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Antibacterial and Antioxidant Effects of the Green Synthesized Silver Nanoparticles from Flowers of *Callistemon lanceolatus*

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Abstract: Silver nanoparticles (AgNPs) synthesized from the flower extract of *Callistemon lanceolatus* (family Myrtaceae). The plant was collected from Thrissur, Kerala. The synthesis of silver nanoparticles was confirmed by visual observation: UV-Vis spectrum and X-ray diffraction (XRD) and the activity of the synthesized nanoparticles from flowers of *C. lanceolatus* was confirmed by antimicrobial and antioxidant study. Synthesis of silver nanoparticles using the flower extract of *C. lanceolatus* leads to the transformation of red-colored flower extract to dark-colored solution. Colour change indicates the formation of silver nanoparticle. The reduction of silver ions in the aqueous solution of silver during the reaction with the ingredients present in the flower extract was observed by UV-visible spectroscopy in the range of 422 nm and XRD analysis of synthesized silver nanoparticles from flower extract which showed major diffraction peaks at 32.5°, 38.3°, 64.6°, and 76.8°, respectively. Antimicrobial activity of the disc diffusion method in the biosynthesized silver particles from *C. lanceolatus* flower extract showed resistance against 2 gram-positive and 3 gram-negative bacteria, and the highest inhibition zone in gram-negative bacteria *P. mirabilis* (10 mm at 50 µl/disc). During DPPH assay study, an aqueous suspension of silver nanoparticles exhibited significant dose-dependent DPPH radical scavenging activity with IC₅₀ value as 49.09 µg/ml. The results are represented as percentage inhibition of DPPH and expressed as the mean ± standard deviation (n = 3). Thus, plant-mediated nanoparticle synthesis is more advantageous as they are free from toxic chemicals and provide natural capping agents.

Keywords: *Callistemon lanceolatus*, Myrtaceae, Green synthesis, Antibacterial, Antioxidant, Nanoparticle

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Introduction

Silver nanoparticles are widely used because of their unique properties in chemical sensing, bio-sensing, catalytic, photonics, electronics, and

pharmaceuticals. These have a good potential as antibacterial activity. Nanoparticles, which are made from comparatively larger bulk material

exhibit new and improved properties depending on their relative size, distribution, and morphology (Li *et al.*, 2012). Nanoparticles present a higher surface-to-volume ratio with decreasing size (Singh *et al.*, 2010). Plant extracts have the ability to reduce metal ions and this has brought about extensive concentration to green synthesis over the years (Marshall *et al.*, 2007; Kumar *et al.*, 2009; Park *et al.*, 2010; Iravani, 2011; Liu *et al.*, 2012). Different plants parts like seeds, flowers, stem, fruits, skin, or even their extracts are now used for nanoparticles production through green synthesis.

Nanoparticles have gained popularity for their application in molecular biology, especially as scaffolds for a variety of biological molecules, such as antibodies, DNA, RNA, and proteins (Arruebo *et al.*, 2009; Robinson *et al.*, 2010; Lukman *et al.*, 2014). The leaves are a tea substitute and have a delightfully refreshing flavor and tan dye is obtained (Cribb and Cribb, 1976). Nanoparticles produced from the green synthetic route are also found to have antioxidant properties; curative potential and antimicrobial activity. Nanoparticles were synthesized from three different leaf conditions – fresh leaves, sun-dried leaves, and hot-air oven-dried leaves (Sondi and Salopek, 2004; Morones *et al.*, 2005; Ruparelia *et al.*, 2008; Zhang *et al.*, 2008; Farooqui *et al.*, 2010; Nabikhan *et al.*, 2010; Muthuswamy *et al.*, 2010; Savithramma *et al.*, 2011; Naveena and Prakash, 2013).

Ravichandran *et al.* (2016) stated that biologically synthesized nanoparticles were 3-30 nm in size and oval or hexagonal in shape. According to Sap-Lam *et al.* (2010), the silver nanoparticles synthesized can be employed in biocontrol of pests including mosquito larvae. According to Salem *et al.* (2017), *Callistemon viminalis* is a plant that has been reported to have various medicinal values such as antibacterial, antifungal, anti-oxidant activities, and other pharmaceutical and insecticidal properties. This is brought about as a result of the diverse plant metabolites like amino acids,

alkaloids, tannins, saponins, flavonoids, enzymes, vitamins, and terpenoids present in the plant which are already established to possess therapeutic activity (Kulkarni and Muddapur, 2014).

