Research Article

Nano Biomed Eng

Green Synthesis of Silver Nanoparticles: Their Characterization, Antimicrobial, Antioxidant Activity and Nanogel Formulation

Aparna Rajurkar[1], Dharmil Gogri[1], Neha Jamdade[1], Anupama Pathak[2]

[1] Department of Microbiology, Sinhgad College of Science, Pune, India

[2] School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, India

Corresponding author. E-mail: aparna7985@gmail.com; aparna.r.scos@sinhgad.edu

Received: Aug. 19, 2022; **Revised:** Jan. 29, 2023; **Accepted:** Mar. 15, 2023

Citation: A. Rajurkar, D. Gogri, N. Jamdade, et al. Green synthesis of silver nanoparticles: their characterization, antimicrobial, antioxidant activity and nanogel formulation. *Nano Biomedicine and Engineering*, 2023.

http://doi.org/10.26599/NBE.2023.9290006

Abstract

This study investigates an efficient and sustainable route of preparing Ag nanoparticles (NPs) using 1–5 mmol/L aqueous silver nitrate with leaf extracts of five plants—*Musa balbisiana* (banana), *Azadiracta indica* (neem), *Ricinus communis* (castor-oil plant), *Tridax procumbens* (tridax), and *Cardiospermum halicacabum* (balloon vine) for their wide availability. These synthesized nanoparticles were characterized with the help of ultraviolet (UV)–visible (Vis) spectrophotometer. The peaks were observed in 418–493 nm. For *M. balbisiana*, *A. indica*, *R. communis*, and *C. halicacabum*, the average size of nanoparticles was in the range of 90–100 nm. For *T. procumbens*, it was 39–60 nm as determined by dynamic light scattering. Energy dispersive X-ray spectroscopy analysis showed the peak in silver region confirming presence of elemental silver. Field emission scanning electron microscopy showed that the particles were of a spherical shape in *M. balbisiana* sample with an average size of 33.87 nm as well as in *T. procumbents* sample with an average size of 28.512 nm. Ag NPs showed effective antibacterial and antifungal activity against representative pathogens of bacteria and fungi. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of Ag NPs was found to be in the range of 8.9%–78.86%. H_2O_2 radical scavenging activity was recorded in the range of 6.036%–57.342%. The nanogel was prepared from synthesized Ag NPs, and its properties like viscosity and stability were evaluated. The results confirmed that this protocol is a simple, rapid, one-step, eco-friendly, nontoxic, and alternative conventional physical/chemical method.

Keywords: Ag nanoparticles (NPs); plants extract; green synthesis; antimicrobial activity

Introduction

The application of nanoscale materials and structures, usually ranging from 1 to 100 nm, is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment [1].

There are various chemical and physical methods for the synthesis of metallic nanoparticles (NPs) like reduction of solutions, photochemical reactions in reverse micelles, electrochemical reduction, heat evaporation and radiation assisted methods. Physical

and chemical methods have usually been successful in the synthesis of nanomaterials in large quantities in short periods of time, as well as for specific size and shape. However, most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals as the stabilizers which may pose potential environmental and biological risks [2]. Phytochemicals (plant-based material) in the synthesis of metallic nanoparticles and their biological applications are one of the most dynamic areas of research in modern material sciences. Utilization of phytochemicals in nanoparticles synthesis is an excellent coalition between nanotechnology and green chemistry, which is often called "phytonanotechnology" [3]. Therefore, green synthesis has been considered as one of the promising methods for synthesis of nanoparticles for being simple, one-step, cost-effective, biocompatible, low toxicity, and eco-friendly in nature [4]. Biological methods of nanoparticles synthesis, using micro-organisms, enzymes, fungi, plants, or plant extracts, have been suggested as possible eco-friendly alternatives to chemical and physical methods. Sometimes, the synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial culture [5]. One of the most promising nanoparticles that act as highly effective antimicrobial agents is silver. Various investigations have been done to study the antimicrobial activity of Ag NPs [6].

In the present study, we have used a rapid, eco-friendly and convenient green method for the synthesis of Ag NPs from silver nitrate using leaf extracts of five Indian plants—*Musa balbisiana* (banana), the fruit, peel, leaves, roots, and stems of these plants have shown antiulcerogenic, antioxidantant, and antimicrobial activity. Some species of *M. balbisiana* possess anti-diabetic, anti-hyperglycemic, and hypoglycemic activity [7]. *Azadiracta indica* (neem) plant is commonly available in India and each part of the tree is used as a household remedy against various human ailments from antiquity and for treatment against viral, bacterial, and fungal infections [8]. *Ricinus communis* (castor-oil plant) is an annual medicinal plant of India, which is the source of bioreductant and stabilizers [9]. *Cardiospermum halicacabum* shows good antioxidant activity as compared to butylated hydroxyl toluene (BHT) and ascorbic acid as a standard antioxidant [10]. For *Tridax procumbens*is, the entire plant is used by indigenous people in Guatemala for the treatment of protozoal infections (malaria, leishmaniasis, vaginitis, dysentery [11]) and gastrointestinal disorders (colic/stomach pains, gastritis/enterocolitis) due to their variable phytoconstituents and especially having high number of ketones, phenols, alkanes, amine, and lactones.

