Green Synthesis and Biological Applications of Silver Nanoparticles Using
Phyllanthus maderaspatensis L. Root Extract

Karuppannan Kokila, Nagaraj Elavarasan and Venugopal Sujatha

Department of Chemistry, Periyar University, Salem, India

ABSTRACT
Biosynthesis of metal nanoparticles is a raising research area because of their vital role in nanomedicines. In the present work, we have synthesized silver nanoparticles (AgNPs) using the aqueous extract of Phyllanthus maderaspatensis L. root. AgNPs are characterized by UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM), XRD – X-ray powder diffraction spectroscopy (XRD), and Energy dispersive X-ray spectroscopy (EDS) techniques. The synthesized AgNPs were also evaluated for its antioxidant, antibacterial, and cytotoxicity activities. The result indicates that the phytoconstituents present in the P. maderaspatensis root extract were mostly accountable for the reduction of Ag+ ions. UV spectrum was observed at 479 nm to the formation of AgNPs. Bioactive compounds were identified by FT-IR. The presence of elements was characterized by EDS. The morphology and size of the AgNPs were determined by SEM, HR-TEM, and X-ray diffraction analysis which showed the average particle size ranging from 3–14 nm. It was more effective against micro-organisms and also, AgNPs might serve as a potent antioxidant as revealed by DPPH and superoxide assays. Furthermore, these AgNPs also showed a potent cytotoxic activity against MCF-7 breast cancer cell lines. Herein, we suggested the green synthesis of AgNPs with potent antibacterial, antioxidant, and cytotoxic activities with feasible biomedical and industrial applications.

KEYWORDS
Phyllanthus maderaspatensis L; silver nanoparticles; antioxidant; antibacterial; cytotoxicity

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CONTACT
Venugopal Sujatha sujah@periyaruniversity.ac.in
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1. Introduction

Nanotechnology is a fast-rising field with probable use in the fields ranging from electronics to cosmetics.[1,2] Nanoscience covers the fundamental understanding of physical, chemical, and biological applications in atomic and subatomic levels.[3] It has opened the doors to pave the way for the rapidly improving technologies involved in the design and growth of novel materials which exhibits unique and improved properties. In this regard, the surface properties with stabilizing agents and functional groups play critical roles in determining bio-compatibility and the destiny of the nanoparticles. A proper selection of these agents not only improves the presentation and quality of the nanoparticles but prevent the side effects such as toxicity, aggregation and contamination.[4]

Developments of the ‘green chemistry’ methods for resourceful synthesis of metal nanoparticles using micro-organisms have drawn remarkable interest in recent years.[5] Biogenic synthesis of metal nanoparticles reduce the environmental issues compared with some physicochemical methods and can be used for large-scale production of nanoparticles with well-defined size and morphology [6] and one of the most useful applications is its antimicrobial activity. Biosynthesis of silver nanoparticles (AgNPs) is a suitable, economic, and environmentally safe approach, compared to chemical synthesis. AgNPs have many applications in the fields of catalysis, dentistry, clothing, mirrors, and food industries.[7]

*P. maderaspatensis* L. (F: Euphorbiaceae) is a widespread medicinal plant, whose species were used in different systems of medicine, mainly for treating liver disorders and urinary infections.[8] It was reported for its chemoprotective, anti-dysenterial, laxative, carminative, diuretic, and immunomodulatory effects.[9] Although, many studies have been carried out on the pharmacology and phytochemistry of this plant, its significance in the areas of nanotechnology is yet to be exposed.

The aim of the present work is to prepare AgNPs using *P. maderaspatensis* root aqueous extract by green synthesis method. The AgNPs are characterized by UV, Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), energy-dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), and high-resolution transmission electron microscopy (HR-TEM). The biosynthesized AgNPs carried out the DPPH and superoxide assays. Furthermore, the antibacterial properties of AgNPs tested *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* using agar diffusion method. Cytocompatibility of the AgNPs nanoparticles was evaluated against breast cancer MCF-7 cell lines. Indeed, our results provide conclusive evidence of the cytotoxicity consequence against cancer cell line.

