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Research Article

Biosynthesis of Phytochemicals Coated Silver Nanoparticles Using Aqueous Extract of Leaves of *Cassia alata* – Characterization, Antibacterial and Antioxidant Activities

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ABSTRACT

Green synthesis of nanoparticles (NPs) using plant extracts is fascinating high research interest and gaining importance in biomedical applications. Silver nanoparticles (SNPs) were crystallized from Ag^+ to Ag^0 using aqueous extract of *Cassia alata* leaves as a reducing agent. Antibacterial, free radical scavenging and antioxidant activities of phytochemicals coated SNPs were evaluated. The formation and the stability of SNPs was confirmed using UV-Vis spectroscopy. The SNPs were exhibited surface Plasmon absorption maxima at 436 nm. The functional groups of phytochemicals participated in the biosynthesis were identified by Fourier transform-infrared spectroscopy. Topography and morphology of synthesized SNPs were examined using field emission scanning electron microscope (FESEM) and high-resolution transmission electron microscopy (HRTEM) respectively. The presence of elemental silver was confirmed energy-dispersive spectrum of the nanoparticles. Images of HRTEM and FESEM confirmed that the synthesized SNPs were monodispersed and spherical in shape. Crystalline nature of the SNPs was evidenced by the selected area electron diffraction patterns with bright circular spots. X-ray diffraction patterns unveiled that the particles were crystalline in nature with face centered cubic structure. The size distribution from 20 to 85 nm and average size 56.5 nm of SNPs were obtained by dynamic light scattering analysis. The inhibition of growth of bacteria tested was highly dependent on the concentration of the extract and the SNPs. The SNPs were exhibited better percentage inhibition at 1000 µg/ml on $DPPH^{\bullet}$ (65.72 ± 0.74), CUPRAC (1.925 ± 0.06) and $DMPD^{\bullet}$ (57.83 ± 1.24) assays. They showed higher activity than the extract at 300 µg/ml on FRAP (1.641 ± 0.07) assay. Among the extract, standard and SNPs tested for antioxidant activity, SNPs showed the highest activity at 300 µg/ml on TAC (0.224 ± 0.01) assay.

Keywords: *Cassia alata*; green synthesis; characterization; Silver nanoparticle; antibacterial; free radical scavenging; antioxidant.

INTRODUCTION

Nanoscience is an inspiring and influential discipline of science which have accessible numerous novel and cost-effective yields and applications. Advancements in nano-structured materials have facilitated several applications of nanoparticles. Nanomaterials are deliberately engineered to direct the enhancement of special properties at the nanoscale and have superior bioavailability than larger particles, resulting in greater utilization in single cells, tissues and organs. When NPs administered into the body, they just penetrate and cause damage to biological membranes, cells, and even cells' nuclei[1]. NPs have applications in diverse fields, including energy conversion and storage, chemical manufacturing, biological applications, and environmental technology. The major types of nanoparticles produced in industrial can be classified as inorganic metals (silver, gold, etc) metal oxides (TiO_2, ZnO_2, CeO_2, iron oxides, etc), quantum dots (CdSe) and carbon based nanomaterials (e.g., Carbon nanotubes, fullerenes, graphene). Several physical properties of metal NPs can be tailored for a specific application by controlling their composition, size, shape, and structure[2]. The main challenge in the development of catalytic NPs is to prepare nanomaterials that are highly active, selective, stable, robust, and inexpensive[3].

There has been a growing need to replace the chemical synthetic procedures with clean, nontoxic, and environmentally acceptable green chemistry methods. Many researchers have turned toward biological systems such as microorganisms and plants to draw inspiration for green technologies. Metal based nanoparticles are synthesized for numerous applications from the extracts of different plant parts such as leaves, roots, flower, seeds, etc. Water soluble plant metabolites and co-enzymes present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process[4]. Synthesis of reproducible and highly stable metal, metal oxides and metal composite of nanoparticles by chemical reduction using biomaterials as a reducing agent is quite rapid, feasible at room temperature and pressure, cleaner, nontoxic, easily scaled up and environmentally benign. Hence, this green chemistry procedure has attracted the

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Table 1: Antibacterial and antioxidant activities of extracts of *cassia* species and SNPs synthesized using their extracts reported in the literature.

