Andrographis paniculata-mediated synthesis of silver nanoparticles: antimicrobial properties and computational studies

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Abstract

The present work reports a simple-economical procedure for the formation of silver nanoparticles by using controlled addition of concentration of cetyltrimethylammonium bromide (CTAB) as a capping agent, taking one-factor-at-a-time into consideration during the optimization process. Wet chemical method for silver(I) reduction has been used to synthesize nanoparticles using *Andrographis paniculata*. During the reaction, antioxidant property of biosystem reduces silver(I) to metallic silver(0) and CTAB encapsulates preventing the agglomeration, acting as barrier for the growth of the particle. The synthesised nanoparticle was characterised by powder X-ray diffraction, Fourier-transform spectroscopy, UV–Vis spectroscopy and SEM. The results showed that the silver nanoparticles doped with CTAB and its high crystallinity can be obtained using simple solvent method. The size of the nanoparticle by XRD, UV–Vis and SEM were found to be 0.897 nm, 45 nm and 18 to 43 nm, respectively. Tauc's plot of optical band gap suggested the movement of size towards quantum confinement for the synthesised nanoparticles was 2.25 eV. An antioxidant activity of *A. paniculata* and synthesized AgNps was studied by series of antioxidant assays. The nanoparticles were having antibacterial and antifungal activities when it was studied using Streptomycin and Ketoconazole as controls.

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Graphic abstract

Keywords Nano silver · Green synthesis · Mie plot · Antioxidant assay · Tauc plot · Disc diffusion assay

Abbreviations

AA	Ascorbic acid
AgNps	Silver nanoparticle
BHT	Butylated hydroxyl toluene
CTAB	Cetyltrimethylammonium bromide
CERAC	Cerric reducing antioxidant capacity
DPPH	2:2-Diphenyl-1-picrylhydrazyl
ET	Electron transfer
FRAP	Ferric reducing antioxidant parameter
GA	Gallic acid
HAT	Hydrogen atom transfer
NOS	Nitric oxide scavenging
SPR	Surface plasmon resonance
XRD	X-ray diffraction

1 Introduction

In the past decade synthesis of nanomaterials had made significant achievement towards catalysis [1], biology and medicine [2], optics [3], and tissue engineering [4]. These

nanomaterials have unique optical and electronic properties different from the bulk due to their high surface to volume ratio. The metal nanoparticle occupies a prominent place in the Hindu mythology due to its stable character [5]. The unique properties such as optical, electrical, thermal, biological associated with AgNps which have made it to venture into different fields including medical, food, health care, consumer and industrial [6]. The properties of AgNps depend upon the factors such as surface chemistry, size, shape, morphology, particle composition, capping, agglomeration and type of reducing agents/antioxidants used in its synthesis [7].

Synthesis of AgNps involves three different approaches namely, physical, chemical and biological. These chemical methods of synthesis are easier and a simplest approach that employs three main components such as metal precursors, reducing agents and stabilizing/capping agents. Basically reduction of silver salts by top-down methods involves two stages viz., nucleation and subsequent growth. Formation of AgNps with or without stabilizers such as surfactants, polymers, co-polymers, long chain

unsaturated carboxylates, solid matrix and hyper branched dendrites has been reported by many investigators [8]. Generally different types of chemical reducing agents are used including sodium citrate [9], ascorbic acid [10], sodium borohydride [11] and hydrazine [12]. It is emphasised that addition of stabilizing agent is to protect the nanoparticles from coagulation. Surfactants in the bulk play a prime role in the surface chemistry of the nanoparticles. The nanoparticles are advantageous due to the large surface area, which can be monitored effectively by the use of UV–Vis spectrophotometry, XRD and SEM. The surface modification can be assessed by FT-IR.