The synthesized Ag NPs were characterized using ultraviolet (UV)–visible (Vis) spectrophotometer, dynamic light scattering (DLS), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The synthesized Ag NPs were found to show effective antibacterial activity against different human pathogens.

Materials and Methods

Selection and collection of plant material

Five different Indian plants were selected for the Ag NPs synthesis. The leaves from *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum* were selected for the study.

Preparation of plant extract

The fresh leaves of *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbents*, and *C. halicacabum* were collected locally. They were washed several times with tap water to remove the dust, and then sun dried to remove the remaining moisture. About 20 g of finely cut leaves was kept in a beaker filled with 200 mL of distilled water and boiled for 30 min. The mixture was cooled down and filtered through Whattman's filter paper No. 1, and the aqueous extract was stored at 4 °C for further use [12]. The phytochemical analysis revealed the presence of carbohydrate, protein, saponins, phenols, terepenoids, and flavonoids [13].

Green synthesis of Ag NPs

Silver nitrate GR is purchased from Merck (India). 100 mL of 1, 2, 3, 4, and 5 mmol/L silver nitrate was prepared in an Erlenmeyer flask. Ag NPs were prepared in bumper tubes by varying concentration of silver nitrate (1–5 mmol/L) and keeping the plant extract concentration constant (1 mL). The mixture was incubated in a dark condition to minimize photo-activation of silver nitrate at room temperature, and Ag NPs synthesis was confirmed by the change of

solution from colourless to reddish brown with reduction of Ag^{+} to Ag^{0} [8].

Characterization of synthesized Ag NPs

UV–Vis spectral analysis of *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum* was done by using Shimadzu UV–Vis spectrophotometer (UV-1800, Japan), which has a resolution of 0.5 nm and wavelength range between 200 to 800 nm. DLS (Spectroscatter 201), which is based on the laser diffraction method with multiple scattering techniques, was employed to study the average particle size of Ag NPs synthesized from of *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum*. The phase variety and grain size of synthesized Ag NPs from *M. balbisiana*, *T. procumbent*, and *R. communis* were determined by XRD (D-8 Advance). The morphological features of synthesized Ag NPs from *M. balbisiana* plant extract and *T. procumbens* were studied by SEM (FEI Nova NanoSEM 450). After the addition of $AgNO_3$ with plant extract, the SEM thin films were prepared by making a smear of the solutions on aluminium foil 2 h later. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 10 kV by using software xT microscope control EDS: Espirit 1.

Assessment of antibacterial assay

The Ag NPs synthesized from *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum* leaf extract were tested for the antibacterial activity by standard Agar Well Diffusion method. *P. aeruginosa, K. pneumonia*, and *S. aureus* human pathogenic bacteria were used as the test specimen. A pure culture of *P. aeruginosa, K. pneumonia*, and *S. aureus* were subcultured in nutrient broth.

Sterile soft agars were inoculated with test organism and were uniformly spread on sterile nutrient agar plate by using agar overlay method. Five circular wells with 6 mm in diameter were made using sterile cork borer. The wells were loaded with 100 µL of synthesized Ag NPs (1–5 mmol/L). To check the antibacterial activity, the plates were incubated at 37 °C overnight, and the zone of inhibition was observed. Control plates were inoculated with $AgNO_3$ solution.

Assessment of assays

The antifungal assay was done using *Aspergillus niger* by Poison Food method. During the process, individual fungal strains were point-inoculated on the sterile potato dextrose agar (PDA) plates containing Ag NPs synthesized from *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum*, and incubated at 28 °C for 48 h. The diameter of Mycelia colony developing on the nanoparticles containing PDA plates (1 mL) was compared with the diameter of colony obtained on the control plates. Control plates were prepared with only plant extract and silver nitrate solutions separately.

DPPH radical scavenging activity of Ag NPs was assessed quantitatively by photometric method. The Ag NPs were tested for the DPPH free radical scavenging activity according to the method [13]. The ability of synthesized Ag NPs to scavenge hydrogen peroxide was determined [14].

