Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2440-2448 | ISSN:2251-6727

Green Synthesis of Silver Nanoparticles Using *Berberis aristata* Leaf Extract with Potent Antifungal Potential

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(Received: 19 June 2025 Revised: 22 July 2025 Accepted: 12 August 2025)

KEYWORDS
Berberis aristata, methanolic extract, Silver nanoparticles, antifungal activity, flavonoid, polyphenols.

ABSTRACT:
Background: Berberis aristata DC. belongs to family Berberidaceae and widely distributed in evergreen regions of temperate and sub- tropical. Berberine has predominant clinical uses in bacterial diarrhea, intestinal parasite infections, ocular trachoma infection, eye infection, skin diseases, jaundice, antifungal, antipyretic and as antiarrhythmic, anti-inflammatory, immunostimulant, antitumor, astringent, tonic, febrifuge, laxative and also for menorrhagia
Aim and Objective: The aim of this study is the green synthesis of silver nanoparticles from the extract of Berberis aristata leaf and assessment of their antifungal activity.
Methodology: Biosynthesis of silver nanoparticles from medicinal plant material is considered as the most suitable method, as it increases the therapeutic effects of the silver nanoparticles. These silver nanoparticles were characterized by Ultraviolet-visible spectroscopy (UV), Scanning electron microscopy (SEM). An antifungal test for candida was also conducted to assess the antifungal efficacy.
Results and Discussion: Total flavonoid and polyphenolic content of the extract was found to be 19.6% and 43.9% respectively. Extract is rich in flavonoids and polyphenols and these outcomes demonstrated potential for anti-psoriatic effects. The findings showed that the antioxidant capacity of the trisodium citrate and AgNP was similar. The zone of inhibition of the nanoparticles was computed and compared with the standard medication in the antifungal effect studies. The SEM results confirm that silver nanoparticles with a spherical and smooth surface may be produced from the methanolic concentrate of Berberis aristata.

INTRODUCTION

Berberis aristata DC. belongs to family Berberidaceae and widely distributed in evergreen regions of temperate and sub- tropical. Berberis has about 650 species worldwide, of which 54 have been reported from Indian Himalaya, especially in state of Himachal Pradesh.(1) Berberis aristata, known as Daruhaldi, is a large deciduous shrub usually in 1.8–3.6 meter height. Its leaves are obovate or elliptic, entire, base gradually narrowed with reticulate nerves and glossy dark green color. Its flowers are numerous and stalked. Its roots are thick woody, yellowish brown, cylindrical, knotty and covered with a thin brittle bark and have valuable isoquinoline alkaloid berberine. *Berberis aristata* is used in ayurveda medicines from very long time (2). An important ayurvedic preparation Rashut is prepared by this plant. Berberine has predominant clinical uses in bacterial diarrhea, intestinal parasite infections, ocular trachoma infection, eye infection, skin diseases, jaundice, antifungal, antipyretic and as antiarrhythmic, anti-inflammatory, immunostimulant, antitumor, astringent, tonic, febrifuge, laxative and also for menorrhagia.(3).

Nanoparticles are particles with dimensions typically ranging from 1 to 100 nanometers. Due to their small size, nanoparticles exhibit unique physical, chemical, and biological properties compared to bulk materials.(4) Silver nanoparticles are tiny particles of

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JCHR (2025) 15(4), 2440-2448 | ISSN:2251-6727

silver which can be used to encapsulate and deliver drugs to specific targets in the body. It offers benefits such as controlled drug release, enhanced drug stability and targeted therapy. (5)The process of creating silver nanoparticles loaded with herbal extracts offers a novel way to combine the beneficial effects of nanotechnology with natural substances. Green synthesis methods offer several advantages over conventional synthesis routes, including environmental friendliness and biocompatibility.(6)

MATERIALS AND METHOD

Extraction by using Soxhlet Extraction Assembly

The plant leaves were washed with water and then dried. Coarse powder was prepared using the dried leaves (50 g). The extraction was done by using methanol as a solvent (250 ml).(14) The coarse powder was filled in porous bag and placed in thimble of Soxhlet apparatus. The round bottom flask was heated using heating mantle.(15) Due to the heat the solvent in the round bottom flask vaporizes into the condenser and then drip back to round bottom flask. Then the extract was evaporated to dryness, dark green sticky mass was obtained.(16). Methanol is polar in nature, and could extract polar secondary metabolites. It is self-preservative at a concentration above 20% (17).

