64.74°, 77.86° and 81.66°. Which corresponds to the facets (111), (200), (220), (311) and (222), respectively. The obtained peaks are comparable with peaks from JCPDS (file No: 89-3722) [42], and can be indexed to the face centered cubic structure of silver. The nanoparticles were crystalline with no such impurities indicated by sharp peaks (Fig. 5). The average nanocrystalline size was calculated from the most intense lattice plane reflection (111) by using (eq. (3)), the Debye-Scherrer equation:

$$D = 0.9\,\lambda / (\beta \cos\theta) \tag{3}$$

From the full width half maximum of (111) reflection, the average crystallite size of Ag NPs, was found to be approximately 17 nm.

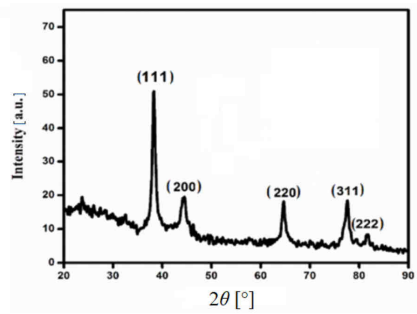


Fig. 5. XRD pattern of *C. tamala* stabilised Ag NPs

FESEM-EDX Analysis

FESEM of the synthesised Ag NPs was done to determine the structural morphology of the nanoparticles and is shown in Figure 6a-6c. From the data it is clear that the Ag NPs were roughly spherical. From the FESEM images, it is clear that the nanoparticles exhibit some sort of agglomeration under lower resolution, and it may come due to weak physical force. The nanoparticles were well separated under high resolution, and were found to be in the nanometer range. The energy dispersive spectrum of synthesised nanoparticles is shown in Figure 6d. It suggested the presence of elemental silver, since elemental silver has a typical strong signal peak at 3 keV. The presence of C and O in the sample is evidenced by the biomolecules attached to the nanoparticles (organic substances) as bio-capping agents. Similar results were reported earlier in the case of phytosynthesis of Ag NPs.

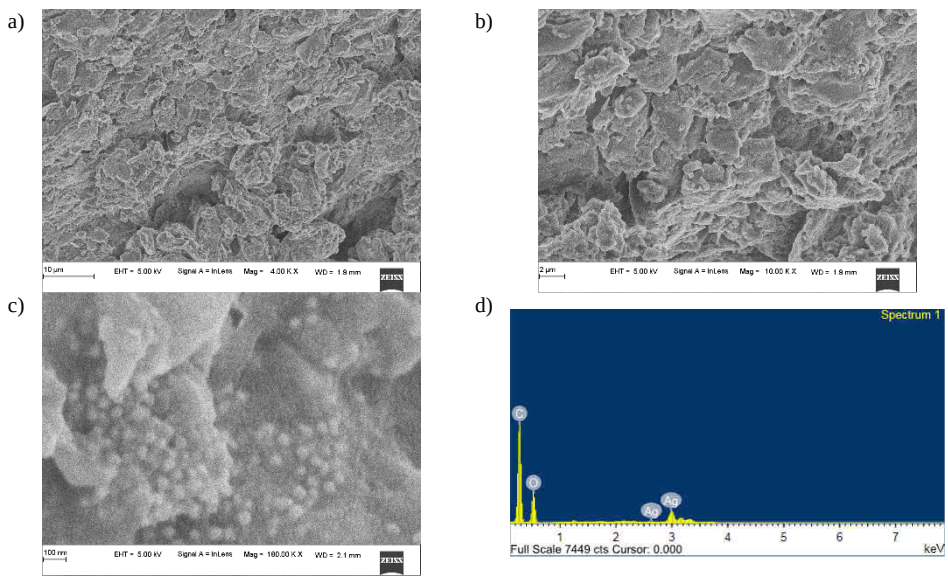


Fig. 6. a-c) FESEM micrograph of Ag NPs, d) EDX spectrum of Ag NPs

TEM Analysis of Ag NPS

The TEM analysis gives more understanding of the size and morphology of synthesised nanostructures. The morphology of the NPs was predominantly spherical. Some notable variation in nanoparticles' size and shape can often be seen in the case of biological synthesis. The Ag NPs were capped with the biomolecules in the extract is evidenced from the lighter particle's edges compared to the centres as reported earlier. The selected area electron diffraction (SAED) pattern shown in Figure 7a confirms the crystallinity of Ag NPs. The Ag NPs showed "d" spacing of 0.21 nm (Fig. 7c), which in turn affirms its crystalline nature. The average particle size of Ag particles was about 38 nm, as manifested in Figure 7d.