Nanogel preparation using Ag NPs

Carbopol 974P was used as the gelling agent, 4 g/mL solution of Carbopol 974 P were prepared and kept overnight. The solution was further neutralized by using saturated NaOH. In the prepared gel, 10 g/mL of prepared synthesized Ag NPs of *A. indica* (5 mmol/L), *M. balbisiana* (2 mmol/L), *R. communis* (3 and 5 mmol/L), *C. halicacabum* (2 and 3mmol/L), and *T. procumbens* (4 mmol/L) were mixed and further stirred for 15 min and filled in laminated tube. The texture, appearance, stability, and viscosity were evaluated.

Results and Discussion

Phytochemical analysis

The preliminary investigation of phytochemical analysis revealed the presence has been given in the Table 1. The results of the qualitative screening of the phytochemical components such as carbohydrate, protein, saponins, phenols, terpenoids, and flavonoids in aqueous extracts of the plant species are shown in Table 1. The extracts of the leaves of *M. balbisiana* revealed the presence of carbohydrate, protein, saponins, phenols, terpenoids, and flavonoids. The extract of *A. indica* showed the presence of carbohydrate, protein, saponins, terpenoids, flavonoids, but absence of phenols. The aqueous

Table 1 Phytochemical analysis of plant extract used in Ag NPs synthesis

Plant extract	Carbohydrate	Protein	Saponins	Phenols	Terpenoids	Flavonoids
Musa balbisiana (Banana)	+	+	+	+	+	+
Azadiracta indica (Neem)	+	+	+	−	+	+
Ricinus communis (Castor)	−	+	+	+	+	+
Tridax procumbens (Tridax)	+	−	+	−	+	+
Cardiospermum halicacabum (Balloon Vine)	+	+	+	−	+	+

extract of *R. communis* showed the presence of protein, saponins, phenols, terpenoids, flavonoids, and absence of carbohydrates. *T. procumbens* extract revealed the presence of carbohydrate, saponins, terpenoids, flavonoids, and absence of proteins and phenol. Extract of *C. halicacabum* showed the presence of carbohydrate, protein, saponins, terpenoids, flavonoids, and absence of phenols

Synthesis of Ag NPs

Ag NPs exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations. In the present study, green synthesis of Ag NPs by the aqueous extract of *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, *C. halicacabum* was studied. Visual observations in Fig. 1 showed a change of color in silver nitrate solution from yellow to brown, whereas no color change was observed in the culture supernatant without silver nitrate or in media with silver nitrate alone. This color change is attributed to the excitation of surface plasmon resonance (SPR) and the color intensity increases with the increase in concentration of silver (i.e. 1–5 mmol/L as shown in Figs. 1(a)–1(e)).

UV–Vis spectrophotometric analysis

Reduction of silver ions into Ag NPs during exposure to plant extracts was observed as a result of the color change. Metal nanoparticles have free electrons, which show the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of Ag NPs were observed around 410–495 nm.

As shown in Fig. 2(a), the peak ranges are for *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum* are 421–480, 420–493, 410–534, 418–464, and 420–470, respectively. Parallel changes in color have been observed when different concentrations (1–5 mmol/L) of silver nitrate were used by keeping plant extract (1 mL) constant. The appearance of the brown color was due to the

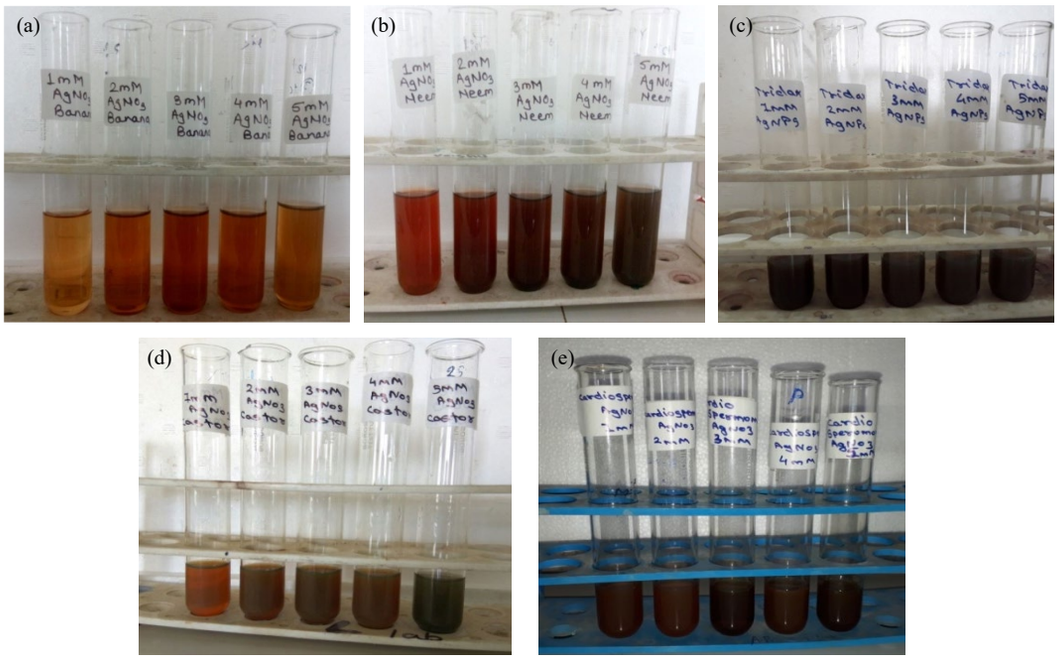

Fig. 1 Visual observation of synthesized nanoparticles. (**a**) *M. balbisiana*, (**b**) *A. indica*, (**c**) *T. procumbens*, (**d**) *R. communis*, and (**e**) *C. halicacabum*.