Total Flavonoid Content

The total flavonoid content of *Berberis aristata* extract was determined by the Aluminium chloride colorimetric method. Rutin was taken as a reference standard. A stock solution of the standard was prepared by dissolving 10 mg of Rutin in 10 ml of methanol (1000µg/ml). Different aliquots of 100-1000µg/ml was prepared in a 10 ml volumetric flask & 4 ml distilled water was added along with 0.3ml 5% $NaNO_2$. After 5 min add 0.3 ml of 10% $AICI_3$, & after 5-6 min add 2 ml IM NaOH (23). After the addition of NaOH, and 30 min incubation in dark, the solution turns red color. Absorbance was measured at 510 nm & calibration curve was plotted as concentration v/s absorbance using a UV-Visible spectrophotometer (24). Blank was prepared by replacing standard through methanol. *Berberis aristata* methanolic extract was prepared by the same procedure. The total flavonoid content was calculated from a calibration curve using Rutin as a reference standard, and the result was expressed as mg Rutin equivalent per g dry weight. (25)

Total Phenolic Content

Berberis aristata methanolic extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of ethyl acetate solution of extract and 2.5 ml of 10% Folin-Ciocalteu's reagent followed by the addition of 2.5 ml of 7.5% NaHCO3, Blank was concomitantly prepared, containing 0.5 ml water, 2.5 ml 10% Folin- Ciocalteu's reagent, and 2.5 ml of 7.5% of NaHCO3). (26)The samples were incubated in a thermostat at 45°C for 45 min.(27) The absorbance of the test sample was determined using a spectrophotometer at Amax 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. (28)A standard solution of Gallic acid at a concentration range of 100-1000 µg/ml was prepared & the calibration curve was constructed as Concentration v/s Absorbance. The content of phenolic compounds in extracts was expressed in terms of Gallic acid equivalent (mg of GAE/g of extract), Gallic acid equivalence (GAE) was calculated using the linear regression equation obtained from standard Gallic acid (29) .

Green synthesis of Silver Nanoparticles

Sodium citrate reduction method was used to prepare silver nanoparticles (AgNPs). A solution of 0.1699 g $AgNO_3$ in deionized water was prepared and was heated until it began to boil.(18) Sodium citrate solution (0.25 g in 20 ml of deionized H_2O) was added drop wise to the above $AgNO_3$ solution, as soon as boiling commences. Continue heating until color change (light yellow) was evident indicating the reduction of Ag+ ion. Heating of the solution was continued for an additional 10 min and then the solution was cooled to room temperature.(19) The mixture was resuspended well and centrifuged (12 000 rpm for 10 min) to get separate the dry mass. Synthesized AgNPs were further redispersed and characterized for UV-absorption spectrum using UV 1700 Shimadzu Spectrometer (Shimadzu Corp., Tokyo, Japan), particle size, zeta potential, and polydispersity index using zeta sizer (Bechman Coulter, UK), X-Ray Powder Diffractometery using Bruker AXS D8 Advance Diffractometer (Bruker, Billerica, MA), and morphology using Hitachi H-7500 transmission electron microscope (TEM, Hitachi, Tokyo, Japan), USA operating at 100 kV(20).

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Evaluation of Silver nanoparticles

Particle size and polydispersity index

The particle size and polydispersity index (PDI) of nanoparticles were measured by a dynamic light scattering technique using a Litesizer (Anton Paar Litesizer 500) at 25 °C in triplicate. From the prepared nanoparticles dispersion, 100 µL was diluted to 5 mL, with double distilled water to measure for size and PDI.

Zeta Potential

The nanoparticles dispersion samples were diluted with unionized water and were further subjected to zeta potential measurement using a Zetasizer. Measurements were done in triplicate at a scattering angle of 90°.