Cassia species	Extract of Parts of the plant & activity		synthesis of SNPs	SNPs synthesized using extract of the plant & activity	
	Antibacterial	Anti-Oxidant		Antibacterial	Anti-Oxidant
Cassia abbreviata	stem bark [12]	bark [13]	×	×	×
Cassia alata	leaf [14], [15],[16]	Leaf [14], [17]	✓	leaf [11]	×
Cassia angustifolia	root[18] leaf [19] [20]	leaf & flower [21]	✓	leaf [22]	×
Cassia auriculata	leaf [23] [24] leaf & flower [25]	leaf [23] flower [26] [27]	✓	leaf [28] [29]	×
Cassia biflora	leaf [30]	×	×	×	×
Cassia didymobotrya	leaf [31]	×	×	×	×
Cassia fistula	flower [32] leaf & root [33] leaf [34]	stem bark, leaf, flower & fruit [35], fruit [36]	✓	leaf [37] fruit [38]	leaf [37] [39]
Cassia grandis L.*f.*	leaf & root [33]	leaf [40]	×	×	×
Cassia hirsute	leaf [41] [42]	leaf [43]	×	×	×
Cassia italic	leaf [44]	root [45]	×	×	×
Cassia javanica L.	leaf [46] bark, leaf and flower [47]	bark and leaves [48]	✓	leaf [5]	×
Cassia mimosoides	plant [49]	plant [49]	×	×	×
Cassia occidentalis L	leaf [50]	leaf [50]	✓	plant [51]	×
Cassia pumila	Pod [52]	×	×	×	×
Cassia Sophera	seed [53]	leaf [54]	×	×	×
Cassia roxburghii DC	leaf [55]	leaf [55]	✓	leaf [56][57]	leaf [57]
Cassia tora	leaf [58] [59]	leaf [60] [61]	✓	leaf [62][63]	leaf [63]

✓ - Reported in the literature, × - not reported in the literature.

Figure 1: a) *Cassia alata* plant

b) *Cassia alata* leaf

attention of biologists and nanotechnologists and has recently emerged as one of the active areas of current nanobiotechnological research[5]. Extracts of a diverse range of plant species have been successfully used to synthesize nanoparticles. Among the metal nanoparticles, SNPs are considered to be of great importance because of their properties such as antiviral, antibacterial, antifungal, electrical conductivity, chemical stability and catalytic activity which have led to a variety of new products and scientific applications.

Cassia alata (Synonym; *Senna alata*) belonging to the family Leguminosae and subfamily of Fabaceae and is widely used as traditional medicine in India and Southeast Asia. This plant possesses insecticidal, anti-inflammatory, hydragogue, sudorific, diuretic, pesticidal properties[6]. Roots, leaves and flowers of this plant possess many biological properties such as antibacterial, antifungal, antitumor, expectorant, urinary tract problems[7], asthma, bronchitis and constipation[8]. Leaves of *C. alata* are used against yellow fever or malaria, and as antiasthmatics, or antidiabetics[9]. Fresh leaf juice is used to treat ringworm, snakebite, scorpion bite, skin diseases, impetigo, syphilis sores, itching, mycosis (washerman's itch), herpes and eczema. The main constituents of *C.alata* are flavonoids,

Figure 2: (a) $AgNO_3$ solution (b) Aqueous extract of *Cassia alata* (c) SNPs solution (after addition of extract to $AgNO_3$ solution)

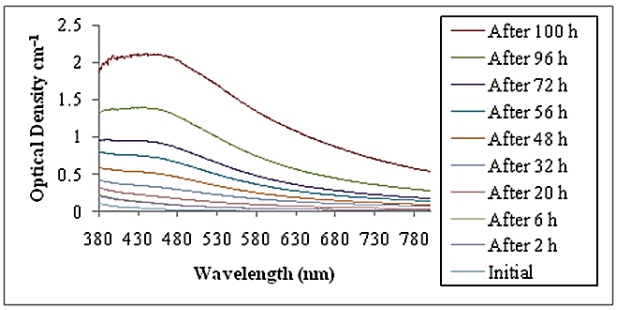


Figure 3: UV-Vis absorption spectra of SNPs (synthesized by reducing 50 mM aqueous $AgNO_3$ with the *C. alata* leaf aqueous extract at 30° C) over reaction time.

alkaloids, anthraquinone derivatives, tannins, sterols and triterpenes[10]. Reports from literature evidence that synthesis of silver nanoparticles (SNPs) using cassia species such as *cassia angustifolia, Cassia auriculata, cassia fistula, cassia italica, and cassia toraI* (Table 1). There is no much study on the synthesis of SNPs using the extract of *C. alata* leaves as reducing agent except[11] and their free radical scavenging and antioxidant activities.

The present work is aimed to i) synthesize SNPs in a spontaneous reduction of aqueous 50 mM $AgNO_3$ at room temperature using aqueous leaf extract of *C. alata* as a reducing as well as stabilizing agent ii) evaluate *in vitro* effect of SNPs on bacteria, capacity to inhibit oxidation of other molecules and power of scavenging free radicals of the extract and the SNPs and iii) characterize the synthesized SNPs using UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX), High-Resolution Transmission Electron Microscopy (HRTEM) with Selective Area Electron Diffraction (SAED), X-ray diffraction (XRD) and Dynamic Light Scattering (DLS).