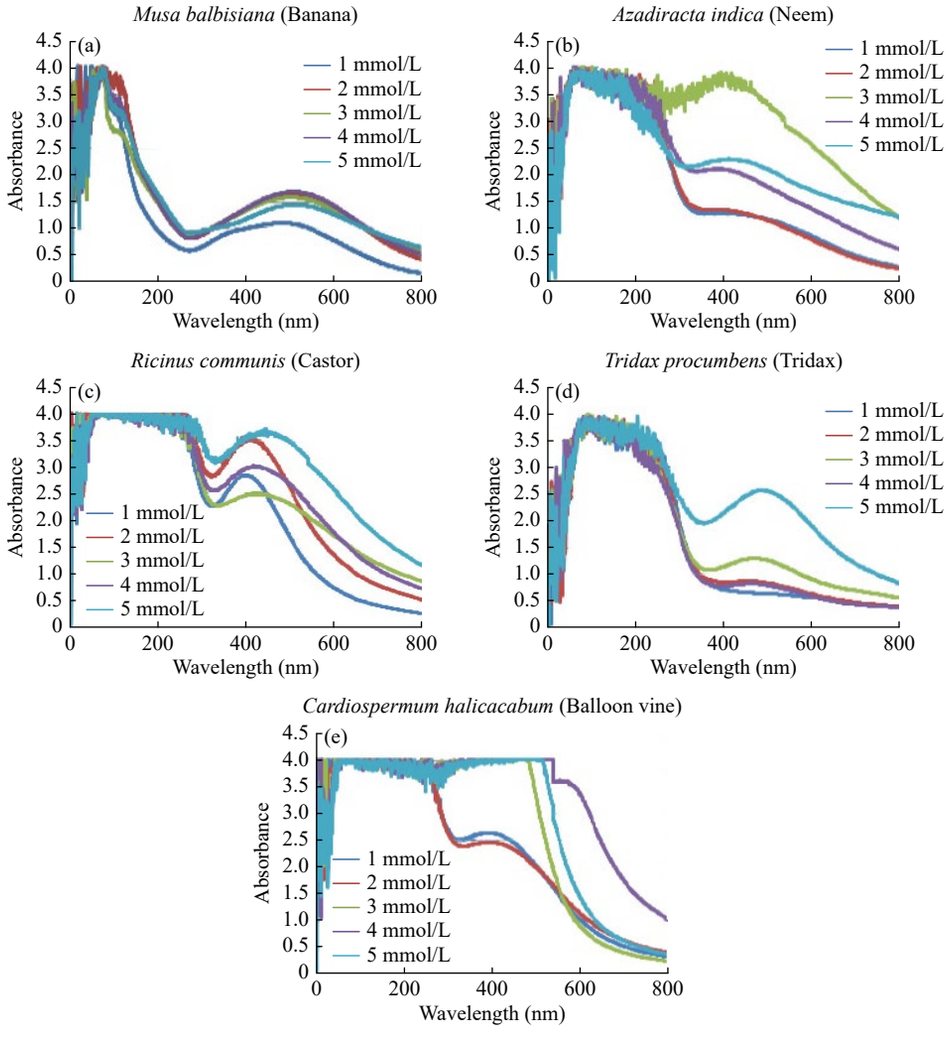

Fig. 2 UV–Vis spectra showing absorbance with different concentration of $AgNO_3$ (1–5 mmol/L).

excitation of the SPR. Similar lines of results were reported in Refs. [8, 15, 16].

DLS analysis

The particle size distribution (PSD) of synthesized Ag NPs of different concentration (3 and 4 mmol/L) was shown in Figs. 3(a)–3(e). The colloidal 3 mmol/L *M. balbisiana* Ag NPs solution with particle sizes of 90–100 nm is shown in Fig. 3(a). Figure 3(b) showed the colloidal 3 mmol/L *A. indica* Ag NPs solution contains particles of sizes ranging from 90 to 100 nm. Figure 3(c) showed the colloidal 3 mmol/L *R. communis* Ag NPs solution contains particles of sizes ranging from 90 to 100 nm. Figure 3(d) showed the colloidal 4 mmol/L *T. procumbens* Ag NPs solution contains particles of sizes ranging from 39 to 60 nm. Figure 3(e) showed the colloidal 3 mmol/L *C. halicacabum* Ag NPs solution contains particles of sizes ranging from 90 to 100 nm

Ahmed et al. reported the size of their Ag NPs synthesized from *A. indica* as 34 nm [8]. Sundararajan et al. reported the size of Ag NPs synthesized from *C. halicacabum* was 74 nm [10].