Entrapment Efficiency

Prepare a suspension of nanoparticles containing the extract to be encapsulated. Place the nanoparticle suspension in centrifuge tubes and centrifuge at a specified speed (3,000 rpm) for 30 min duration (42,43). After centrifugation, collect the supernatant, which contains any unencapsulated or free extract. Measure the concentration of the flavanoid in the supernatant by using UV-Vis spectroscopy).(44)

The entrapment efficiency is calculated using the formula:

$$\text{EE (\%)} \frac{Total\ Drug - Free\ Drug}{Total\ Drug} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR)

Ensure the silver nanoparticles are in solid form by drying them at a controlled temperature (below 45 °C) to avoid degradation of biomolecules.(33). Grind 1–2 mg of dried silver nanoparticles with 100–200 mg of potassium bromide (KBr) powder using an agate mortar and pestle. Compress the KBr-nanoparticle mixture into a thin, transparent pellet using a hydraulic press(34). Use an FTIR spectrometer equipped with a transmission or attenuated total reflectance (ATR) mode(35). Record the spectrum in the mid-infrared region (4000–400 cm−1−1) at a resolution of 4 cm−1−1.(36, 37, 38)

Field emission scanning electron microscopy (FESEM)

Centrifuge the colloidal solution at 14,000 rpm for 4 minutes to separate the nanoparticles from the solution. Discard the supernatant and re-dispersed the pellet in deionized water. Dry the purified silver nanoparticles at a controlled temperature (e.g., under a lamp or in an oven at low temperature) until completely dry.(39). Use a carbon-coated copper grid for mounting the nanoparticles. This helps in providing conductivity during SEM analysis. Place a small amount of the dried silver nanoparticle sample onto the carbon-coated grid.(40). Allow it to air dry completely to ensure proper adhesion. Set the desired operating voltage (typically between 5 kV and 20 kV. Carefully load the carbon-coated grid with silver nanoparticles into the SEM chamber. Capture images at various magnifications to analyze particle morphology, size, and distribution. Analyze SEM images to determine particle shape, size distribution, and aggregation. Record findings, including particle morphology and any notable characteristics observed in SEM images.(41)

Microbial assay of silver nanoparticles

Take a 1µg of *Berbaris aristata* (reference standard) using a pipette and dilute in a 10 ml of methanol. Pipette out the solution from above solution and add in 10 ml of methanol (30). Grow Candida albicans overnight in Sabouraud dextrose broth (SDB) at 28 °C with shaking at 150 rpm. Use Sabouraud dextrose agar (SDA) as the medium. Ensure the plates are sterile and allow them to solidify properly. Dip a sterile cotton swab into the prepared fungal suspension. Streak the agar plate evenly in three directions to create a uniform lawn of fungal growth. Allow the inoculated plate to air dry for about 15 minutes. Place gradient diffusion strips (or antifungal discs) containing specific antifungal agents (e.g., fluconazole, amphotericin B) onto the surface of the agar plate. Ensure the strips/discs are in full contact with the agar but do not touch the edges of the plate. Incubate the plates at 35 °C for 24 hours.(31)

If growth is insufficient, extend incubation for another 24 hours. After incubation, examine the plates for clear zones around the antifungal strips/discs where fungal growth has been inhibited. Measure the diameter of these zones using a ruler or caliper. Compare measured inhibition zones to standard interpretive charts to determine whether Candida is susceptible, intermediate, or resistant to the antifungal agent. (32)

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RESULT AND DISCUSSION

Total flavonoid content

Figure 1 illustrates flavonoid content across various samples. The x-axis represents concentration of samples, while the y-axis represents absorbance. We got the absorbance of 0.153 at the unknown concentration which came to be 19.6 micro gram. Thus, our total flavonoid content was obtained to be 19.6%

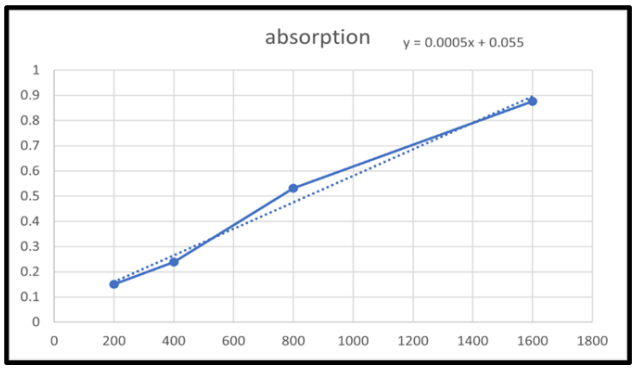

Figure 1. Calibration Curve of Total Flavonoid Content

Total flavonoid content (per gm of extract) was found to be 19.6 %.