MATERIALS AND METHODS

All the analytical grade chemicals were purchased from HiMedia Laboratories (Mumbai, India). The bacteria were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

Collection of plant material

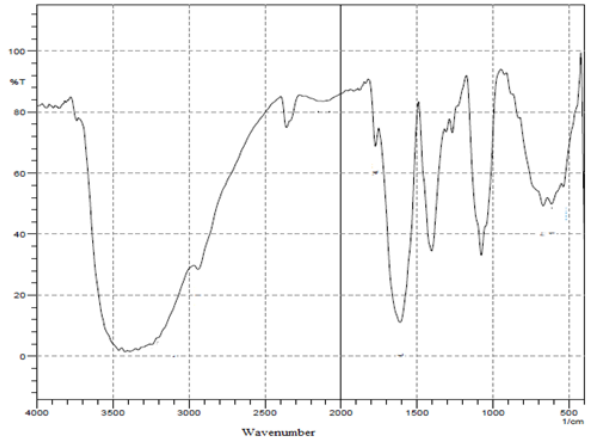


Figure 4 a: FTIR spectrum of *C. alata* leaf extract

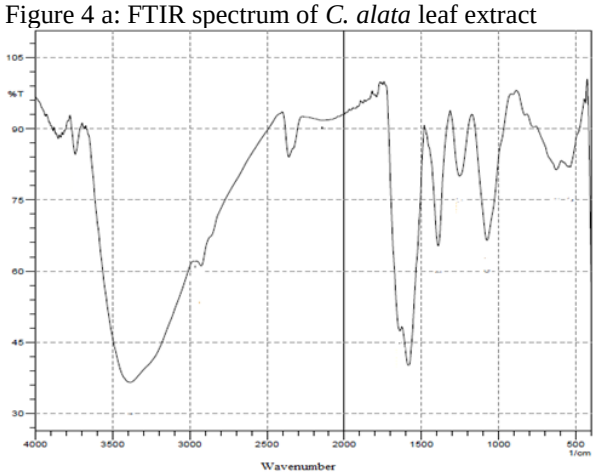


Figure 4 b: FTIR spectrum of SNPs

The healthy leaves of *C. alata* were collected in Chennai, Tamil Nadu, India. They were washed thoroughly with water and then rinsed with distilled water to remove dust particles. Then the leaves were dried in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms for 10 days at room temperature to avoid damage to the active chemical constituent. The leaves were stirred or turned frequently in order to secure adequate air circulation and the drying frames were located at a sufficient height above the ground.

Test organisms

The following test organisms were obtained as pure culture to determine the antimicrobial activity using disc diffusion method: (i) Gram positive bacterial strains (two reference cultures) - *Staphylococcus aureus* (*S. aureus*) (ATCC 25923) and *Bacillus subtilis* (*B. subtilis*) (MTCC 441) (ii) Gram negative bacterial strains (two reference cultures) - *Escherichia coli* (*E. coli*) (ATCC 25922) and *Proteus vulgaris* (*P. vulgaris*) (MTCC 1771) (iii) Gram positive bacterial strains (two clinical isolates) - Methicillin-resistant *Staphylococcus aureus*, (MRSA, clinical pathogens) and MRSA-ATCC 29213; (iv) Gram negative bacterial strains (two clinical isolates) - *E. coli* Extended spectrum beta-lactamases(ESBL), and *E. coli* Cipro R ICMR-24.

Preparation of aqueous leaf extract and synthesis of silver nanoparticles

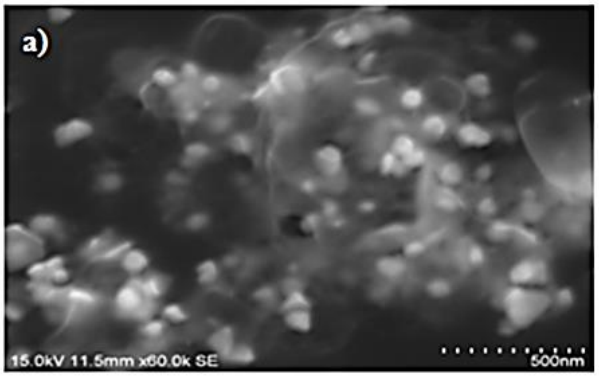


Figure 5 a: FESEM image

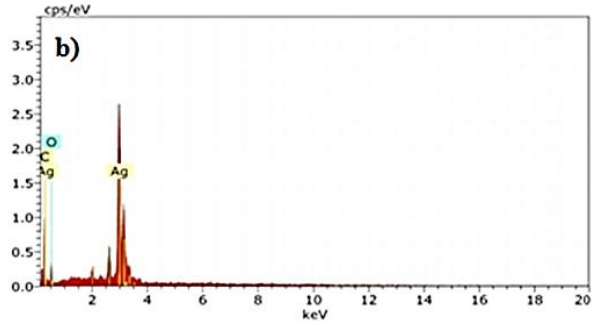


Figure 5 b: EDX spectrum of SNPs.

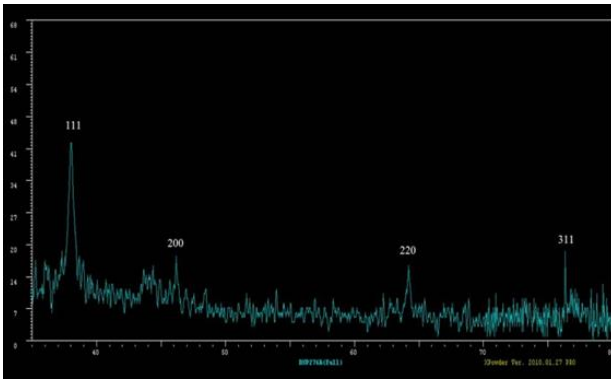


Figure 6: XRD pattern of 50mM-SNPs.