Green synthesised extracts adopted by different researchers for the synthesis of AgNps includes cocoa bean extract [13], miracle fruit plant [14], *Petiveria allia- cea* L. Leaf extract [15], agriwastes, enzymes and pigments [16], *Buchholzia coriacea*, Cocoa pod husk [17, 18], *Cola nitida* [19, 20], *Persea Americana* fruit peel [21], *Opuntia ficus-indica* [22], *Chasmanthera dependens* [23] and so on. The biological method has several advantageous features for instance non-toxic, ecofriendly acceptable route, economical, very much feasible reaction conditions and such others. Bimolecular systems that are associated with plants like protein, Flavanoids, polyphenols not only acts as antioxidants but also as capping agents in overlaying the nanoparticles.

Andrographis paniculata, native to India, although used in many parts of southeast Asia, is one of the most popular medicinal plants traditionally used in the treatment of cancer, diabetes, blood pressure, ulcer, leprosy, skin diseases, influenza and malaria in central Asia, America and African continents [24]. The bitter nature of *A.paniculata* makes it an herbal candidate against the overgrowth of parasitic organisms, especially in the gastrointestinal tract [25]. A study is conducted by Indian journal of pharmaceutical sciences showed, it has choleric, immunostimulant, hepatoprotective, antidiarrhoeal, anti-inflammatory, antibacterial, antitubercular, antihypertensive, antipyretic antifungal, antimalarial action as well as antidote for

snake bites [26]. Phytochemical research reveals that *A. paniculata* contains diverse compounds that includes 55 ent-labdane diterpenoid lactones, 30 flavanoids, 8 quinic acids, 4 xanthones, 5 rare noriridoids, tannins, alkaloids and such others that possess a wide spectrum of biologically active compounds of pharmacological significance [27–29]. This plant derived phytomedicines provide an economical source instead of chemotherapeutic agents. The photographic image of the *A. paniculata* is provided in the Fig. 1.

Basically, the present work reports a green, fast, economical and reliable strategic approach to synthesize silver nanoparticles by using *A. paniculata* and CTAB as a reducing and capping agent, respectively. Furthermore antimicrobial activity on Gram-positive, Gram-negative and fungi was tested using Streptomycin and Ketoconazole as standards and also computational studies have been carried out to study the morphology of AgNps.

2 Materials and methods

All chemicals purchased were of analytical grade and used without further purification. Entire preparations were carried out using double distilled water to avoid any contamination of the synthesised silver nanoparticles. Whole reactions were carried out in dark chamber to minimise the photo-activation of silver nitrate at room temperature.

A SYSTRONICS model 2202 PC, based on double beam ultraviolet–visible (UV–Vis) spectrophotometer with 1.0 cm matched cells were used for all absorbance measurements. A water bath shaker (NSW 133, New Delhi, India) was used to maintain a constant temperature for colour development. All synthetic procedures were initiated by REMI cyclomixer. FT-IR studies were carried out by Agilent FTIR spectrophotometer. An Analytical XRD instrument was used by Xan'pde high score plus software operating at 40 kV voltages and a current of 30 mA with Cu K α radiation. Scanning of biosynthesised silver nanoparticles was exercised in 2 θ region and the data was analysed. Scanning electron microscope (EVO LS 15, Carl Zeiss Microscopy, Jena, Germany) was used to determine the morphologies. Samples were prepared by placing a drop of the working solution on a carbon coated copper grid operating at 5 kV. Particle size determination was carried out by ImageJ software. Tauc plot and curve fitting was done by Origin 16 software. An Antibacterial activity was carried out by using disc diffusion method. Extinction coefficient of different sized nanoparticles was carried out by using Mie plot software. As per the Mie scattering procedure which assumes a complex solution form. Theoretically to determine the size of the nanoparticle several calculations were performed and comparison has been

Fig. 1 Photographic image of *A. paniculata*

made with experimental data. The particle size is the best fit spectrum with the experimental molar extinction coefficient. The particle size is varied from 5 to 60 nm with a periodical span of 5 nm.