Total phenolic content

Figure 2 illustrates phenolic content across various samples. The x-axis represents concentration of samples, while the y-axis represents absorbance. we got absorbance of 0.127 at the unknown concentration which came to be 439.7 microgram. Thus, our total phenolic content was obtained to be 43.9%

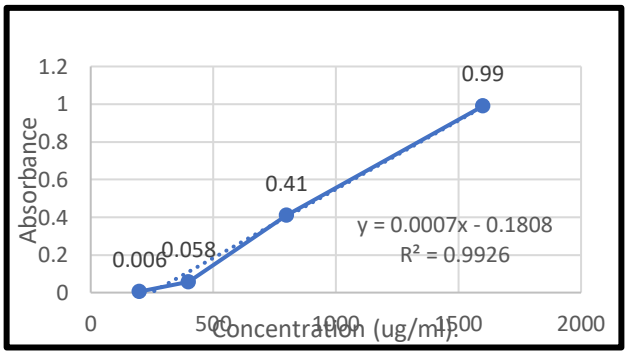

Figure 2. Calibration Curve for Total Phenolic Content

Total polyphenolic content (per gm of drug) was found to be 43.9%.

Characterization of silver nanoparticles

Particle size

The particle size represents the average diameter of particles in nanometers (nm). A smaller particle size indicates a more uniform and finer particle distribution. The average particle size was found to be 100.86 nm (figure 3).

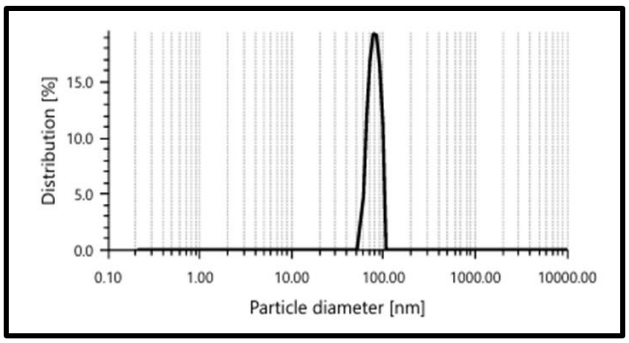

Figure 3. Graph of Particle Size

Polydispersity index

The PDI measures the particle size distribution, ranging from 0 (monodisperse) to 1 (polydisperse). A lower PDI value (<0.5) indicates a narrow particle size distribution, while a higher value (>0.5) indicates a broader distribution. PDI was found to be 0.331.

Entrapment efficiency

Higher entrapment efficiency values indicate better incorporation and retention of the compound within the formulation. The entrapment efficiency was found to be 90.9%.

Field emission scanning electron microscopy (FESEM)

Nanoparticles shape was found to be spherical with smooth surface. Size of nanoparticles observed by FESEM was found between 65 to 161 nanometers range, which are belonging to standard range.