Paosen *et al.* (2017) studied the green synthesis of silver nanoparticles using plants from the Myrtaceae family and characterization of their antibacterial activity. Phytochemical studies of different *Callistemon* species revealed the presence of different monoterpenes, sesquiterpenes flavonoids (Chowdhury *et al.*, 2012; Haque *et al.*, 2013). Moreover, phytochemical explorations of members of this genus resulted in the identification of C-methyl flavonoids, triterpenoids, and phloroglucinol derivatives (Varma and Parthasarathy, 1975; Huq and Misra, 1997; Wollenweber *et al.*, 2000). It is a well-established fact that plant-derived compounds offer potential sources of new antibiotics, anticancer agents, and anti-HIV agents among other pharmaceutical agents (Gurib *et al.*, 2005). The use of silver ions as antimicrobial agents is limited by the solubility of silver ions in biological and environmental media containing Cl⁻, because AgCl has very low solubility and rapidly precipitates (Marambio *et al.*, 2010).

Callistemon lanceolatus is rich in polyphenols and is used as anticough, antibronchitis and insecticide in folk medicine (Marzouk, 2008). *Callistemon* is sometimes considered a synonym of *Melaleuca* (Goyal *et al.*, 2012). Kumar *et al.* (2011) reported that the essential oils from leaves of *Callistemon lanceolatus* possess antimicrobial, antinociceptive and anti-inflammatory, Cardio-protective, Antiaflatoxin, Antioxidant activity, etc. The fruit of *C. lanceolatus* showed calcium channel blocking activity and flavonoids isolated from the aerial parts showed effectiveness against Alzheimer's disease (Kim *et al.*, 2009; Ali *et al.*, 2011; Firoz *et al.*, 2011). It has been postulated that terpenes in the leaves may be responsible for the efficacy of *Callistemon* in traditional treatments (Jirovetz *et al.*, 1997). Larayetan *et al.* (2019) found that three biosynthesized silver

nano-particles (AgNPs) were obtained from the reduction of silver nitrate (AgNO_3) by the aqueous crude extracts of aerial parts of *C. citrinus* plant (Larayetan *et al.*, 2019).

Antimicrobial and antioxidant activity of the biosynthesized silver particles from *C. lanceolatus* flower extract was investigated in the present study.

Materials and Methods

In the present study, we used an eco-friendly synthesis of silver nanoparticles using an aqueous flower extract of *Callistemon lanceolatus* belonging to the family Myrtaceae. Fresh flowers of *C. lanceolatus* were collected from Thrissur, Kerala and the identity of the plant was verified. *C. lanceolatus* interceded combination of AgNps was done dependent on green methodology with slight alterations. *C. lanceolatus* flowers (20 g) were washed thoroughly with distilled water for 15 min to dispose of surface debasements, cleaved finely with sanitized scissors under aseptic conditions, and dried in shade. Crushed flowers were stirred with 200ml distilled water at room temperature for 15 min and filtered using Whatman no. 2 filter paper. This filtrate or aqueous flower extract was used as a green chemical reducing as well as the stabilizing agent. Flower extract was stored at 4°C for further use.

Synthesis of silver nanoparticles using *C. lanceolatus* flower extract

For the synthesis of AgNPs, 2 ml of flower extract and 15 ml of AgNO_3 were used. The reaction mixture was prepared by adding 15 ml of AgNO_3 (0.4 g AgNO_3 in 250 ml water) and 2 ml of flower extract gradually on the magnetic stirrer and kept for 2 h. A colour change pattern was noticed from red to dark brown which indicates the formation of AgNPs (Fig. 1). This stoichiometric proportion was fixed based on initial testing with UV – Visible spectroscopy.

UV – visible spectral analysis

The colour change observed in the solution showed the formation of silver nanoparticles.

Incorporated silver nanoparticles were affirmed by testing the response combination at customary spans and assimilation maxima were examined by UV-Vis spectra, at the frequency 200 - 600 nm. The reaction mixture was subjected to centrifugation at 1500 rpm for 10 min. Following which the pellet was re-dispersed in sterile distilled water was repeated thrice to ensure the better separation of free entities from the metal nanoparticles.