Five grams of shade dried and finely powdered *C. alata* leaves were mixed with 100 ml of deionized water.

The mixture was boiled for five minutes, cooled and filtered through Whattman No. 1 filter paper. Forty milliliters of freshly prepared extract were added to 60 ml of 50 mM aqueous $AgNO_3$ solution in 250 ml Erlenmeyer flask for the reduction of Ag^+ ions. The flask was incubated at room temperature in the dark. The change in the color of the reaction mixture solution from yellow to dark brown indicated the formation of the SNPs (Figure 2). The SNPs formed were purified by repeated dispersion in water and centrifugation at 12,000 rpm for 30 minutes. The purified SNPs were kept under room temperature for drying, weighed and stored.

Characterization of SNPs

Optical properties of NPs depend on the size, shape, concentration, agglomeration, and refractive index near the surface of NPs, which make UV/Vis/IR spectroscopy as a valuable tool to identify and characterize these NPs. The structure, composition and size distribution of the synthesized SNPs were studied using FESEM, EDX, HRTEM, SAED and DLS.

UV–Vis spectroscopy is an important technique for analyzing the formation of SNPs in aqueous solution. A coupled state between oscillation of free electrons in the conduction band of SNPs and electromagnetic wave give rise to a surface plasmon resonance (SPR) absorption band. The bio-reduction of the silver ions in the reaction mixture (aqueous extract and $AgNO_3$) was monitored periodically by measuring in the wavelength region 380 to 800 nm using UV–Vis spectroscopy (SL 218 UV-Visible Spectrophotometer, ELICO Ltd, Hyderabad, India).

FTIR analysis was performed to identify the functional groups of biomolecules which are responsible for the reduction of the Ag^+ ions and capping of the reduced SNPs present in the aqueous leaf extract of *C. alata*. FTIR spectra were recorded in the range of 400–4000 cm^{-1} by a Perkin-Elmer spectrophotometer (Perkin-Elmer Co, Germany).

The topographical information of purified SNPs was examined using a field emission scanning electron microscope (Hitachi SU6600, Japan). EDX (DMAX 8121-H, Horiba Co., Japan) coupled with FESEM was used to carry out elemental analysis of the sample to confirm the presence of silver. Tecnai-F20 high resolution transmission electron microscopy (HRTEM, FEI, Netherlands) was operated at an accelerating voltage of 200 kV and fitted with a charge-coupled device (CCD) camera was used to identify the morphology of the synthesized SNPs. For HRTEM analysis, a drop of synthesized SNPs was placed on the carbon coated copper grid, allowed to dry at room temperature and then to ensure complete dryness the sample-loaded copper grid was placed under an IR-lamp for 15 minutes. The high resolution images were captured and SAED pattern was obtained by the Tecnai-F20microscope.

XRD (powder x-ray diffractometer XRD 3003 TT, GE Inspection Technologies, Germany) was used to investigate the crystalline nature of the SNPs. It was operated at a voltage of 40 kV and a current of 30 mA with Cu-Kα radiation (λ = 0.1540598 Å). The data were obtained over the range of 30 to 80° (2θ) with a scanning rate of 0.005°/s and step size of 0.02°. DLS (DynaPro Plate Reader II - Wyatt technology) was used to study size distribution and zeta potential (related to the magnitude of the electrical charge at the particle surface) or stability of the SNPs dispersed in water.