2. Materials and Methods

2.1. Plant Collection

The fresh root of *P. maderaspatensis* was collected around the University campus, Salem district, Tamil Nadu, India. At the time of collection, a pressed specimen was prepared and authenticated by a Botanist (BSI/SRC/5/23/2013-14/ Tech-704), Botanical Survey of India, TNAU, Coimbatore.

2.2. Preparation of Plant Extract

*P. maderaspatensis* roots were washed thoroughly two to three times with double-distilled water. About 10 g of crushed root powder was taken in a 500-mL beaker and boiled with 100 mL of distilled water at 70 °C in a water bath for 30 min. The mixture was filtered through Whatman No. 40 filter paper. The filtrate was stored in refrigerator at 4 °C until further use. These aqueous extract was used as reducing as well as stabilizing agent.

2.3. Phytochemical Qualitative Screening Analysis

The root aqueous extract of *Phyllanthus* maderaspatensis was subjected to qualitative preliminary phytochemical screening to analyze the presence of the secondary metabolites according to the usual methods.[10]

2.4. Biosynthesis of AgNPs

For the synthesis of AgNPs, 5 mL of the aqueous root extract was added to 100 mL of 10-mM silver nitrate (AgNO₃) solution. The reaction mixture was stirred at 10 min and incubated at room temperature for 24 h. The developments of AgNPs were studied by visual observation of the color change from light yellow to deep brown color. At the end of the process, the colloidal suspensions were centrifuged at 10,000 rpm for 10 min. The formation of silver nanoparticles was characterized by spectral techniques.

2.5. Characterization of AgNPs

2.5.1. UV–Vis Spectroscopy

The synthesized AgNPs was established by sampling the aqueous component behind the reaction and the absorption maxima was scanned by T80 UV–Vis spectrometer at the wavelength of 200–800 nm.

2.5.2. FT-IR Spectroscopy

FT-IR spectrum to be obtained using Thermo Nicolet, Avatar 370 model ranges from 400 to 4000 cm⁻¹. Air-dried powder of *P. maderaspatensis* root and synthesized AgNPs was mixed with KBr to prepare pellets which
were obtained the FT-IR spectrum, the KBr was used as a reference.

2.5.3. XRD Studies
The particle size and nature of the AgNPs were determined using XRD. This was carried out using Bruker AXS D8 Advance model by 30 kv and 30 mA with Cu ka radi-ans at 2θ angle. X-ray powder diffraction analysis is a fast analytical technique primarily used for phase identification of a crystalline material and can give the information on unit cell dimensions. The analyzed material is finely ground and average size of composition is determined.

2.5.4. EDS Analysis
Energy-dispersive X-ray analysis (EDX; Bruker) takes advantage of the photon nature of lamp. In the X-ray range, the power of a single photon is just sufficient to generate an assessable voltage pulse X-ray, the output of a very low noise preamplifier linked to the low noise are a statistical measure of the equivalent quantum energy. By digitally recording and counting a huge number of such pulses through Multi Channel Analyzer, a total image of the X-ray spectrum is building up almost concurrently. This digital quantum counting system makes the energy-dispersive spectrometry very reliable. A semiconductor material is used to discover the X-rays mutually with processing electronics to analyze the spectrum.

2.5.5. SEM Analysis
The morphology of synthesized AgNPs was observed by scanning electron microscope (SEM, VEGA 3 TESCAN) under an acceleration voltage of 30 kV. SEM analysis was done by preparing thin films of suspension of AgNPs on carbon-coated copper grid by dropping little amounts of sample on the grid. Further sample was removed using blotting paper and the film on grid was dried in mercury lamp for 5 min.

2.5.6. HR-TEM Analysis
HR-TEM (Model-Tecnai, G2 20 Twin) was used to study the surface morphology and size of the synthesized nanoparticles. For HR-TEM analysis, the sample was prepared by dissolving 2 mg of the nanoparticles in 10 mL of methanol by sonication process. Two drops of this solution were placed on the carbon-coated copper grids and were allowed to evaporate the solvent.