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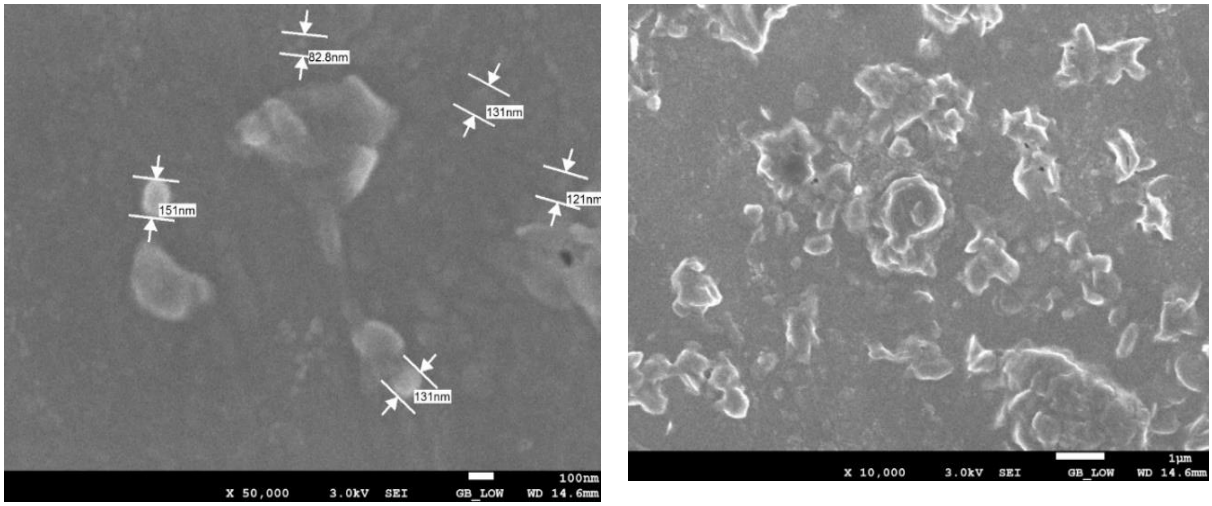

Figure 4. FESEM Images of Silver Nanoparticles

FTIR (Fourier Transform Infrared Spectroscopy) Analysis

The IR spectrum silver nanoparticles showed characteristic peaks at about 3300-3700 cm^{-1} due to O-H and N-H stretching of OH and NH group, and 1,650 cm^{-1} due to C=O stretching of the amide group.

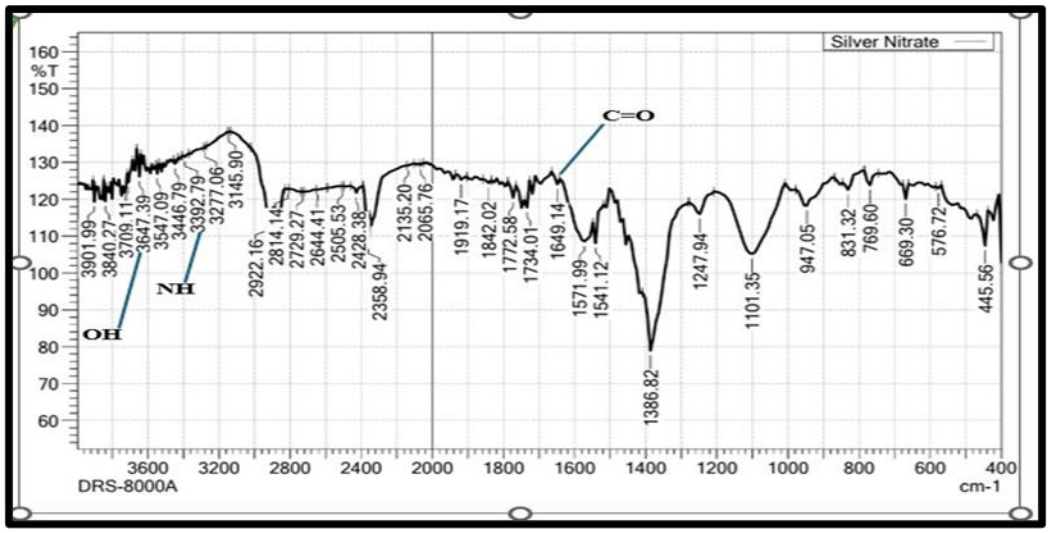

Figure 5. FTIR Spectra of Silver Nanoparticles

Table 1. FTIR Spectral Analysis of Silver Nanoparticles

Characteristic functional group	Standard wave number	Observed wave number
OH stretching	3300-3700	3647.39
NH stretching	3300-3400	3392.79
C=o	1650	1649.14

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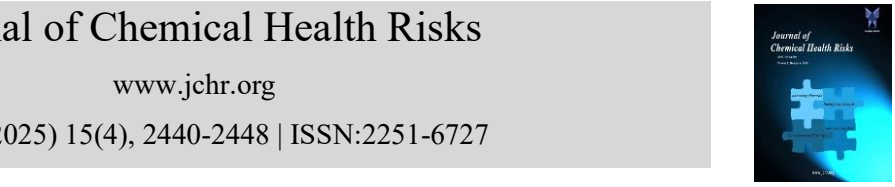