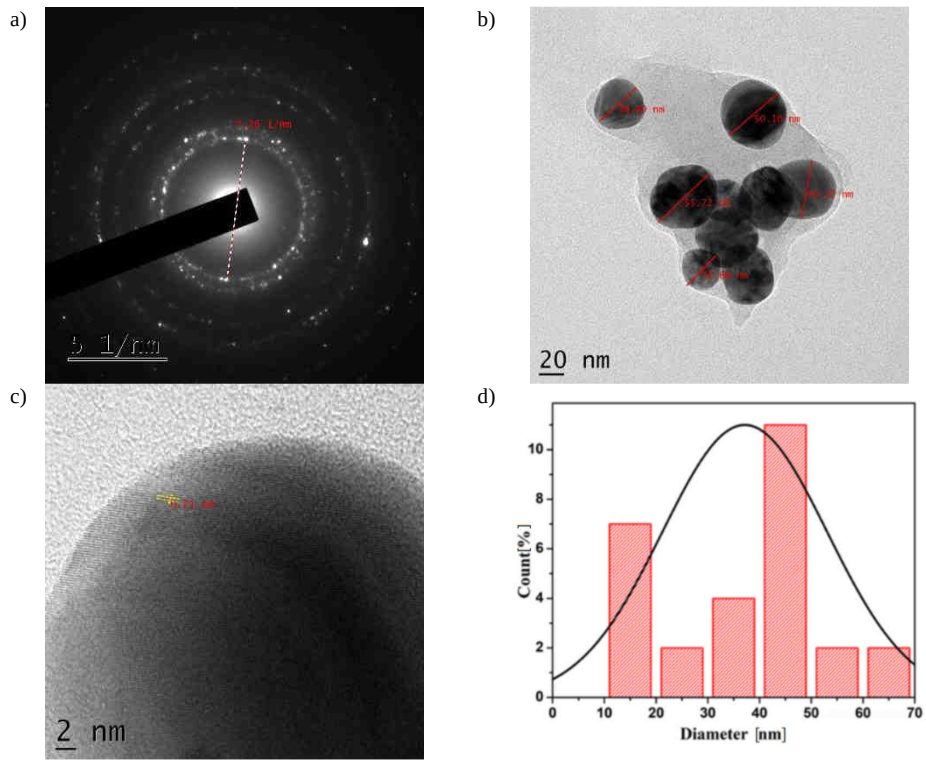


Fig. 7. a) SAED pattern, b) TEM image of Ag NPS at 20 nm range, c) HR-TEM images at 2 nm range, d) histogram table of Ag NPs

Antioxidant activity on DPPH assay

The antioxidant activity of a substance arises due to the capability to donate hydrogen (which could be taken by oxidants by hydrogen atom transfer [43]). DPPH is a stable free radical with intense violet colour, having a strong absorption at 517 nm. The *C. tamala* extract showed significant antioxidant activity, by the bioactive components present in it. The antioxidants present in the *C. tamala* extract can account for the reduction of Ag NPs [44]. Green synthesised Ag NPs showed antioxidant activity against DPPH since the Ag