2.6. Biological Applications of AgNPs
2.6.1. In vitro Antioxidant Activities of AgNPs
2.6.1.1. DPPH Radical Scavenging Activity of AgNPs. Various concentrations of AgNPs were assayed using a methanol solution containing DPPH radicals (6 × 10⁻⁵ mol/L) at an absorbance of 517 nm. Ascorbic acid was used as a standard. The radical scavenging activity (% RSA) was calculated using the following formula: % RSA = \([A_{\text{DPPH}} - A_{\text{AgNPs}}]/A_{\text{DPPH}}\] × 100, where \(A_{\text{AgNPs}}\) is the absorbance of the AgNPs, and \(A_{\text{DPPH}}\) is the absorbance of the DPPH solution.[11]

2.6.1.2. Superoxide RSA of AgNPs. Measurement of superoxide anion scavenging activity of AgNPs was based on the method described by Vishwanath et al. [12] Superoxide radicals are generated within PMS-NADH systems by oxidation of NADH and assessed by the reduction of nitroblue tetrazolium. Ascorbic acid was used as a control. The decreased absorbance of the solution mixture indicated increased superoxide radical anion scavenging activity. The inhibition of superoxide anion percentage (%) was calculated using the formula.

\[
\text{Scavenging activity} (\%) = 1 - \frac{\text{absorbance of AgNPs}}{\text{absorbance of control}} \times 100
\]

2.6.2. Antibacterial Activity
Peptone-10 g, NaCl-10 g, and Yeast extract 5 g, Agar 20 g in 1000 mL of distilled water. At first, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37 °C for 18 h. The agar disks of the above media were prepared and wells were complete in the plate. These plates were inoculated with 18-h old cultures (100 μL, 10⁴ cfu) and spread equally on the plate. After 20 min, the wells were filled with various concentrations of AgNPs. The control wells were packed with gentamycin along with solvents. All these plates were treated with 37 °C for 24 h and the diameter of inhibition zones were noted. The assay [13] was repeated two or three times and the mean of the three experiments were recorded.

2.6.3. In vitro Assay for Cytotoxicity (MTT assay)
MTT Assay: Cytotoxicity studies of the AgNPs were carried out on human breast cancer cell line (MCF-7), which was got from National Centre for Cell Science (NCCS), Pune, India. The cell viability was obtained using 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide [MTT] assay method.[14] The cell was grown in Eagle’s minimum essential medium containing 10% fetal bovine serum (FBS) and maintained at 37 °C, 5% CO₂, 95% air, and 100% relative moisture for 24 h prior to addition of compound. The mono layer cells were fractionated by EDTA to make single-cell suspensions and viable cells were counted using a haemocytometer, diluted with medium containing 5% FBS to give last density of 1 × 10⁵ cells/mL. One hundred H₁/well of cell suspension were seeded into 96 well of plates at plating density
following formula and the graph was plotted among percentage of cell inhibition and concentration, from this IC$_{50}$ value was calculated.

\[
\text{Cell Inhibition (\%) = } 100 - \left( \frac{\text{ABS}(\text{AgNPs})}{\text{ABS}(\text{Control})} \right) \times 100
\]

### 2.6.4. Statistical Analysis

All the tested experiments were carried out in triplicates and the results are communicated as mean ± SD with one-way analysis (ANOVA) through SPSS software (16.0 version).

### 3. Results and Discussion

#### 3.1. Phytochemical Qualitative Screening Analysis

The qualitative phytochemical screening analysis of the aqueous root extract of *Phyllanthus maderaspatensis* shows the presence of alkaloids, flavonoids, amino acids, proteins, tannins, steroids, and terpenoids (Table 1). Phytochemical study was made to check the possible bioactive molecules concerned in the reduction of silver ions.

#### 3.2. Characterization of AgNPs

##### 3.2.1. UV–Vis Spectroscopy

The formation of AgNPs was initially identified by the color changes, from yellow to brown color. From Figure 1, the result obtained for UV spectrum for the AgNPs prepared from *P. maderaspatensis* root reveals that the maximum absorption was found to be at 479 nm. The maximum absorption due to the excitation of Surface Plasmon Resonance, typical of AgNPs having absorbance values, which were reported earlier in the visible range of 446–480 nm.[15,16] It reveals the presence of AgNPs.