XRD Analysis

XRD analysis was carried out to confirm the crystalline nature of the particles, and the pattern is shown in Figs. 4(a)–4(c), which has 4 intense peaks in the whole spectrum of 2θ values of 20°–80°. This pattern reveals the diffraction peaks at 2θ values of 39.01°, 46.48°, 64.69°, and 77.62° [15, 17], corresponding to the lattice planes (111), (200), (220), and (311), respectively. The XRD pattern was verified using the Joint Committee on Powder Diffraction Standards (JCPDS) file No. 01-071-4612 database. Using the Scherrer equation, the average crystalline size of Ag NPs was determined from the DiffractOgram, with the crystalline size as 25 nm for Ag NPs from *M. balbisiana*, 26 nm for Ag NPs from *T. procumbens*, and 21 nm for Ag NPs from *R. communis*. The nanophase Ag NPs demonstrated high crystallinity, and there are no other extra peaks.

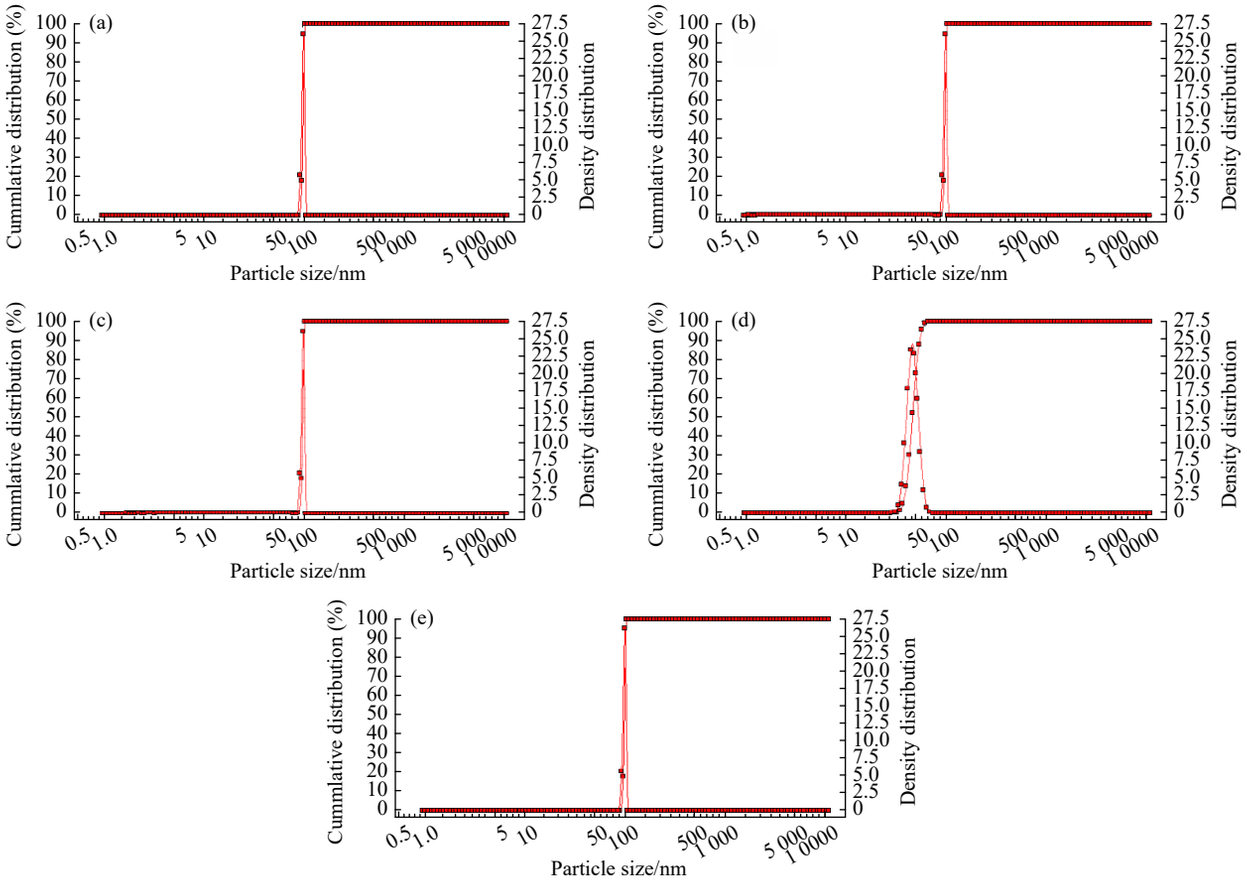

Fig. 3 Particle size analysis of synthesized nanoparticles. DLS result for 3 mmol/L (**a**) *M. balbisiana*, (**b**) *A. indica*, (**c**) *R. communis*, (**d**) *T. procumbens*, and (**e**) *C. halicacabum.*