2.1 Preparation of the plant extract

A. paniculata extract was used to prepare silver nanoparticles based on its cost effectiveness, ease of availability and high medicinal value. Fresh leaves were collected from nearby agricultural fields adjacent to Mysore city, Karnataka, India in the month of September (average temperature around 25 °C). These were cleaned orderly with running tap and double distilled water to remove debris along with other organic contaminants in addition to dried sunlight. These leaves were powdered. About 1.0 g of the powdered plant was kept in a beaker containing 50 mL of double distilled water. An extract was cooled and filtered with Whatman (no. 540) filter paper and stored at 4 °C for subsequent use.

3 Antioxidant activity: a substantial proof in the reduction of Ag (I)

Although there are different instrumental methods to provide a substantial proof for the formation of nanoparticle that include XRD, and UV–Vis spectra. But still it is further justified by the antioxidant power of the *A. paniculata*. The antioxidant capacity of *A. paniculata* could be done by analytical assays such as metal reducing antioxidant capacity assay involving dyes like indigo carmine and amaranth, scavenging assays for instance 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide, total phenolic content assay by Folin-Ciocalteu reagent, phosphomolybdenum method by using standards antioxidants namely Ascorbic acid (AA), Butylated hydroxyl toluene (BHT) and Gallic acid (GA) as described below.

3.1 DPPH radical scavenging activity

The DPPH radical scavenging activity of the extract and AgNps was done by Blois [30] method with slight modification. 1 mL of 0.2 mM DPPH (prepared by methanol) was mixed with 0.9 mL of double distilled water and 0.1 mL sample with varying concentrations including 0.01, 0.025, 0.05, 0.1, 0.2 mg/mL of AgNps. The reaction mixture was incubated for 20 min at 28 °C under dark. The control contained all the reagents but without the sample while methanol was used as a blank. The DPPH radical scavenging activity was determined by measuring the absorbance at

517 nm using spectrophotometer. The scavenging DPPH activity (%) of the tested sample was calculated as

$$= (1 - \text{absorbance of the sample} / \text{absorbance of control}) \times 100.$$

3.2 Nitric oxide scavenging (NOS) activity

Nitric oxide generated from sodium nitroprusside interacts with oxygen to produce nitrite ions, assayed by Griess reaction with slight modification as suggested by Green et al. [31]. Scavenging of NO for extract and AgNps was determined by incubating sodium nitroprusside (5 mM, in Phosphate Buffer Saline) with various concentrations of the extract at 25 °C. After 2 h, 0.5 mL of Griess reagent was added. The absorbance was measured at 550 nm. The percent inhibition of NO was calculated by using the formula

$$= (1 - \text{absorbance of the sample} / \text{absorbance of the control}) \times 100$$

3.3 Total phenol content

Total phenol content of the leaf extract of *A. paniculata* was determined by the Folin-Ciocalteu method [32]. 150 µL of extract, 2400 µL of ultrapure water and 150 µL of 0.25 N Folin-ciocalteu reagent were combined, mixed well and was allowed to react for 3 min. To this 300 µL of 1 N sodium carbonate solution was added and mixed well for uniform concentration. The solution was incubated at room temperature in the dark for 2 h. The absorbance of the solution was measured at 725 nm by using a spectrophotometer and the results are expressed in mg of GA equivalents per gram of the extract fraction.

3.4 Determination of total antioxidant activity

Total antioxidant activity was evaluated by phosphomolybdenum method as described by Prieto et al. [33]. The procedure for determination is based on standard calibration which involves mixing 1.0 mL of extract with 1.0 mL of standard reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After cooling, the absorbance was measured against the reagent blank at 695 nm. The antioxidant capacity is expressed as mg of AA equivalence per gram of extract.