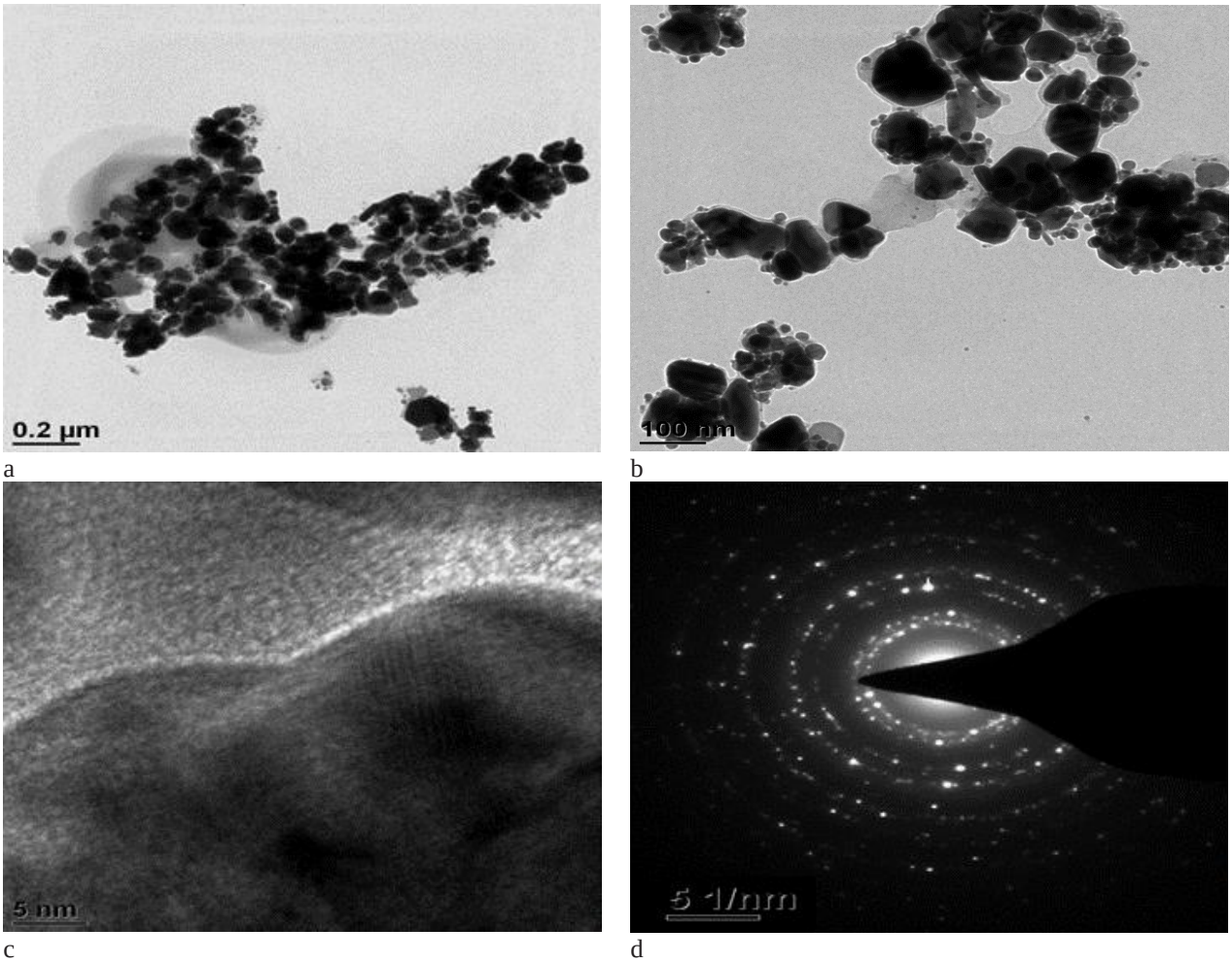
Antibacterial Activity

Preparation of bacterial inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHB) (Himedia, Mumbai, India) for 24 h at 37°C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 1×10^4 CFU/ml.

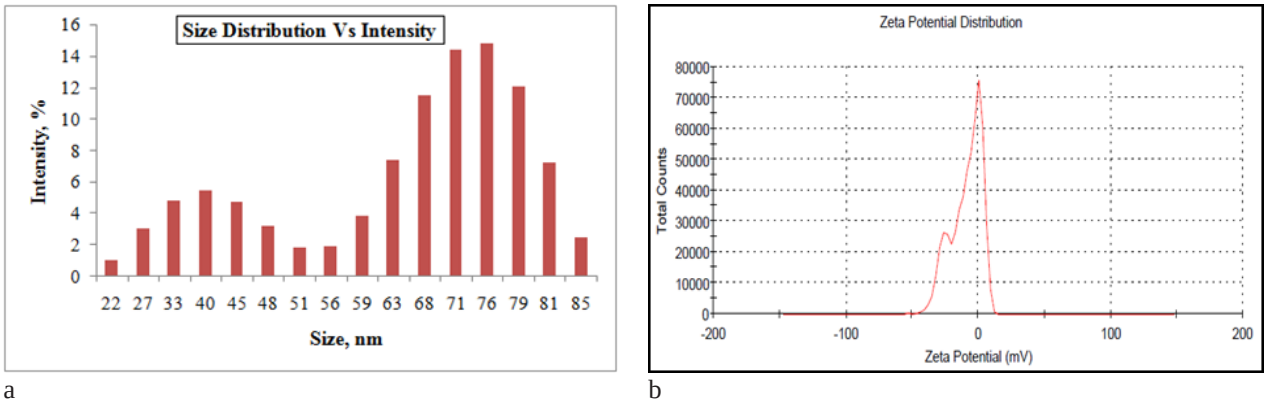
Disc diffusion method

Antibacterial activity was performed using disc-diffusion method[64]. Petri plates were prepared with 20 mL of Mueller Hinton Agar (MHA) (Hi-media, Mumbai, India). The 24-hour test cultures were swabbed on the solidified



a b c d

Figure 7: a) & b) HRTEM images c) HRTEM image of lattice fringes d) SAED pattern of synthesized SNPs.



a b

Figure 8: a) Histogram distribution of SNPs (DLS) b) Zeta potential of synthesized SNPs

media and allowed to dry for 10 min. The discs were loaded with SNPs at the concentration 250, 500 and 1000 µg/disc and with the extract at the concentration 1.25, 2.5 and 5.0 mg/disc separately. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Streptomycin (25 µg/disc) and blank discs impregnated with the solvent (water) were used as positive control and negative control respectively. The plates were incubated for 18 h at 37 °C and zone of inhibition was recorded in millimeters.

Antioxidant activity

The Antioxidant activity of aqueous extract of *C. alata* and synthesized SNPs was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, cupric ion reducing antioxidant capacity (CUPRAC) assay, N,N-Dimethyl-p-phenylenediamine (DMPD) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and total antioxidant capacity (TAC) assay.