SEM analysis

SEM analysis provided further insight into the morphology and size of the Ag NPs. The size of the synthesized Ag NPs was 1–100 nm. The result showed that the particles were of spherical shape in *M. balbisiana* sample with an average size of 33.87 nm as well as in *T. procumbens* sample with an average size of 28.512 nm. (Figs. 5(a) and 5(b)), similar to the results reported in the previous literature [15]. Although the agglomeration of Ag NPs was found at some places, there was no direct fusion, it is mainly because of the presence of capping agents.

Antibacterial activity

The results of antibacterial activities of Ag NPs evaluated from the Well Diffusion method are given in Table 2. Ag NPs synthesized by using *M. balbisiana* plant extract effectively inhibited *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

S. aureus, Klebsiella pneumonia, and *P. aeruginosa* showed susceptibility to Ag NPs synthesized from plant extract of *A. indica, T. procumbens*, *R. communis*, and *C. halicacabum.* Ibrahim et al. reported the antibacterial activity of Ag NPs synthesized from *M. balbisiana* against *S. aureus* and *P. aeruginosa*, while control and plant extract alone did not exhibit any antibacterial activity [7]. Although, it is presumed that the extract of the plant leave possess the antibacterial activities and must be reflected through greater inhibition zone, but it alone shows very low activity due to its medium of extraction as well as lower concentration during experimentation [8].

Ag NPs synthesized from *C. halicacabum* in this report showed better growth inhibition of *P. aeruginosa* as compared to results of Sundararajan et al. [10]. Our study showed superior antibacterial activity of silvar nanoparticles synthesized from *T. procumbens* against *P. aeruginosa* compared to results of antibacterial activity of Ag NPs reported by Dhanalakshmia and Rajendran [11].

Antifungal activity of Ag NPs

The results of antifungal activities of prepared Ag NPs evaluated from the poison food method are given in Table 3. Ag NPs synthesized by using plant extract

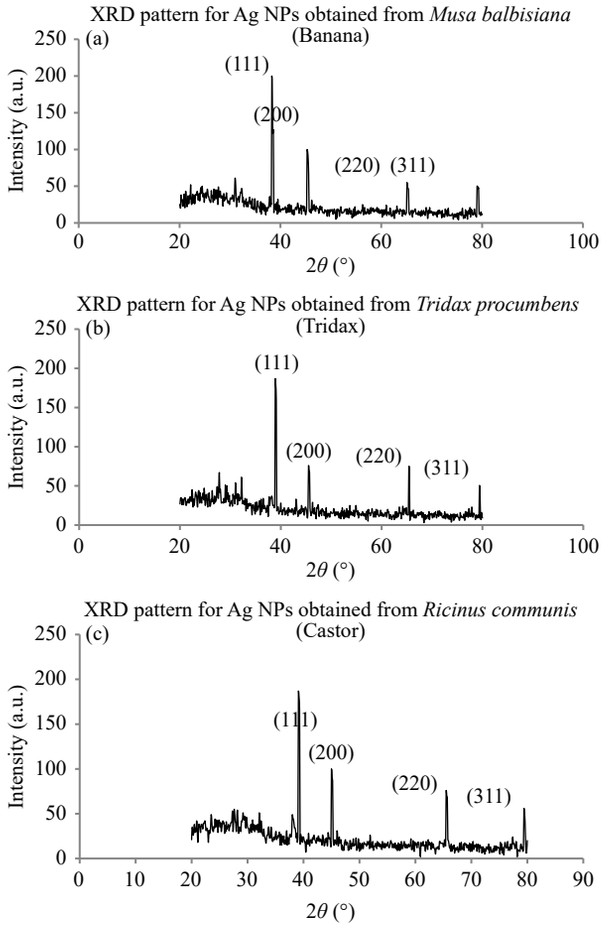

Fig. 4 XRD Results of Ag NPs synthesized from (**a**) 3 mmol/L $AgNO_3$ *and M. balbisiana*, (**b**) 4 mmol/L $AgNO_3$ *and T. procumbens*, and (**c**) 3 mmol/L $AgNO_3$ *and R. communis*.

of *M. balbisiana, A. indica*, and *T. procumbens* showed good antifungal activity against *A. niger*, *Fusarium*, *Collectotrichum*, and *Penicillium.*