X-ray Diffraction analysis

The synthesized silver nanoparticles were centrifuged at 10,000 rpm for 15 min and the pellets were re-dispersed in sterile double distilled water and centrifuged at 10,000 rpm for 10 min. The purified pellets were dried at 50°C in an oven and analyzed by X-ray Diffraction Unit (XRD). The presence, crystalline nature, phase variety, and grain size of synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy.

Antibacterial activity of silver nanoparticle against pathogenic bacteria

The antibacterial activity of *C. lanceolatus* and green synthesized silver nanoparticles were tested by the disc diffusion method for the determination of antibacterial activity against *Escherichia coli*, *Pseudomonas putida*, *Streptococcus* species, *Bacillus thuringiensis* and *Proteus mirabilis*. The paper disc was sterilized by autoclaving at 30° for 15 min. The disc was impregnated with 100µl samples of synthesized AgNps solution were added to individual discs in separate plates followed by drying. Agar plates were made by using Muller Hinton (MH) agar. 200 ml of distilled water is taken in a beaker and 5.6 g of MH agar was dissolved in it. It was autoclaved for 30 min at 120°C for disinfection. When it was cooled, poured into Petri dishes and allowed to solidify. Petri dishes were prepared and these five bacteria were inoculated on Muller-Hinton agar plates. Streptomycin was used as the positive control. After incubation at 37°C for 24h, the zone of inhibition was measured.



Fig. 1: (A) Solution of Silver Nitrate before, (B) after the addition of flower extract, and (C) silver nanoparticle formation after three hours.

Antioxidant activity

The antioxidant potential of the silver nanoparticles was determined by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity. To prepare 100 ml 0.2 mM DPPH solution, 7.83 mg DPPH was accurately weighed and dissolved in 100 ml methanol. The crude extracts were prepared at different concentrations. The samples were made up to 1 ml using methanol. To this, 1ml DPPH solution was added. A control was also prepared with 1ml methanol and 1ml DPPH solution. Absorbance was read at 517 nm in a UV – Visible spectrometer. Radical scavenging activity was expressed as the percentage and was calculated using the formula:

$$\text{Inhibition \% (I)} = 100 * (A \text{ blank} - A \text{ sample} / A \text{ blank})$$

Where I (%) is the inhibition per cent, A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

The pink colour of the DPPH solution diminishes to light pink or changes to yellowish-brown or decolourized depending on activity. Yellow spot formed due to bleaching of the purple colour of DPPH reagent was recorded as positive antioxidant activity of the radical scavenging capacity of different extracts was estimated. The antioxidant capacity was expressed as radical scavenging activity. The percentage of the radical

activity was plotted against the corresponding antioxidant substance concentration to obtain the IC₅₀ value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay system. IC₅₀ values are inversely proportional to the antioxidant potential.

Results and Discussion

After the addition of the flower extract to the AgNO₃ solution, it was observed that the colour of the solution changed gradually from red to dark brown. The colour change indicated the formation of nanoparticles. *C. lanceolatus* flower extract without silver nitrate did not show any change in the colour. The reduction of silver ions in the aqueous solution of silver during the reaction with the ingredients present in the flower extract was observed by UV-visible spectroscopy in the range of 422 nm (Fig. 2). The colour of the flower extract changed to light brown within an hour and then later changed to dark brown during the 2h incubation period. No significant change occurs after two hours. The brown colour could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles. Reduction of silver ions present in the aqueous solution of the silver complex during the reaction with the ingredients present in the flower of *Callistemon* extract observed by the UV-visible spectroscopy revealed the presence of silver nanoparticles which may be correlated with UV-