DPPH radical scavenging assay

DPPH radical scavenging activity of aqueous extract of *C. alata* and synthesized SNPs was determined based on the method described[64]. 40 µL of various concentrations (125–

Table 2: Antimicrobial activity of aqueous extract of the leaves of *C. alata* against reference cultures (Zone of inhibition in mm).

Name of the Microbe (References)	*C. alata* leaves						Streptomycin 25 (µg/disc)
	Extract (mg/disc)			SNPs (µg/disc)			
	1.25	2.5	5.0	250	500	1000	
S. aureus (ATCC 25923)	-	-	8	-	8	10	18
B. subtilis (MTCC 441)	-	9	10	8	9	11	21
E. coli (ATCC 25922)	-	-	10	-	8	13	14
P. vulgaris (ATCC 1771)	8	-	8	-	-	8	10

-; no activity

Table 3: Antimicrobial activity of aqueous extract of the leaves of *C. alata* against clinical isolates (Zone of inhibition in mm).

Name of the Microbe (Clinical isolates)	*C. alata* leaves						Streptomycin 25 (µg/disc)
	Extract (mg/disc)			SNPs (µg/disc)			
	1.25	2.5	5.0	250	500	1000	
ESBL, *E.coli*	-	8	9	-	8	9	14
MRSA	-	-	-	-	-	8	9
ICMR-24 [*E.coli*] Cipro R	8	-	8	8	8	10	19
ATCC – 29213 [MRSA]	-	-	9	8	9	11	17

-; no activity

1000 µg/mL) of extract/SNPs was added to ethanolic solution of DPPH (0.1 M, 2,960 µL). The absorbance of the reaction mixture was measured at 517 nm after 30 min of incubation (30 ºC) in the dark. Ascorbic acid (AA) was used as the control. The free radical scavenging activity was calculated as follows:

DPPH radical scavenging activity = $[(A_C – A_S /A_C) \times 100]$ where A_C is the absorbance of the control and A_S is the absorbance of the extract/SNPs/AA.

CUPRAC assay

The cupric ion reducing capacity was measured according to the method[64]. The control Butylatedhydroxytoluene (BHT)/extract/SNPs was mixed with $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM), and ammonium acetate buffer (pH 7.0, 1 mL, 1 M), adjusted to total volume of 4 mL. The absorbance was measured against the blank at 450 nm after 30 min incubation (30 ºC). In the assay, Cu (II) was reduced to Cu (I) by the electron donating antioxidants.

FRAP assay

The FRAP assay was performed according to a procedure described[64] with some modifications. FRAP reagent (50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 10 mMTPTZ (2,4,6-tripyridyl-s-triazine) in 40 mMHCl and 5 mL 20 mM$FeCl_3 6H_2O$) was prepared. FRAP reagent (2960 µL) was mixed with 40 µL of extract/SNPs/AA and the mixtures were incubated at 37°C for 4 min. The absorbance was measured at 593 nm and the results were expressed as Fe^{2+} equivalents per gram dry mass.

TAC assay

The total antioxidant activity of the extract/SNPs/AA was determined according to the method[64]. Briefly, an aliquot 300 µg/ml of sample was combined with 3ml molybdenum reagent (50 ml of 0.6 M sulfuric acid, 50 ml of 28 mM sodium phosphate and 50 ml of 4 mM ammonium molybdate). The reaction mixture was incubated in a water bath at 95 ºC for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm. The total antioxidant activity of the sample was expressed as mg gallic acid equivalents (GAE)/g of extracts.

DMPD Radical scavenging assay

DMPD radical (DMPD˙) scavenging ability was performed by the method[64]. The coloured DPMD radical solution was prepared by adding 1:10 ratio of ferric chloride and the DMPD (Fe^{3+}: DPMD ratio 1:10). 50 µl of the extract/SNPs/AA (125-1000 µg/ml) was added to 2.95ml DMPD˙ solution and the absorbance at 505 nm was measured after 10 min. The percentage scavenging activity was calculated.

Statistical analysis

The antioxidant data were analyzed using Microsoft Excel (2007) and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

UV Visible

The relative percentage of scatter or absorption from the measured extinction spectrum depends on the size, shape, composition, and aggregation state of the sample. UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions.

Plasmons are the oscillations of free electrons that are the consequence of the formation of a dipole in the material due to electromagnetic waves. In metal, coupled state arises between a plasmon and a photon which is known as plasmon polariton. Surface plasmon resonance (SPR) is the coherent excitation of all the free electrons within the conduction band, leading to an in-phase oscillation. The resonance falls into the visible region for SNPs and hence SNPs have characteristic optical absorption spectrums. In the present study the absorbance of the reaction mixture was measured at the different intervals of time (Figure 3). The highest SPR absorption was observed at 436 nm after 100 hrs with the color change of the reaction mixture from light yellow to brown indicating the completion of the reaction. In the previous report it has been reported that the