NPs can reduces the radical into its non-radical form. The antioxidant activity of Ag NPs is due to the biomolecules capped on the surface, especially polyphenols. On reduction, the colour of the DPPH solution changes to pale yellow and decreases the absorption of the resulting solution. The percentage of DPPH radical scavenging activity was increased linearly with an increase in concentration from 5 µg/mL to 400 µg/mL. The *C. tamala* extract show maximum percentage inhibition of 91 % (Fig. 8a). The Ag NPs was also shown to have antioxidant activity in a concentration-dependent manner. The secondary metabolites in the extract tend to reduce the DPPH and scavenge the free radical. The Ag NPs shows a maximum antioxidant potential of 83 % for the same concentration (Fig. 8b). The calculated EC_{50} values for *C. tamala* extract and Ag NPs were 130.86 µg/mL and 96.55 µg/mL, respectively. At low concentration, the Ag NPs have higher DPPH scavenging compared to *C. tamala* extract. At the same time, the scavenging ability increased drastically at higher concentration of *C. tamala* extract, by the presence of a higher amount of biocomponents in it. The phenolics present in the extract clearly justify its better antioxidant activity.

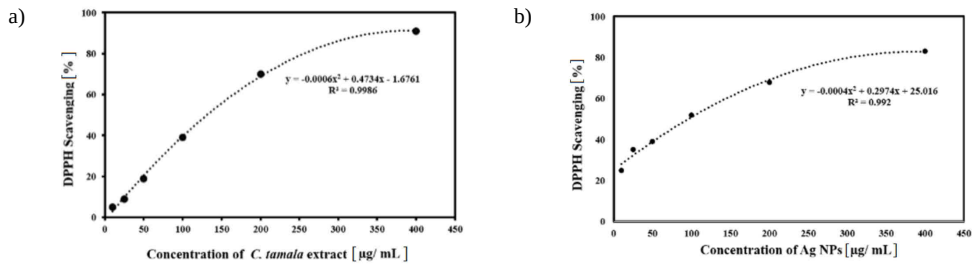


Fig. 8. Antioxidant potential of: a) Ag NPs, b) *C. tamala* leaf extract

Antibacterial activity

The *C. tamala* leaf extract and synthesised Ag NPs were tested for bactericidal activity against human pathogens including both gram negative (*E. coli, K. pneumonia* and *P. aeruginosa*) and gram positive (*S. aureus*) strains of bacteria. The exact mechanism of action of Ag NPs on bacteria remains to be not fully elucidated. However, these Ag NPs can bind with the bacterial cell and disrupt cell functioning. The antibacterial potency of Ag NPs was assessed by the zone of inhibition formed. The biosynthesised Ag NPs display superior bactericidal effect against all bacteria, and it is higher in the case of *S. aureus* and *E. coli* (Fig. 9). The leaf extract did not show noticeable antibacterial activity on all the tested bacteria. The results revealed that Ag NPs itself having antimicrobial activity, and the capping agents bound with the biosynthesised Ag NPs enhance its activity. The Ag NPs demonstrated better antimicrobial effects in comparison to the *C. tamala* leaf extract, due to large surface area nanoparticles. Since the substantial surface area ensures better contact of Ag NPs with the microorganism's cell wall.