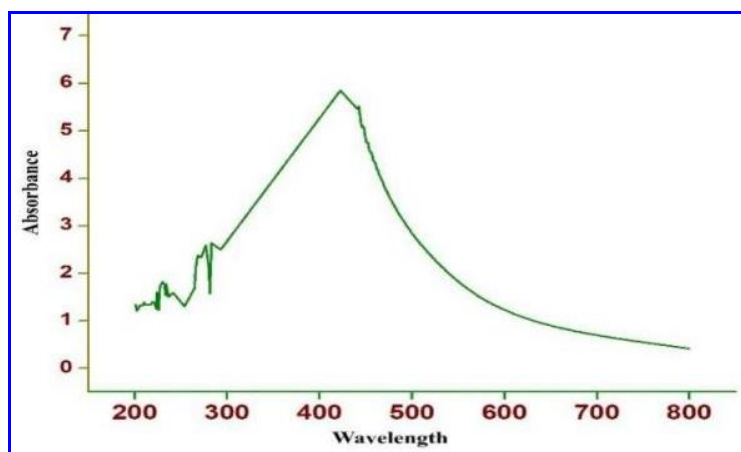


Fig. 2: UV Visible spectra of Silver nanoparticles of *Callistemon lanceolatus*.

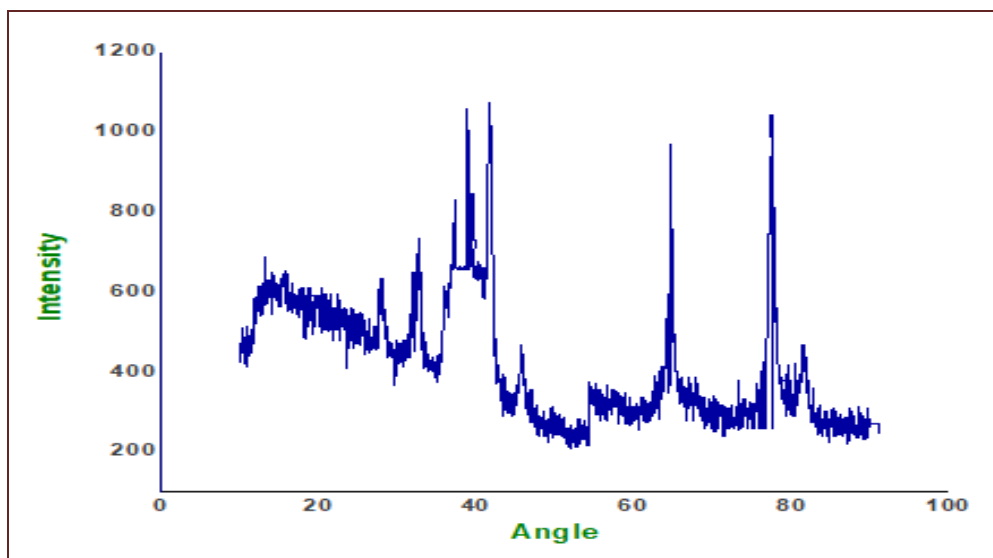


Fig. 3: XRD Silver nanoparticles of *Callistemon lanceolatus*.

visible spectra. These changes were attributed to the excitation of surface plasmon resonance (SPR) in the metal nanoparticles. Ag nanoparticles were observed to be stable in solution and show very little aggregation. Besides, the plasmon bands are broadened with an absorption tail in the longer wavelengths which may be due to the size distribution of the particles.

XRD Analysis

The study on structural details of the synthesized silver nanoparticles from *C. lanceolatus* was carried out by XRD. The XRD analysis of synthesized silver nanoparticles from flower

extract showed major diffraction peaks at 32.5°, 38.3°, 64.6°, and 76.8°, respectively. The obtained XRD spectrum confirmed that the synthesized silver nanoparticles were in nanocrystal form and crystalline in nature. The major peaks can be assigned to the planes 122, 111, 220, and 311 facet of silver crystal, respectively. The high peaks in the XRD analysis indicated the active silver composition with the indexing (Fig. 3).

Antimicrobial properties of silver nanoparticles of Callistemon lanceolatus

The antimicrobial activity of biosynthesized silver nanoparticles from *Callistemon lanceolatus* flower

Table 1: Antibacterial activity of silver nanoparticles

	Name of organism	Diameter of Zone of Inhibition(mm) SNP		
		5 µl /disc (mm)	10 µl /disc (mm)	50 µl /disc (mm)
1.	<i>Proteus mirabilis</i>	9 ± 0.25	9 ± 0.4	10 ± 0.25
2.	<i>Pseudomonas putida</i>	7 ± 0.35	8 ± 0.35	8.5 ± 0.35
3.	<i>Streptococcus</i>	6 ± 0.25	7 ± 0.44	7.5 ± 0.35
4.	<i>Bacillus thuringiensis</i>	6 ± 0.45	6.8 ± 0.42	7.5 ± 0.45
5.	<i>Escherichia coli</i>	7 ± 0.25	7.5 ± 0.46	8 ± 0.45