##### 3.2.2. FT-IR spectroscopy

The FT-IR spectroscopy measurement was studied to identify the possible biomolecules for the formation and stabilization of AgNPs. The peaks appeared at 3388.21, 2922.24, 2854.69, 1732.12, 1623.49, 1446.78, 1236.87, 1043.71, 602.89 cm$^{-1}$ in the root extract of *P. maderaspatensis* was shifted to 3430, 1606, 1311, 1079, 767, 591 cm$^{-1}$ to the formation of AgNPs (Figure 2). A strong absorption peak at 3430 cm$^{-1}$ reveals the presence of phenols and alcohols with free –OH group. The peak obtained at 1606 cm$^{-1}$ is perhaps assigned to the amide group of proteins. However, the native proteins was observed to the peak at 1606 cm$^{-1}$ by the interactions of AgNPs through biosynthesis and the secondary structure was not affected for the period of reaction with before Ag$^+$ ions or after binding with Ag$^0$ nanoparticles. The symmetrical stretching of carboxylate group can be attributed to the peak

![Figure 1. UV–Vis absorption spectrum of AgNPs.](image1)

![Figure 2. FT-IR spectrum of AgNPs.](image2)

| Phytoconstituents | *Phyllanthus maderaspatensis* aqueous root extract |
|-------------------|--------------------------------------------------|
| Alkaloids         | +                                                |
| Flavonoids        | +                                                |
| Phenolics         | +                                                |
| Tannins           | −                                                |
| Saponins          | −                                                |
| Carbohydrate      | +                                                |
| Proteins          | +                                                |
| Steroids          | +                                                |
| Terpenoids        | −                                                |
| Glycosides        | −                                                |
| Fats/oils         | −                                                |

Note: +; presence; – absence.

Table 1. Phytochemical qualitative screening analysis.
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Peaks observed in the pattern are well agreed with the previously reported values (JCPDS No. 04-0783). The sharp peak as well as broad diffraction pattern concludes that the synthesized system possess nanodimensional state. The particle or granule size of the particles on the AgNPs was calculated by Debye Sherrer's equation.

$$D = \frac{0.94\lambda}{\beta \cos \theta}$$

where, $D$ is the average crystallite size, $\theta$-diffraction angle, $\beta$-full width at half maximum, and $\lambda$ is the X-ray wavelength. [18–20] The average crystallite size was found to be 10.14 nm.

### 3.2.4. EDS Analysis

The EDS pattern obviously shows that the AgNPs are mediated by the reduction of silver ions using *P. maderaspatensis* aqueous root extract. The EDS analysis confirmed the presence of silver nanoparticles of the plant and mostly showed highest peak at 3 keV (Figure 4) and the presence of silver (74.88%) and oxygen (25.12%) without any contaminants.

### 3.2.5. SEM Analysis

The morphology and size of the synthesized AgNPs were obtained from SEM analysis. The SEM images revealed the presence of spherical shape in AgNPs with particle size ranging from 3 to 14 nm (Figure 5(a) and (b)). Thus, the AgNPs synthesized are on equal sharing throughout the process and also the sizes, stability of nanoparticles are consistent on the process. The synthesis of AgNPs has been reported by various researchers from different plant sources. The reports shows that the AgNPs obtained from the variety of sources are mostly spherical shape and different sizes.[21–23]

### 3.2.6. HR-TEM Analysis

Figure 6(a) and (b) shows the TEM analysis of the synthesized AgNPs. It was obtained that most of AgNPs were spherical in shape. A little agglomerated AgNPs were also observed in some places, thereby indicating possible sedimentation at a later time. The TEM analysis was used to investigate the microstructure and crystallinity nature of AgNPs synthesized using the root aqueous extract of *P. maderaspatensis*. It was also studied that AgNPs were observed the maximum size of particles in the ranges from 3 to 12 nm, which coincides with the SEM analysis results.

### 3.3. Biological Applications of AgNPs

#### 3.3.1. In vitro Antioxidant Activities of AgNPs

The results of the antioxidant potential against DPPH (20.20 μg/mL) and superoxide (35.30 μg/ml) radicals exhibited by AgNPs were measured in terms of IC$_{50}$ values as shown in Table 2. The percentage of RSA increases present at 1311 cm$^{-1}$. The peak appeared at 1079 cm$^{-1}$ was assigned to the stretching vibration of (C–O stretching) carbonyl group. The peak obtained at 767 cm$^{-1}$ was assigned to the C–H aromatic group. The frequency peaks in the range of 591–571 cm$^{-1}$ are capable to the structural vibrations which confirm the presence of metal oxide stretching vibrations. Singhal et al. [17] based on the characteristic features of the infrared vibrational peak in the spectrum, phenol and flavonoids were found to be the possible bioactive compounds to obtain the AgNPs.