Antioxidant activity

Antioxidants naturally protect individuals from a variety of degenerative illnesses. Phenolic chemicals are universal antioxidants that absorb oxygen-derived free radicals by giving a hydrogen atom or an electron to the free radicals [16]. Green synthesized Ag NPs were tested for antioxidant activity against the DPPH radical and compared to normal ascorbic acid. Ag NPs synthesized by using plant extract of *Ricinus communis* showed 78.86% DPPH scavenging activity, *Cardiospermum halicacabum* showed 71 % higher antioxidant activity compared to *Tridax procumbens* 45 %, *Musa balbisiana* 8.9 % and *Azadiract aindica* 26.29 % (Fig. 6).

Hydrogen peroxide scavenging (H_2O_2) Assay

The scavenging ability of Ag NPs synthesized from *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum*on hydrogen peroxide is shown in Fig. 7. These nanoparticles were capable of scavenging hydrogen peroxide in an amount-dependent manner. Ag NPs of *A. indica* exhibited 57.342% scavenging activity on hydrogen peroxide. On the other hand, Ag NPs of *M.*

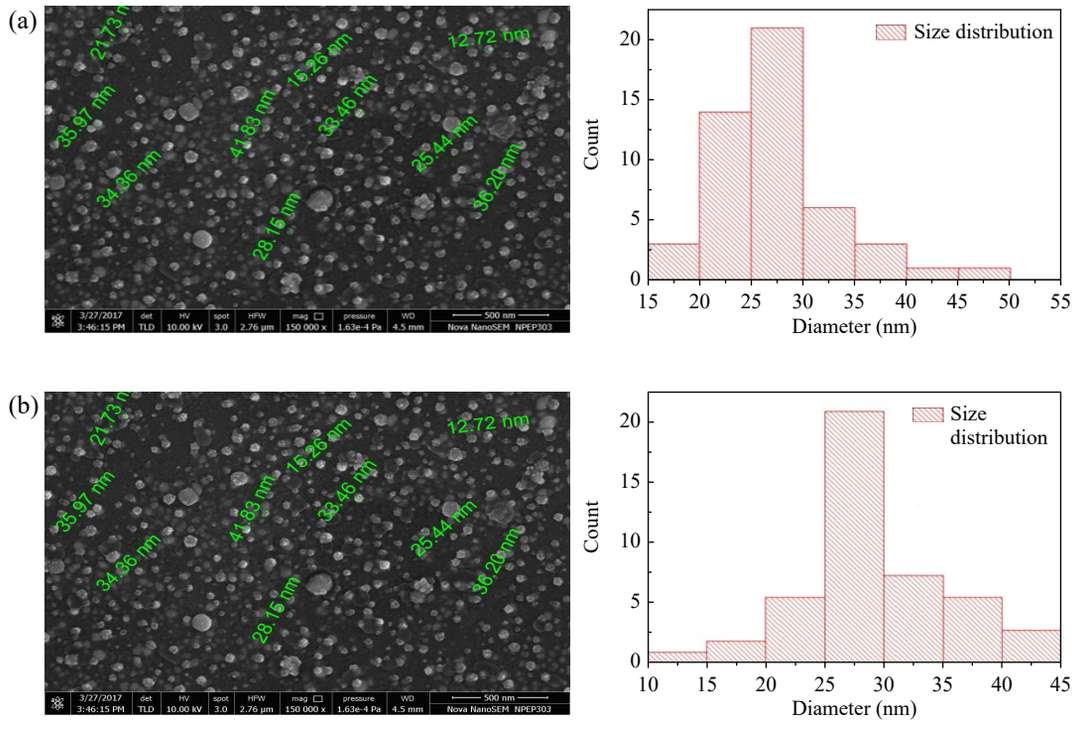

Fig. 5 FESEM micrograph and particle size distribution histograms of Ag NPs synthesized from (**a**) *M. balbisiana* and (**b**) *T. procumbens*.

Table 2 Antibacterial activities of synthesized Ag NPs

Ag NPs (mmol/L)	Zone of inhibition (mm)														
	M. balbisiana			*A. india*			*R. communis*			*T. procumbens*			*C. halicacabum*		
	SA	PA	KN	SA	PA	KN	SA	PA	KN	SA	PA	KN	SA	PA	KN
1	7	10	—	—	13	—	11	15	—	—	14	—	—	21	—
2	8	12	—	—	14	10	10	16	11	14	20	16	—	22	—
3	8	12	—	—	14	16	8	17	—	12	21	18	—	20	—
4	9	9	—	10	17	17	12	19	8	12	20	18	—	23	—
5	10	12	—	19	19	15	15	15	—	12	15	17	—	19	—