3.5 Determination of total ascorbic acid content

Total ascorbic acid content in the extract was evaluated by the method reported by Zhang et al. [34] with slight

Table 1 Antioxidant activity of *A. paniculata* by selected HAT and ET methods

SI No	Mechanism	Assay methods	Quantification/parameter	Antioxidant capacity/reducing ability
1	HAT	DPPH*	Scavenging	0.01 mg/mL
2		NOS*	EC ₅₀ /IC ₅₀	0.02 mg/mL
3	ET	FRAP**	Linearity/Beer's law	17.175 µM
4		CERAC**		1.825 µM
5		FCR***		0.23 µM
6		Ferricyanide, ferric chloride method**		6.87 µM
7		Phosphomolybdenum**		5.68 µM

Standards selected as *BHT, ** AA, *** GA

modification. Ascorbic acid in the range of 0.02, 0.04, 0.06, 0.08, 0.10 mg was mixed with 1.0 mL of 15 mM potassium ferricyanide, 1.0 mL of 15 mM ferric chloride and the final volume was made to 25.0 mL with distilled water. The reaction mixture was maintained at room temperature for 25 min, after which its absorbance was read at 720 nm against the reagent blank. The antioxidant activity is expressed as mg of ascorbic acid equivalents per gram of extract.

3.6 Metal reducing antioxidant parameter

3.6.1 Ferric reducing antioxidant capacity (CERAC)

The CERAC was studied involving indigocarmine [35] and amaranth [36] with slight modification to previously suggested methods. Extracted sample in the range from 1 to 10 mg was mixed with 0.48 mM Ce(IV) and 0.085 mM of indigocarmine dye in a final volume of 10 mL. After shaking for uniform concentration the absorbance of the solution was measured at 610 nm with reference to control. The content is expressed as mg of AA equivalents to per gram of extract.

3.6.2 Ferric reducing antioxidant parameter (FRAP)

The stock solution was prepared as suggested by Nagaraja et al. [35]. To 1 mL of the mixture containing ferric ammonium sulphate and 1,10-phenanthroline different concentrations of extract were added. The absorbance of the resulting dark red coloured solution was measured at 510 nm against corresponding control. The antioxidant reducing parameters were evaluated as AA equivalents. Table 1 depicts the antioxidant activity evaluated by different concentration using hydrogen atom transfer (HAT) and electron transfer (ET) methods.

4 Eco-synthesis of silver nanoparticles

In enhancing the precision of the experimental results and to optimize the synthetic procedure, the experiment was repeated by taking one-factor-at-a-time method. Experimental factors were designed in such a way that any one concentration was varied by keeping remaining factors at constant. AgNps were prepared by the reduction of silver nitrate solution with antioxidant in the presence of CTAB. After performing a series of experiments,

Fig. 2 Photographic images of the **a** silver nitrate, **b** *A. paniculata* plant extract alone and **c** AgNps **A** Silver nitrate **B** *A. paniculata* **C** AgNps

by varying the concentration of reductant a perfect silver sol was obtained.

Finally, 5.0 mL of the reaction mixture was mixed with silver nitrate solution of 1.6 mM and 0.02 mM CTAB (Himedia, Mumbai, India). The colourless reaction mixture was reacted with 1.0 mL of *A. paniculata*. Removal of the uncapped biochemical compounds or CTAB from the synthesised AgNps was done by centrifuging 50 mL of the residual solution of AgNps at 10,000 rpm for 30 min and followed by first resuspending in double distilled water subsequently in sterile distilled water in an order. This centrifugation and resuspension procedure were repeated for 4–5 times.

5 Results and discussion

5.1 Optimisation of the reaction condition

A brown coloration which confirmed the reduction of silver ions to reduced silver in elemental form. The appearance of colour was indicated with the formation of AgNps. It was seen during the optimization process, decrease in the concentration of CTAB effectively, increased the formation of the sol which may be due to the formation of precipitate of silver bromide at higher concentration resulting by common ion effect. The CTAB has different roles to play in the formation of AgNps; it is not only acts as a stabiliser in avoiding the coagulation, but also as a capping agent to the AgNps due to its surfactant capability. The extract, being a strong antioxidant by itself acts as

Fig. 3 DPPH scavenging activity of AgNps/BHT/*A. paniculata*

Fig. 4 NO scavenging assay of AgNps/BHT/*A. paniculata*

a reducing agent in the formation of reduced elemental form of silver. Further, the volume of *A. paniculata*, CTAB and silver nitrate were ratio metrically increased in the bulk preparation of the nanoparticle sample. Care should be taken for the homogeneity of the sample. The photographic differentiation between the blank and the AgNps are shown in the Fig. 2. The purified suspension was dried in an oven for a requisite period to obtain the powder and further analysed by FTIR, XRD, SEM and UV-visible spectrophotometer.