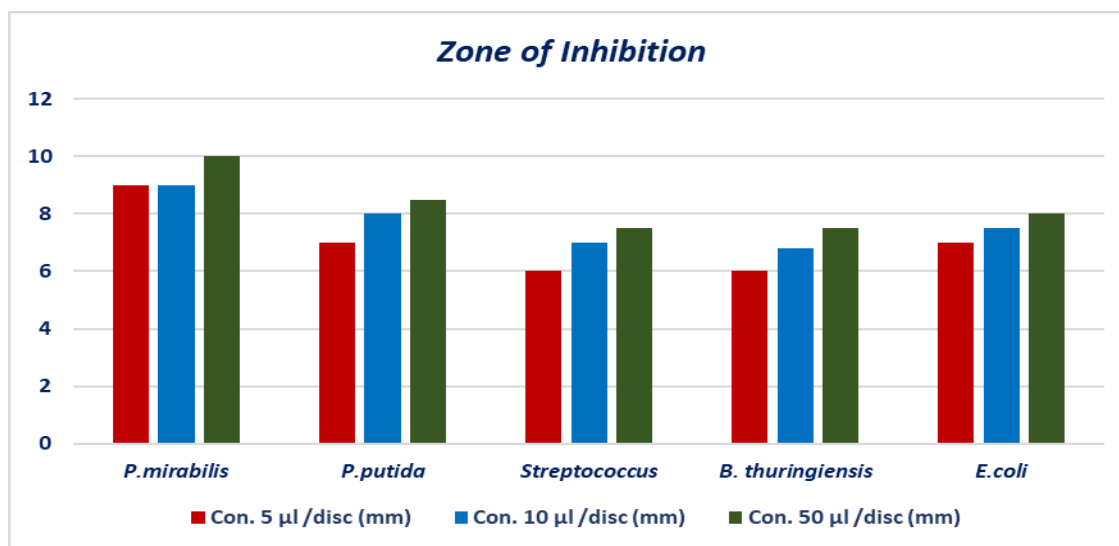


Fig. 4: Zone of inhibition.

extract was evaluated against various bacterial strains. The results demonstrated that the highest zone of inhibition was observed against the Gram-positive bacterium *Proteus mirabilis* (10 mm), followed by the Gram-negative bacterium *Pseudomonas putida* (8.5 mm). Moderate inhibition was recorded for *Escherichia coli* (8 mm), while *Bacillus thuringiensis* and *Streptococcus* exhibited the lowest inhibition zones of 7.5 mm each. Notably, the maximum inhibition was achieved at the highest tested concentration of silver nanoparticles (50 µl/disc) across all bacterial strains (Table 1, Fig. 4).

The results were observed in terms of IZ around the disc caused by the diffusion of antibacterial properties from the plant-based

silver nanoparticles impregnated disc into the surrounding medium. The diameter of the zone indicates the strength of effectiveness of the nanoparticle. The antibacterial activity of silver nanoparticles may be due to the property that it can continually release silver ions, which may be considered the mechanism of killing microbes.

AgNPs were successfully synthesized using plant extract of *C. lanceolatus*. It has been demonstrated that phytochemical constituents of the plant extracts could act as reducing agents in the conversion of silver ions to AgNPs. It may be due to the excitation of surface plasmon resonance vibrations in AgNPs. Negatively charged surfaces of AgNPs help in preventing the aggregation of nanoparticles and controlling shapes and sizes.