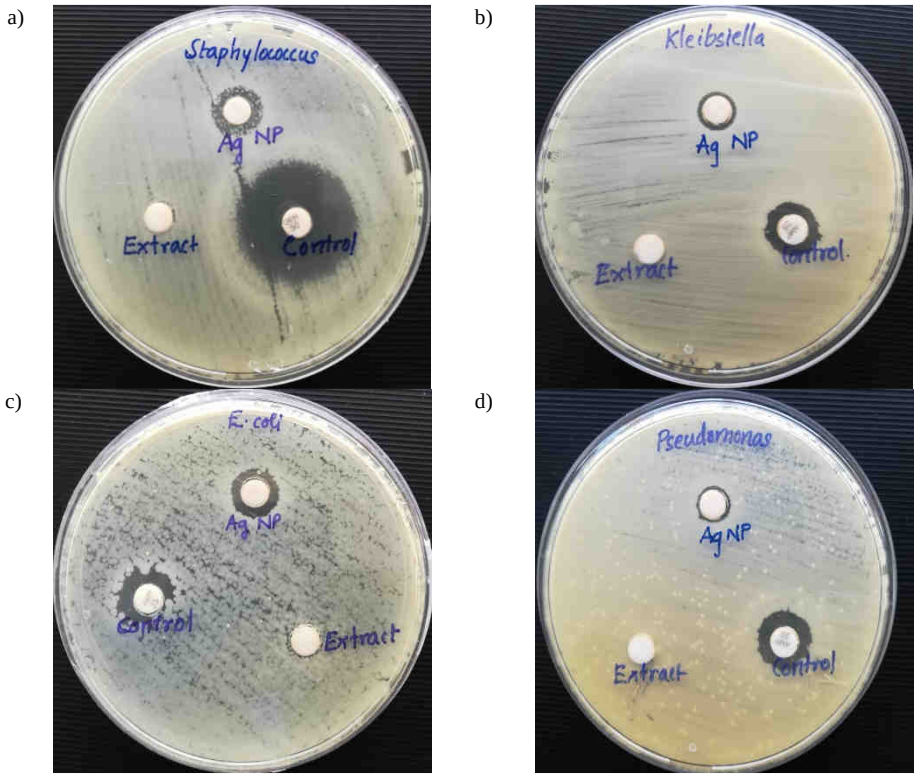


Fig. 9. Bactericidal activity of green synthesised Ag NPs against: a) *S. aureus*, b) *K. pneumonia*, c) *E. coli*, d) *P. aeruginosa*

Cytotoxic assessment of Ag NPs

Cytotoxic effects of the biosynthesised Ag NPs were studied on human embryonic kidney 293T (HEK-293T) cell lines by XTT assay. The HEK-293T cell lines are normal human cells derived from embryonic kidney, widely used standard cell. Nanoparticles, especially nanosilver, are known cytotoxic agents towards normal cells. The percentage cell viability of Ag NPs was calculated and shown in Figure 10. At lower concentrations of Ag NPs, the cells appear similar to the normal untreated cells. But cell damage arises on exposure to a higher concentration of Ag NPs. The cytotoxicity of Ag NPs on HEK-293T cell lines was dose-dependent; i.e., cytotoxic potency of Ag NPs increases with Ag NP's concentration. The NPs impart cell proliferation at lower concentration leading to a rise in cell viability. The toxicity profile of Ag NPs may depend on characteristic parameters like size, shape, agglomeration rate and others. The synthesised Ag NPs were effective against normal kidney cells, HEK-293T. And in the future, it can give insight into cancer nanomedicine using these Ag NPs.

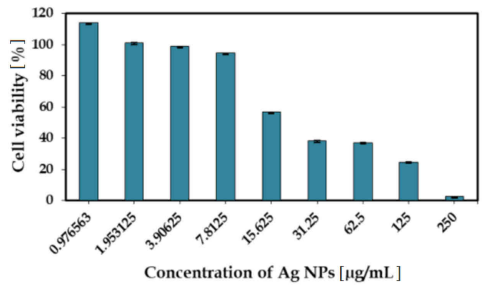


Fig. 10. Effect of Ag NPs concentration on HEK-293T cell viability

Conclusion

An eco-friendly synthesis of stable Ag NPs was proposed based on the bio-platform, *C. tamala* leaf extract. The green synthesised Ag NPs were characterised by various analytical techniques. The purity and crystallinity of synthesised particles were analysed by XRD analysis. The obtained TEM images confirmed the formation of spherical Ag NPs of approximately 38 nm size. The radical scavenging potential of the synthesised Ag NPs was demonstrated by DPPH assay, and the antioxidant activity may arise due to the biomolecules capped with the Ag NPs. The HEK-293T cell lines was adopted as a model system to evaluate the cytotoxicity of green Ag NPs. Moreover, the Ag NPs can be suited as a potent bactericide to human pathogens. The proposed work implements an easy method for the biosynthesis of multifunctional Ag NPs. The *C. tamala* extract has great medicinal importance. Thus, the Ag NPs formed are environmental benign and can be applicable for multifarious applications in the biomedical field and further research.

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