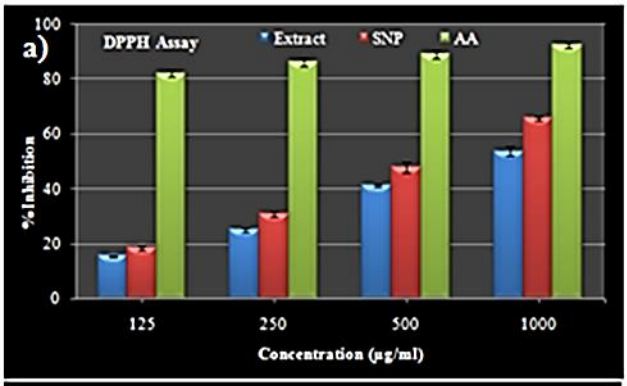


Table 4. FRAP and TAC methods

Sample	FRAP mM Fe(II) / g	TAC mg GAE/g
Extract	0.989 ± 0.23	0.048 ± 0.02
SNP	1.641 ± 0.07	0.224 ± 0.01
AA	2.270 ± 0.05	0.043 ± 0.01

Figure 9. a) free radical scavenging ability by DPPH· b) antioxidant activity by CUPRAC c) free radical scavenging ability by DMPD. Table 4) antioxidant capacity by TAC and FRAP methods. Each value represents the mean ± standard deviation of triplicate experiments. (GAE- Gallic acid equivalent, AA-Ascorbic acid, BHT-Butylatedhydroxytoluene).

particles in the SPR region of around 410–450 nm could be attributed to spherical nanoparticles[65,66]. As the particles were stabilized, the extinction peak increased in intensity due to the augmentation of stable nanoparticles, and there was no peak broadening or formation of a secondary peak at longer wavelengths due to the formation of aggregates. The observed absorption peak at 436 nm confirmed the synthesis of SNPs.

FTIR Analysis

FTIR spectra of the extract and SNPs are shown in Figure 4a and b respectively. In Figure 4a, the appeared six prominent peaks at 3385 (broad), 2941, 2358, 1774, 1610, 1404 and 1078 cm^{-1} are representative of functional groups –OH stretching, –C-H Stretching, –C≡N Stretching, carboxylic Acid –C=O Stretching, –C=C Stretching (α, β-unsaturated ketones), –OH bending (carboxylic acid) and –C-O stretching respectively of various compounds available in extract.

These characteristic functional groups of phytoconstituents present in the leaves of *C. alata* are responsible in the bioreduction process for synthesis of SNPs. Eight noticeable peaks at 3741, 3387, 2926, 2358, 1693, 1579, 1392 and 1076 cm^{-1} are in the spectrum of SNPs (Figure 4b), predict the existence of the functional groups such as –OH stretching (free), –OH stretching (phenols),–C-H Stretching, –S-H stretching, –C=O Stretching, N-H bending (amide), –OH bending (carboxylic acid) and –C-N stretching (amine) respectively. The peaks present at 3741 and 3387 cm^{-1} indicate the binding of free –OH and hydroxyl group of polyphenols on the SNPs surface respectively[67]. The peaks at different wavenumbers in the FTIR spectrum of SNPs proves that the adsorption of phytoconstituents on the surface, possibly by interaction through carbonyl groups or π-electrons and responsible for capping and efficient stabilization of SNPs binding on the surface of the synthesized SNPs.

FESEM and EDX analysis

The FESEM image confirms the presence, uniform distribution and nearly spherical shape of the SNPs (Figure 5a). The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer. The energy of the X-rays emitted from element is characteristic of the difference in energy between the two shells and the atomic structure of the element which enables the EDX to analyze the elements present in the material. EDX spectrum shows peaks corresponding to the elements making up the true composition of the sample. The elemental profile of synthesized nanoparticles shows the highest x-ray energy peak at 3 keV due to silver and confirms the presence of silver (Figure 5b).

XRD Study

The XRD technique was used to determine and confirm the crystal structure of synthesized SNPs. The XRD pattern of the SNPs is shown in Figure 6 which clearly shows that the eco-friendly synthesized SNPs are crystalline in nature and supports the results obtained by SAED analysis (Figure 7d). There are four well-defined characteristic diffraction peaks corresponding to (111), (200), (220) and (311)

planes of the face-centered cubic (fcc, Bravaise lattice) crystal structure of metallic silver. The broadening of peaks in the XRD patterns of solids is attributed to particle size. Broader peaks signify smaller crystallite/diffracting domain size and reflect the effects due to sample contribution[69]. Constant values of $\sin^2\theta$ / $h^2 + k^2 + l^2$ confirm the fcc structure of silver in all the cases of SNPs. The lattice constant (a = 4.08 Å) and the inter-planar spacing (d_{hkl}) values calculated from the XRD spectrum of the synthesized SNPs were in good agreement with[70] and the value reported in the standard powder diffraction card to Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04–0783.

HRTEM and SAED

Atomic structures and growth directions of nanomaterials can be directly investigated by HRTEM[71]. Images of SNPs with 0.2 µm, 100 and 5 nm magnifications are presented in Figures 7a, b and c respectively.

SAED pattern of nanocrystals gives ring patterns analogous to those from X-ray powder diffraction and can be used to identify texture and discriminate nanocrystalline from amorphous phases[72]. SAED pattern (Figure 7d) with bright circular spots corresponding to the diffraction planes (111), (200), (220) and (311) of fcc silver, indicates that these NPs are polycrystalline in nature[73].