5.2 Antioxidant activities of *A. Paniculata* and AgNps

A. Paniculata and synthesised AgNps were subjected to antioxidant assays. The inhibition assays such as DPPH and NOS assays were carried out for both the samples, whereas antioxidant capacity assays based on the reduction metal ion such as Folin-Ciocaltaeu, phosphomolybdenum, ferricyanide-ferric chloride, CERAC and FRAP were performed for *A. Paniculata*. The results are presented in the Table 1, Figs. 3 and 4. The results indicate that the *A. Paniculata* and AgNps electron reduction capacity assays follows

the order highest for BHT and least for the extract. The HAT based capacity assays carried out for AgNps suggest that the electronic system around the silver metal are free enough to react with DPPH and NO scavenging systems.

5.3 Mechanistic steps in the formation of AgNps

As the positively charged nitrogen on CTAB cannot supply electron for the reduction, silver nitrate forms a Ag^+ -CTAB complex. It is quite logical to say that Ag^+ reacts with Br^- in CTAB to result in $[\text{Ag-CTAB}] \text{Br}^-$. Addition of sufficient amount of *A. Paniculata* to the mixture reduces the silver to atomic state. The FT-IR data confirms that the silver forming a complex with CTAB. Liu et al. have reported that the silver coordinated complex have a stronger Ag-Br complex and the weak Ag-N coordination bonds through XPS characterisation [37]. The complexing process of CTAB in the AgNO_3 -*A. Paniculata* system is probably basis of the below reactions. Hence stepwise formulation of the reaction takes place in the following order

- The Br^- in CTAB provide electron forming Ag-Br coordination and then the CTA- Br^- - Ag^+ complex.

Scheme 1 Formation of AgNps from *A. Paniculata* extract and CTAB

- Subsequently, *A. Paniculata* reduces silver to zero oxidation state thus resulting in the CTA-Br⁻Ag⁰ formulation
- The Ag atoms grows, a part CTAB is released and other adsorb on the atomic silver

In this way, more CTAB molecules surround the silver particles, thus avoiding the coagulation and sufficiently acting as a capping agent. The mechanistic process is presented in the Scheme 1.

5.4 Visual and UV spectral outcome

In addition to extracts of *A. paniculata* to the mixture of silver nitrate and CTAB effectively led to change in colour from mud red to light red and finally to dark red, the colour of AgNPs within the reaction duration was due to the Surface Plasmon Resonance (SPR). SPR of light can be thought as the change in the direction of incident light when it encounters an obstacle or non-homogeneity of a scattering nanoparticle. As the electromagnetic radiation interacts with quantized electronic energy levels on surface, an oscillating electric field is developed which results in periodic separation of charge within the molecule called the induced dipole moment. Majority of scattering light has the same intensity as that of the incident light called elastic scattering.

Different parameters that have been identified for formation of silver sol include concentration of CTAB, *A. paniculata* extract, silver nitrate and time. The concentrations tested were in the range from 0.1 to 0.02 mM (CTAB), 0.5 to 3.0 mL (plant extract) and 0.4 to 2.8 mM (AgNO₃). It was found that the formation gets optimised within 10 min. Based on optimisation, it was found that 0.02 mM of CTAB, 1.0 mL of extract and 1.6 mM silver nitrate were sufficient for getting maximum colouration. SPR band was observed at 450 nm which is similar to that reported earlier in literature [38, 39]. The so prepared AgNPs was found to be highly stable without undergoing coagulation or deterioration, periodically confirmed by UV-Vis spectral data.