### 3.2.3. XRD Studies

The X-ray diffraction pattern of biosynthesized AgNPs was confirmed by the characteristics peak observed in XRD image (Figure 3). In the XRD pattern, four prominent diffraction peaks were observed at $2\theta = 37.80^\circ$, 43.96°, 64.22°, and 77.09°, which corresponds to (111), (200), (220), and (311) Bragg reflections of fcc structure (face-centered cubic) of metallic silver, respectively.
inhibition was higher in the case of *B. subtilis* (17 mm) and *S. aureus* (14 mm) followed by *E. coli* (16 mm), when compared to Gentamycin as a standard (Table 3). The inhibition of bacterial growth reported in this study was dependent on the concentration and number of AgNPs in the medium. It has been reported that antibacterial effect was size, dose-dependent, and was more significant against Gram –ve bacteria than the gram +ve bacteria. [27,28] The present study also clearly indicates the synthesized AgNPs have potent antibacterial activity against Gram +ve bacteria than Gram –ve bacteria. The antibacterial studies of colloidal AgNPs are partial by the dimensions of the particles. The smaller the particles lead to the better antibacterial effects. [29]

with increasing concentrations (50–250 μg/mL) (Figure 7(a) and (b)). The actual efficiency shall be ascribed from the highest % RSA at minimum concentration thereby revealing its electron donating capacity. The scavenging activities of the AgNPs used in this work are related to those previously reported.[24,25] The free radical scavenging activities of AgNPs have been attributed to the functional groups of bioreductant molecules attached to the surface of the particles.[26]

### 3.3.2. Antibacterial Activities of AgNPs

The biologically synthesized AgNPs showed the excellent antibacterial activity against the bacterial pathogens like, *S. aureus, B. subtilis,* and *E. coli* (Figure 8). The zone of inhibition was higher in the case of *B. subtilis* (17 mm) and *S. aureus* (14 mm) followed by *E. coli* (16 mm), when compared to *Gentamycin* as a standard (Table 3). The inhibition of bacterial growth reported in this study was dependent on the concentration and number of AgNPs in the medium. It has been reported that antibacterial effect was size, dose-dependent, and was more significant against Gram –ve bacteria than the gram +ve bacteria. [27,28] The present study also clearly indicates the synthesized AgNPs have potent antibacterial activity against Gram +ve bacteria than Gram –ve bacteria. The antibacterial studies of colloidal AgNPs are partial by the dimensions of the particles. The smaller the particles lead to the better antibacterial effects. [29]
confirmed a considerable cytotoxicity against the MCF-7 cell line. The result showed that MCF-7 cells proliferation was potentially inhibited by AgNPs with an IC$_{50}$ value of 67.23 μg/mL of the concentration. Camptothecin was used as a control. The percentage of cytotoxicity increases with the increase in concentration of AgNPs suggests that biosynthesized silver nanoparticles could be of immense use in medical field to certain extent as anticancer agent.

From the results indicated in Figure 9(b), it is seen that the percentage of viability decreases with concentration, whereas cytotoxicity increases with concentration demonstrating a direct dose-dependent relationship. The results were also reported by Vasanth et al. [30] and Prabhu et al. [31], which further support the anticancer potentials of the green synthesis AgNPs.

### 3.3.3. Cytotoxicity of AgNPs

The in vitro cytotoxicity of the AgNPs was evaluated against MCF-7 breast cancer cell line at various concentrations (50–250 μg/mL) (Figure 9(a)). The samples was confirmed a considerable cytotoxicity against the MCF-7 cell line. The result showed that MCF-7 cells proliferation was potentially inhibited by AgNPs with an IC$_{50}$ value of 67.23 μg/mL of the concentration. Camptothecin was used as a control. The percentage of cytotoxicity increases with the increase in concentration of AgNPs suggests that biosynthesized silver nanoparticles could be of immense use in medical field to certain extent as anticancer agent. From the results indicated in Figure 9(b), it is seen that the percentage of viability decreases with concentration, whereas cytotoxicity increases with concentration demonstrating a direct dose-dependent relationship. The results were also reported by Vasanth et al. [30] and Prabhu et al. [31], which further support the anticancer potentials of the green synthesis AgNPs.