Antifungal assay

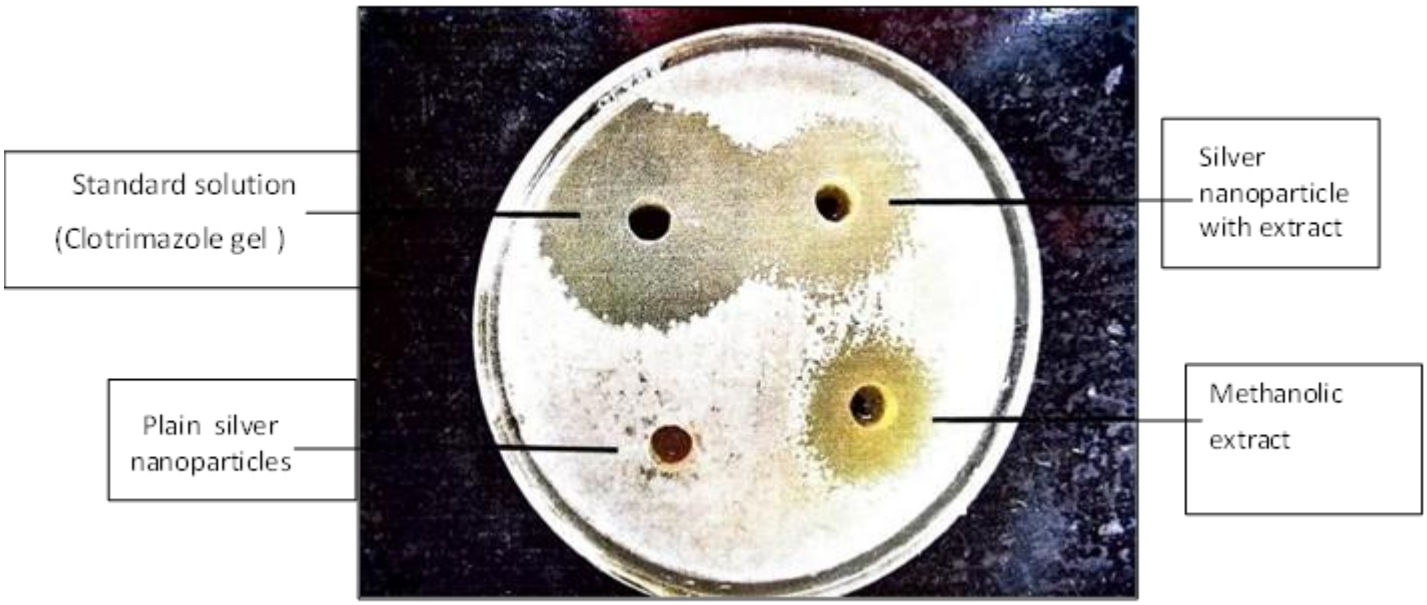

Figure 6. Antifungal Activity

Table 2. Antifungal Assay Analysis

Sample	Diameter of zone of inhibition
Standard solution (Clotrimazole gel)	20 mm
Extract loaded Silver nanoparticle	15 mm
Silver nanoparticles	9 mm
Methanolic extract	12 mm

CONCLUSION

The study synthesized silver nanoparticles using *Berberis aristata* leaf extract and evaluated their antimicrobial, ant psoriatic properties. The results indicated that the nanoparticles exhibited strong antifungal activity against certain strains of candida. This suggests the potential of these nanoparticles for various biomedical application. The synthesis of silver nanoparticles using *Berberis aristata* root extract showcases promising outcomes, revealing potent antifungal activity. This novel approach the natural compounds present in the plant extract to produce nanoparticles with multifaceted therapeutic potential. The ant psoriatic activity of berberis aristata was confirmed by antifungal study on candida fungi. These findings underscore the significance of utilizing plant-based synthesis methods for nanoparticles production, offering sustainable and bio-friendly alternatives with diverse biomedical application.

Acknowledgement: Authors are very much thankful to the Management of Dadasaheb Balpande College of Pharmacy, Besa, Nagpur for providing the facilities for the smooth conduct of this research.

Conflict of Interest: Nil

Funding: NA

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