5.5 Mie scattering theory

The theories of Rayleigh and Mie are identical with reference to the specific turbidity in the range $0 < \pi d/\lambda < 0.3$. The theories indicate limit for angle independent Rayleigh and Mie scattering lies in the range of $d < d/20$, $\lambda > d > \lambda/20$ and $d > \lambda$ [40]. Since the SPR originates from the extinction spectrum which depends on the size, shape and aggregation of nanoparticle, UV-Vis spectroscopy is very useful for the estimation of nanoparticle size [41]. The computational calculation for retro theory is to find out the size of the nanoparticle based on the practical value which

means analyst seems to do infinite number of graphical data in finalising the size.

In the theoretical calculation, which can be taken as computational, Mie according to following method; a plane harmonic wave hits a smooth spherical metallic particle surrounded by a medium with real refractive index, M_o . The metallic particle has a complex refractive index, $M = N_1 + iK_1$ (where N_1 is refractive index of the core and K_1 is imaginary part of the refractive index) since the incident light is not only scattered but also absorbed. Mie used to measure values of the reflection and transmission coefficients of metals to calculate the refractive index. Both real and imaginary parts strongly depend on the wavelength of the light. The smallest size investigated by Mie was 20 nm, and he mainly concentrated on sizes between 40 and 180 nm [42].

Mie presented a solution to Maxwell's equation describing the extinction spectra of spherical particles in which extinction efficiency can be obtained by sum of scattering and absorption efficiency [43]. In these conditions, the extinction, scattering and absorption cross section of a spherical particle with a radius ' r_o ' complex electric permittivity $\epsilon = \eta^2$ embedded in a dielectric medium permittivity $\epsilon_m = \eta_m^2$. This particle is illuminated by a plane wave of angular frequency $\omega = 2\pi C/\lambda_o = kC/\eta_m$. Defining set of useful dimensionless parameters such as

$$m = \frac{n}{n_m}$$

$$v = kr_o$$

$$\omega = mv$$

In this condition, the extinction, scattering and absorption cross section are given by the following formulae [44–46].

$$\sigma_{ext} = \frac{2\pi}{k^2} \sum_{j=1}^{\infty} (2j+1) \text{Re}(a_j + b_j)$$

$$\sigma_{sca} = \frac{2\pi}{k^2} \sum_{j=1}^{\infty} (2j+1) \text{Re}(|a_j|^2 + |b_j|^2)$$

$$\sigma_{abs} = \sigma_{ext} - \sigma_{sca}$$

where $k = 2\pi \sqrt{\epsilon_m}/\lambda$ is the wave vector, $a_j(R, \lambda)$ and $b_j(R, \lambda)$ are scattering coefficients in terms of Ricatti-Bessel functions and $(\eta_j(x)$ and $\psi_j(x))$ are defined by the following equations

$$a_j = \frac{m\psi_j(\omega)\omega'_j(v) - \psi_j(v)\psi'_j(\omega)}{m\psi_j(\omega)\xi'_j(v) - \xi_j(v)\psi'_j(\omega)}$$

and

Fig. 5 Mie scattering plot of AgNps involving theoretical back calculation. The absorbance spectra from 1 to 12 (bottom to top) refer to dimension from 5 to 60 nm with a periodical span of 5 nm. The

13th spectra mentions the absorption of AgNps scanned in the wavelength range of 350–700 nm

Fig. 6 Tauc plot in the determination of quantum confinement

$$b_j = \frac{\psi_j(\omega)\psi_j'(\nu) - m\psi_j(\nu)\psi_j'(\omega)}{\psi_j(\omega)\xi_j'(\nu) - \psi_j(\nu)\xi_j'(\omega)}$$

The size of the nanoparticle based on Mie scattering, involving reverse calculation by Mie plot is shown in the graphical plot and the size of AgNps is found to be 45 nm (Fig. 5).