### Table 2. IC$_{50}$ values of AgNPs analyzed through in vitro radical scavenging assays.

| In vitro radical scavenging property | IC$_{50}$ values of AgNPs (μg) | IC$_{50}$ values of ascorbic acid (standard) (μg) |
|--------------------------------------|-------------------------------|-----------------------------------------------|
| DPPH radical scavenging property     | 20.20                         | 12.98                                         |
| Superoxide radical scavenging property| 35.30                         | 25.77                                         |

Table 2. IC$_{50}$ values of AgNPs analyzed through in vitro radical scavenging assays.

Notes: Standards were used for a comparative assessment to determine the 50% inhibition of the extracts against the radicals formed at the reaction system. The IC$_{50}$ values are expressed in mean ± SD, significance $p^*$<0.05.
Figure 7. (a) DPPH radical scavenging activity of AgNPs, (b) superoxide radical scavenging activity of AgNPs.

Figure 8. Antibacterial property of AgNPs against pathogens like Staphylococcus aureus (a), Bacillus subtilis (b), Escherichia coli (c) at various concentrations such as 10, 20, 30, 40, 50 μg/mL with control (C).

Figure 9. (a) Cytotoxic activities of AgNPs against the human breast cancer cell lines (MCF-7) at various concentrations ([1] 50, [2] 100, [3] 150, [4] 200, [5] 250 μg/mL), (b) the percentage cell viability of AgNPs against the human breast cancer cell lines (MCF-7) at various concentrations (50–250 μg/mL).
Table 3. Antibacterial property of AgNPs against various micro-organisms.

| Micro-organisms     | Zone of inhibition at various concentrations (μg/mL) |
|---------------------|-----------------------------------------------------|
|                     | 10        | 20        | 30        | 40        | 50        | Control   |
| Staphylococcus aureus\(^a\) | 5 ± 0.583 | 8 ± 1.240 | 11 ± 0.486 | 12 ± 0.638 | 14 ± 0.475 | 17 ± 0.243 |
| Bacillus subtilis\(^a\)    | NA       | 8 ± 0.752 | 12 ± 0.598 | 14 ± 0.102 | 17 ± 0.769 | 16 ± 0.458 |
| Escherichia coli\(^a\)     | 6 ± 0.745 | 9 ± 0.428 | 10 ± 1.014 | 13 ± 1.047 | 16 ± 0.326 | 19 ± 0.563 |

Notes: \(^a\)Gram +ve bacteria, \(^a\)Gram –ve bacteria, NA – no activity rendered by AgNPs against the tested micro-organisms. The results presented above indicate the efficiency of the AgNPs being active against the tested micro-organisms.

4. Conclusion

The present study was concluded that the root extract of *P. maderaspatensis* can be used as a tremendous source for synthesizing the silver nanoparticles. The primary confirmatory for the AgNPs was indicated by color change and UV–Vis absorption spectra of AgNPs formed (479 nm). The FT-IR spectrum revealed the presence of various functional groups like amine, hydroxyl, and carbonyl groups present in root extract are responsible for reduction of Ag\(^{+}\) ions to Ag\(^{0}\) nanoparticles biosynthesis. The spherical-shaped AgNPs have been synthesized using *P. maderaspatensis* root with sizes ranging from 3 to 14 nm. The SEM and HR-TEM image reveals that the nanodimensions of synthesized AgNPs, while XRD peaks were deduced (fcc) crystalline structure. The biosynthesized AgNPs shows a potent antioxidant as revealed by DPPH assay. The green syntheses of AgNPs have more effective antibacterial activity against the pathogens. Furthermore, these AgNPs significantly reduced the viability as well as increased cytotoxicity on MCF-7 breast cancer cell line. Hence, the result reveals that AgNPs synthesized from *P. maderaspatensis* L. root may serve as a potential antioxidant, antibacterial, and cytotoxicity activity, forecasting its pharmacological properties.

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Disclosure statement

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