SA: *S. aureus*, PA: *P. aeroginosa*, KN: *K. pnemoniae*

Table 3 Antifungal activities of synthesized Ag NPs

Fungi	Percent inhibition by Ag NPs (3 mmol/L)					
	M. balbisiana		*R. communis*		*T. procumbens*	
	Control $AgNO_3$	Control plant extract	Control $AgNO_3$	Control plant extract	Control $AgNO_3$	Control plant extract
A. niger	70.58	77.27	50	56.25	84.21	56.25
Fusarium	73.07	50	70	64.7	68.75	64.7
Collectotrichum	53.84	—	58.33	—	61.11	—
Penicillium	57.14	75	69.23	—	73.33	—

balbisiana, *R. communis*, *T. procumbens*, and *C. halicacabum* exhibited 6.036%, 11.569%, 6.036%, and 6.036 % hydrogen peroxide scavenging activity, respectively. Sundararajan et al. reported DPPH radical scavenging activity and H_2O_2 radical scavenging activity of Ag NPs synthesized from *C. halicacabum* [10].

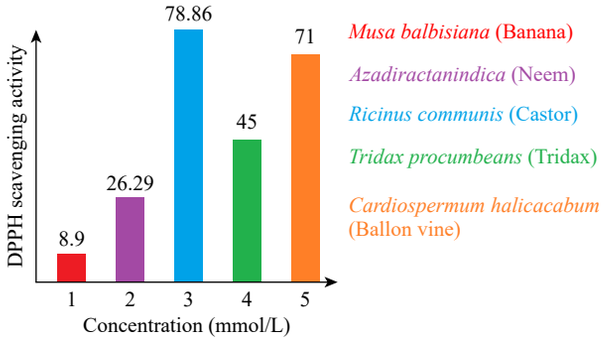

Fig. 6 DPPH scavenging activity of synthesized Ag NPs.

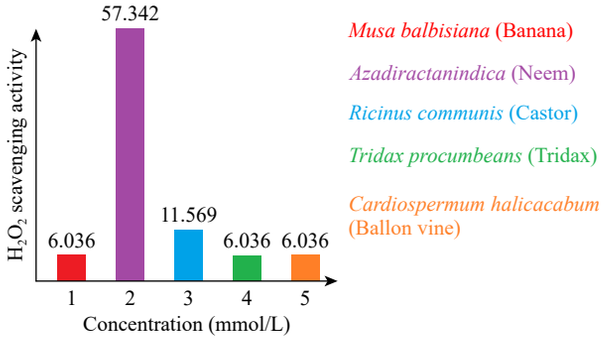

Fig. 7 H_2O_2 scavenging activity of synthesized Ag NPs.

Stability study of gel

The nanogel sample was stored at room temperature to reach their stability. After 21 days, samples were withdrawn and retested. Nanogel was observed for their stability for 21 days at a 5-day interval, and it was observed that nanogel prepared from Ag NPs of *A. indica*, *R. communis*, and *T. procumbens* did not show any change in color, while nanogel containing Ag NPs of *M. balbisiana* and *C. halicacabum* showed color change. The viscosity of the prepared nanogel was analyzed by using Viscometer DV III. The viscosity of nanogel prepared from synthesized Ag NPs of *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, *C. halicacabum* plant extract was found to be 230 350 cP (centiPoise)

Conclusion

The current study describes the eco-friendly, simple, and efficient route for synthesis of Ag NPs using the leaves from *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum*, which has strong antioxidant and antibacterial properties against DPPH free radicals and pathogens. The synthesis of nanoparticles is confirmed by UV–Vis spectrophotometry. Structural analysis of Ag NPs by XRD strongly suggests the formation of elemental Ag NPs. XRD analysis and SEM analysis revealed the average size of Ag NPs and it is found to be in

between 21–26 nm and the synthesized nanoparticles were of spherical size. The Ag NPs synthesized in the present study displayed antibacterial and antifungal activity against selected human and plant pathogens, which suggested that they may play a role in new drug development. The synthesized nanoparticles show good antioxidant activity as well as H_2O_2 scavenging activity. A nanogel was formulated by using synthesized Ag NPs and its stability and viscosity were evaluated. Metal nanoparticles by herbal approach in the present study using *M. balbisiana*, *A. indica*, *R. communis*, *T. procumbens*, and *C. halicacabum* may have persuasive application in medicine therapeutics and diagnostics.

CRediT Author Statement

Aparna Rajurkar: Conceptualization, Investigation, Methodology, Project administration, Supervision, Visualization, Writing-original draft, Writing-review & editing. **Dharmil Gogri**: Experimentation, Data collection, Preliminary analysis. **Neha Jamdade:** Experimentation, Data collection, Preliminary analysis. **Anupama Pathak:** Supervision, Guidance.

Conflict of Interest

The authors declare that they have no conflict of interest.

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