5.6 Tauc plot

Quantum confinement refers to the change in the electronic and optical properties when the sample is

sufficiently nano sized. A phenomenal increase in the band gap with decrease in the size of nanoparticle can be seen. This results in squeezing of electrons and holes into a critical quantum dimension which in turn is called Bohr radius. Several models were used to describe the quantum confinement of nanoparticles, the most widely used is Tauc model which allows the determination of band gap energy E_g from $E(\epsilon)_{1/2}$ as a function of incident energy E . The Tauc optical gap associated with the nanoparticles is determined through the extrapolation of the linear portion of plot of $(\alpha h\nu)_{1/2}$ over a limited range of photon energies $h\nu$. The Tauc optical gap is defined as occurring at the intercept of this linear extrapolation with the abscissa axis which is found to be 2.25 eV (Fig. 6)

5.7 FT-IR studies

The *A. paniculata* extract and CTAB have different roles to play. The extract acts as a reducing and capping agent which was confirmed by FT-IR studies done for the presence of functional groups on the AgNps (Fig. 7). A broad band at 3200–3600 cm^{-1} showing overlapping of the stretching vibration of –OH due to water, shows a strong interaction between water and silver atoms. The bands at 2920 and 2851 cm^{-1} are attributed to methyl and methylene groups of CTAB. The bands at 1996 and 1871 cm^{-1} contribute to the polymeric nature of CTAB. The observed peaks at 907, 1019.4 and 1200 cm^{-1} denotes C–O–C linkages or C–O bonds which are mainly attributed to the

Fig. 7 FT-IR spectra of AgNps capped with CTAB

Table 2 Peak indexing from d-spacing and evaluation of miller indices

2 θ	θ	Sin θ	Sin ² θ	Sin ² θ_h /sin ² θ_1 (A)	A $\times$ 3	Round off	d	A (Å ⁰)	hkl
17.96	8.98	0.15609	0.02436	1.09995	3.29986	3	4.93883	8.97164	111
19.64	9.82	0.17055	0.02909	1.31325	3.93975	4	4.51999	8.97164	200
21.12	10.56	0.18327	0.03359	1.5163	4.54891	5	4.20647	8.97164	210
23.74	11.87	0.20569	0.04231	1.91012	5.73036	6	3.74784	8.97164	211

Fig. 8 XRD pattern of AgNps

flavonoids and terpenoids present in the extract of the plant. Vibrational bands at 510 and 452.9 cm^{-1} , in far IR region may be due to the presence of heavy silver atom. Hence some of the bioorganic active compounds from the *A. paniculata* act as capping agents and contribute in increasing the stability of AgNps.

5.8 XRD studies

The X-ray diffraction pattern of wet chemical method for synthesised AgNps is shown in the Fig. 5. The hkl indexing pattern for the four 2θ peaks at values of 17.96, 19.64, 21.12, and 23.74 was assigned (111), (200), (210) and (211) planes of silver, respectively. The details are provided in Table 2. The average dimension of the synthesised AgNps capped with a surfactant was found to be 8.97 Å⁰. No

Fig. 9 Reitveld analysis of AgNps using Fullprof software **Table 3** Inhibitory antibacterial and antifungal activity

Compounds	Zone inhibition in mm					
	Gram positive bacteria		Gram negative bacteria		Fungi	
	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhimurium</i>	<i>Candida albicans</i>	<i>Botrytis cinerea</i>
Silver nitrate- <i>A. paniculata</i> -CTAB	17	19	22	27	8	17
Silver nitrate- <i>A. paniculata</i>	11	8	13	12	16	14
Streptomycin	19	23	21	24	–	–
Ketoconazole	–	–	–	–	25	15

Fig. 10 Typical SEM image of AgNps prepared by using 16 mM AgNO₃, 2 mM CTAB and 10 mL of *A. Paniculata* extract

spurious diffraction patterns were predominant compared to (111) indicating lack of crystallographic impurities. The intensity of the peak reflects high degree of crystallinity of the AgNps. The intensity of XRD plot was correlated by Fullprof software and it was found that theoretical calculation was in good correlation with that of the experimental XRD data (Fig. 8 and 9).