DLS and Zeta potential

DLS measures the light scattered from a laser that passes through a colloidal solution and by analyzing the modulation of the scattered light intensity as a function of time, the hydrodynamic size of particles and particle agglomerates can be determined.

It was observed that the size distribution of SNPs ranges from 20 to 85 nm (Figure 8a) and the average size of SNPs were found to be 56.5 nm. The zeta potential of the biosynthesized SNPs was found as a peak at -8.98 mV (Figure 8b). The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. The negative value suggests that the surface of the SNPs is negatively charged. The negative charge confirms the repulsion between the particles thereby prevents the particles from agglomeration in the medium and increases the stability of the SNPs[74].

Antibacterial Activity

The encouraging results have recently been reported regarding the bactericidal activity of SNPs of either simple or composite nature. Their application as topical antimicrobial agents to control colonization and proliferation of microbial pathogens including multidrug-resistant organisms is envisaged to revolutionize burn wound care therapy[75]. The present study reveals the antibacterial activity of aqueous extract of *C. alata* leaves and synthesized SNPs at different concentrations. The results were obtained from disc diffusion method against eight different microorganisms (four reference and four clinical) indicated that SNPs inhibited growth of many of the microorganisms tested and it was observed that the amount of inhibition highly dependent on the concentration of SNPs (Table 2 and Table 3). Earlier studies demonstrated that aqueous extract of *C. alata* leaves was active against *S. aureus* (15 mm), *B. subtilis* (12 mm) at the concentration of 50 mg/mL[76] and *E. coli* (11 mm) at 200 mg/mL concentration[77].

The other study demonstrated the moderate antibacterial activity of crude acetone extract of *C. alata* leaves against Grampositive *B. cereus*, methicillin resistant *S. aureus*, *S. aureus* and weak activity against Gram-negative *E. coli*, *P. aeruginosa*, *S. typhimurium*[78].

It is well known that the activity of SNPs is associated with various physical and chemical properties such as shape, size, surface area, surface energy, modified surface, reactivity, composition, dispersion, time of exposition, dose, administration way and others[79]. The SNPs naturally interact with the cell membrane of bacteria and disrupt the membrane integrity. Thus, silver ions bind to sulfur, oxygen and nitrogen of essential biological molecules and inhibit bacterial growth[80]. The present findings are in agreement with the earlier studies and suggest that the effectiveness of SNPs against bacteria can be related with the concentration.

Antioxidant Activity

Two or more methods are required to reliably evaluate the antioxidant activity of compounds[81]. We used five different assays to evaluate the antioxidant activity of the SNPs. The SNPs exhibited higher ability (65.72 ± 0.74) to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine than the extract (53.73 ± 1.58). The ascorbic acid showed the highest activity among the tested samples (Figure 9a). The Antioxidant activity (DPPH) of a crude acetone extract of *C. alata* leaves at a concentration of 100 µg/mL has been reported with an IC_{50} value of 41.80 µg/mL[82].

The cupric ion (Cu^{2+}) reducing capacity of aqueous extract of *C. alata* leaves, BHT and the SNPs was found to be concentration dependent (Figure 9b) and followed the activity order: BHT > SNPs > extract. In the presence of an oxidant solution at acidic pH, $DMPD^{\cdot}$ is converted to stable and colored. The SNPs and extract were able to transfer a hydrogen atom to $DMPD^{\cdot}$ and caused discoloration which was proportional to their concentration. The scavenging activity of SNPs and extract found to be in the order AA > SNPs > extract (Figure 9c).

In the total antioxidant capacity measurement, the test samples (AA/extract/SNPs) were able to reduce Mo (VI) to green phosphate-Mo (V) compounds and was found in the following order: SNPs (0.224 ± 0.01) > extract (0.048 ± 0.02) > AA (0.043 ± 0.01) at the concentration 300 µg/mL. The synthesized SNPs exhibited strongest antioxidant activity among the test samples.

The amount of formation of Fe^{2+}-TPTZ complex decides the antioxidant effect on the FRAP method. The *C. alata* leaf extract and SNPs were able to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II). Increasing absorbance indicated an increase in reductive ability[83]. The calculated FRAP values at 300 µg/ml revealed that the SNPs showed a higher antioxidant capacity than the leaf extract (Table 4). In most of the assays, the SNPs showed better free radical scavenging and antioxidant activity than the extract.

CONCLUSION

Nanobiotechnology represents a new field of innovative approach to develop and test modern drug formulations based on biosynthesized NPs with different biological activities. Physical characteristics of SNPs are important for augmenting antimicrobial activity, but also for reducing free radicals and cancer cell toxicities. The synthesized SNPs using *C. alata* leaf aqueous extract demonstrated excellent antibacterial activity and antioxidant activity. SNPs can be useful for the development of newer and more potent antioxidants. The data presented in our study contribute to a novel and unexplored area of nanomaterials as alternative medicine. The results strongly recommend the application of SNPs as useful antioxidants for health preservation against different oxidative stress associated with degenerative diseases. In fact, antioxidant evaluation is essential for SNPs before its use *in vivo* models and also human applications.

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