Table 2: DPPH Activity of Silver nanoparticles obtained from the flowers of *Callistemon lanceolatus*

S. No	Conc. (μl)	Optical Density			Average
		A	B	C	
1	20	0.190	0.180	0.183	0.184
2	40	0.158	0.151	0.157	0.155
3	60	0.128	0.124	0.133	0.128
4	80	0.118	0.094	0.112	0.108
5	100	0.078	0.073	0.081	0.077

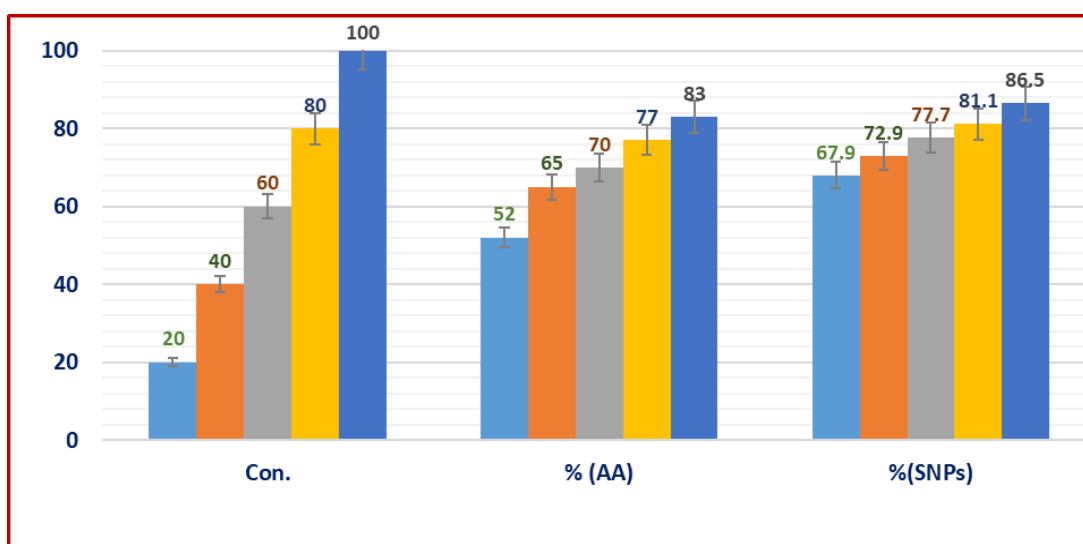


Fig. 5: Percentage of Ascorbic acid and DPPH inhibition of Silver nanoparticles obtained from the flowers of *Callistemon lanceolatus*.

Antioxidant activity

The DPPH assay is used to predict antioxidant activities by a mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radicals and therefore determine free radical scavenging capacity. The method is widely used due to the relatively short time required for the analysis. The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical is very stable, reacts with compounds that can donate hydrogen atoms and has a UV-vis absorption maximum at 517nm. The method is based on the scavenging of DPPH by antioxidants, which upon a reduction reaction

decolorizes the DPPH methanol solution. The assay measures the reducing ability of antioxidants toward the DPPH radical (Table 2).

The lowest concentration i.e. at 20 μl the standard curve gives the % of DPPH inhibition as 52, while at the highest concentration i.e. at 100 μl, % of DPPH inhibition was 83 (Fig. 5). We calculated the IC₅₀ value as 30 μg/ml. The DPPH radical scavenging activity was increased by increasing the concentration of the sample extract. We can correlate the species IC₅₀ values with the standard curve. The results are represented as percentage inhibition of DPPH and expressed as

the mean $\pm$ standard deviation (n = 3).

We calculated IC₅₀ value as 49.09 μ g/ml. As the DPPH concentration increases the percentage of inhibition increases (Fig. 5). We can see that at least concentration (20 μ l) % of DPPH inhibition is 67.9, while at the highest concentration (100 μ l) it is about 86.5% (Fig. 5). We can conclude that compared with the standard, silver nanoparticles of *C. lanceolatus* possess antioxidant activity.

Conclusion

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and disease preventive properties. Ecologically, *Callistemon* species as farm trees are planted for forestry plantations or ornamental purposes. The present study concluded that the extracts, as well as the silver nanoparticles from this plant, have a potent biological activity (antibacterial and antioxidant) and a good media for nanoparticle synthesis. The research needs to use these extracts on a commercial scale in the production of pharmaceutical purposes. Hence, this study reports a novel, rapid, economical, and environmentally friendly method for the production of biologically active silver nanoparticles.

Ethical Statement

This study was in accordance with the Research Advisory Committee (RAC) of the Vimala College (Autonomous), Thrissur, Kerala, India

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Conflict of Interest

The authors declare no conflicts of interest.

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