5.9 Disc diffusion assay

Petri plates were prepared with 20 mL of sterile Mueller–Hinton Agar (MHA) (Himedia, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at 1000 µg/disc. The loaded discs were placed on the surface medium and left for 30 min at room temperature for compound diffusion. Gram positive strains (*Staphylococcus*

Fig. 11 Frequency distribution curve of the AgNps

aureus and Methicillin-resistant *Staphylococcus aureus*), gram-negative organisms (*Klebsiella pneumonia*, *Salmonella typhimurium*) and fungi (*Candida albican* and *Botrytis cinerea*) strains were used. Negative control was prepared. Streptomycin and Ketoconazole (10 µg/disc) were used as positive control. The plates were incubated for 24 h at 37 °C for bacteria and 48 h at 27 °C for fungi. A zone of inhibition was recorded in millimeters and the experiment was repeated thrice. Microbial suspension added during antibacterial activity is 10^6 cells/mL. Table 3 depicts the inhibition study results with reference to the control.

5.10 Size and shape by SEM images

Typical SEM images for the solution containing 16 mM AgNO_3 , 2 mM CTAB and 10 mL of *A. Paniculata* extract are shown in the Fig. 10. The images show the presence of AgNp of various size and shape. The dimension range of

nanoparticle is found to be 18–43 nm. In addition to spherical dimension, some irregular shapes are also noticed, which may result in multiplasmon excitation and hence unexpected broadening of absorption peak. Shape directing properties of cationic surfactant is responsible for the non-specific growth of AgNps [47]. The frequency distribution curve of the dimension range is provided (Fig. 11).

6 Conclusion

Nanoparticles can be produced by several physical and chemical methods by using hazardous chemicals and many sophisticated instrumentation. In the present study, *A. paniculata*, a green bio natural species was effectively used in the quick production of nanoparticles, consuming less time, with less cost and without the involvement of hazardous chemicals. The characterisation was done by UV-Vis spectrophotometer with intervention of computational procedure such as Mie plot. In comparison with reported method, the proposed method has produced nanoparticle of similar dimensions as justified by SEM images and dimension calculation (Table 4). The reduction property of *A. paniculata* as an antioxidant was assessed by different method like DPPH, NO scavenging, and metal reduction. Tauc plot was used for studying quantum confinement of AgNps. XRD reveals high intensity counts at lesser 2θ values compared to other reported methods exposing to the fact that atomic reflecting nature of AgNps. The positive charge of CTAB capped AgNps reveals the wrapping CTAB over silver core by hydrophobic tail compartment of the surfactant with its hydrophilic counterpart pointing outwards. This may result the decrease in the size of the nanoparticle due to the creation of charges and hence avoid coagulation which overrules the formation of monolayer of CTAB, enveloping the silver cluster. The relatively different size of the nanoparticle may thus better insight to the capping mechanism. Furthermore,

Table 4 Comparison of the proposed method with reported biosynthetic procedures

Sl No	Extract	Size (nm)	λ_{max} (nm)	Morphology	References
1	Cocoa bean extract	8.96–54.22	438.5	Spherical	[13]
2	miracle fruit plant	4–26	438.5	spherical and crystalline	[14]
3	<i>Petiveria alliacea</i> L.	16.70–33.74	410	Mono dispersed	[15]
4	Cocoa pod	4–32	428.5	Spherical	[17]
5	<i>Buchholzia coriacea</i>	15–50	413	Spherical	[18]
6	Cola nitida	12–80	431.5	Spherical	[19]
7	Persea americana fruit	18–80	455.5	Spherical	[21]
8	<i>Opuntia ficus-indica</i>	27–38	462	Spherical	[22]
9	<i>Chasmanthera dependens</i>	24.53–92.38	418	Cubically shaped	[23]
10	<i>A.Paniculata</i>	18–43	450	Multi dimensional	Proposed method

the green synthesised AgNps has an effective inhibition growth of both gram positive and gram negative bacteria with reference to antibacterial Streptomycin and antifungal Ketoconazole. Hence, in the near future the synthetic process based on *A. paniculata* can be explored in best efficient and economical way.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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