Volume 7 Issue 2 2021

ISSN 2454-3055

**INTERNATIONAL
JOURNAL OF
ZOOLOGICAL
INVESTIGATIONS**

***Forum for Biological and
Environmental Sciences***

Published by Saran Publications, India

Characterization and Antibacterial Potential of Green Synthesized Silver Nanoparticles of *Cissampelos pareira* L. Collected from Kolli Hills, Tamil Nadu, India

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Received: 7th November, 2021; **Accepted:** 9th December, 2021; **Published online:** 14th December, 2021

<https://doi.org/10.33745/ijzi.2021.v07i02.081>

Abstract: This study was focused on a cost efficient, eco-friendly and safe technique for the synthesis of silver nanoparticle using leaves of medicinally important plant *Cissampelos pareira* and its antibacterial activity. This approach showed that leaf extract of *Cissampelos pareira* reduced silver nitrate into Ag⁰ for the formation of silver nanoparticles. The generated silver nanoparticles were characterized by using UV-Vis spectroscopy, Fourier Transform Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) with EDAX. The biosynthesized silver nanoparticles were confirmed by UV-Vis spectrophotometer peak at 430 nm. SEM images of Silver nanoparticles leaf extract of *Cissampelos pareira* were spherical in shape with size ranges from 10 to 50 nm. Antibacterial activity of Silver nanoparticles of *Cissampelos pareira* leaf extract was carried out against bacterial pathogens like *Escherichia coli*, *Salmonella typhii*, *Proteus mirabilis* and *Staphylococcus aureus* by discs diffusion method. Maximum zone of inhibition were noticed in the *P. mirabilis* followed by *Escherichia coli*, *Salmonella typhii* and *Staphylococcus aureus*. All the values showed p<0.001 level of significance.

Keywords: Silver nanoparticles, FT-IR, SEM, UV-Vis Spectrophotometer, Bioreduction, Antibacterial activity, Bacterial pathogens, *Escherichia coli*, *Salmonella typhii*, *Proteus mirabilis*, *Staphylococcus aureus*

Citation: Seetharaman S., Indra V., Sheela D. and Abdul Rahim M.: Characterization and antibacterial potential of green synthesized silver nanoparticles of *Cissampelos pareira* L. collected from Kolli Hills, Tamil Nadu, India. Intern. J. Zool. Invest. 7 (2): 902-910, 2021.

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Introduction

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level (Albrecht *et al.*, 2006). Nanotechnology is expected to be the basic of many technological innovations in the 21st century. The synthesis of metal nanoparticles is a promising research field due to the possible

applications for the extension of novel technologies. Production of nanoparticles can be achieved mainly through three methods such as Chemical, Physical and Biological methods. Noble metal nanoparticles such as gold, silver and platinum are extensively useful to human. There is a growing interest to develop ecofriendly route for nanoparticles synthesis that do not use toxic

chemicals (Ahmad *et al.*, 2004). In recent times, efficient nanosilver materials are progressively used in diverse fields of biotechnology and medicine; having major focus for researchers over their biological applications (Gong *et al.*, 2007; Cardoso *et al.*, 2014; Henr *et al.*, 2019; Singh *et al.*, 2021).

Cissampelos pareira Linn (family: Menispermaceae) is a perennial climbing herb/shrub with small greenish-yellow flowering plant. It belongs to the genus *Cissampelos*, of which 30-40 species are distributed in the tropical and subtropical world. It has medicinal properties to cure various ailments such as antibacterial, anti-inflammatory, antispasmodic, diuretic, antihistamine, antioxidant, hypotensive, muscle relaxant, uterine relaxant, antiseptic, analgesic, febrifuge, hepatoprotective, antihemorrhagic, cardiogenic, diaphoretic, expectorant and tonic. It is also used for women ailments viz. menstrual problems, hormonal imbalance, ease childbirth, postpartum pain, prevent threatened miscarriage and control uterine hemorrhages, hormonal acne and premenstrual syndrome (Kritikar and Basu 1975; You *et al.*, 2012; Vidya *et al.*, 2018; Nakamura *et al.*, 2019).

In recent years, multiple drug resistance in human pathogenic organisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of diseases. The increasing interest on traditional ethno-medicine may lead to discovery of novel therapeutic agents. Therefore, this study was mainly focused on the synthesis and characterization of silver nanoparticles of *Cissampelos pareira* leaves and its antibacterial activity.

Materials and Methods

Identification and Authentication of plants:

The Fresh plant leaves were collected from Kolli hills, Namakkal District, India. The plant was identified as *Cissampelos paareira* L. and authenticated by Prof. Jayaraman, Plant

Taxonomist at Plant Anatomy Research Centre, Tambaram, Chennai, India and a voucher specimen no. PARC/2016/3266.

Preparation of leaf extract:

The fresh leaves were peeled off and were cleaned with running tap water and finally with Double distilled water to remove all the unwanted dust particles. Cleaned leaves were shade dried at room temperature for 1 week and then they were grind well using a mortar pestle. The materials were kept at room temperature in a dark bottle for further analysis.

Biosynthesis of silver nanoparticles:

Three grams of powdered *C. pareira* was boiled in 100 ml of de-ionized distilled water in a 250 ml conical flask. 80 ml of 1 mM AgNO₃ solution is added followed by addition of 5 ml plant extract and the final volume was adjusted to 100 ml by adding the appropriate amount of de-ionized water. The sample was incubated at room temperature for 24 h (Kasthuri *et al.*, 2009).

UV-Vis -Absorbance spectroscopy analysis:

The bioreduction of AgNPs by the aqueous leaf extract of *C. pareira* was recorded by using a UV-Vis (3000+ double beam, scanned between 200 to 800 nm) spectrophotometer. The same extract was further diluted and observed under UV-Vis absorbance, graph could be obtained. The deionized water was used as a blank.

FT-IR analysis:

FT-IR analysis was carried out at wavelength range from 400- 4000 cm⁻¹ to find out the active functional groups present in the green synthesized AgNPs.

SEM with EDAX analysis of Silver NPs:

The synthesized AgNPs were characterized by using high resolution SEM analysis (JSM-5600LV; JEOL, Tokyo, Japan). The control and experimental samples were prepared by a simple drop coating of suspended gold solution to an electric clean glass and allowed the solvent (water) to

evaporate. The samples were kept to dry at room temperature for 24 h.

The EDAX analysis was carried out for the detection and confirmation of elemental silver elements present in the synthesized silver nanoparticles of *C.pareira*.

Bacterial strains:

The Antibacterial effect of green synthesized AgNPs of *C. pareira* was examined against various pathogenic strains such as *E. coli* (G⁻), *P. mirabilis* (G⁻), *S. typhii* (G⁻) and *S. aureus* (G⁺) using disc diffusion method of Bauer *et al.* (1966). All the strains were procured from the Department of Biotechnology, University of Madras, Guindy campus, Chennai-25, Tamil Nadu, India.

Antibacterial activity by disc diffusion method:

The organisms were sub-cultured using nutrient agar broth at 37 C on rotary shaker at 200 rpm for 24 h. Then the strains were swabbed uniformly on the individual plates using sterile swab. Each 6 mm of disc prepared from Whatman no. 1 filter paper with the help of disc cutter and were sterilized by autoclaving at 15 lbs pressure for 15 min at 121 C under aseptic conditions. After sterilization discs were dipped in a 20 µg/ml of aqueous extract, AgNPs, distilled water (Negative Control), AgNO₃ (1 mM) and 5 µg/ml of Ampicillin (Standard) were placed in five different places in a petriplate with 25 ml of nutrient agar medium. The petriplates were maintained in an incubator for 24 h at room temperature. The zones of inhibition were observed and measured (mm). The same experiments were repeated for six times to know the level of significance.

Results and Discussion

Green synthesis of C. pareira AgNPs and UV-Vis Spectrophotometry:

The reductions of metal ion in plant leaves were solely responsible for ignition of Ag⁺ formation at nano level (appearance of colour change from yellow to yellowish brown confirms the nanoparticles synthesis). White color AgNO₃ and

green color of aqueous solution when react together then color change was observed from yellow to yellowish brown based on the incubation at 24h (Fig. 1) (Prabhu *et al.*, 2010; Ramya *et al.*, 2017). The Silver nanoparticles showed absorption peak at range of 440 nm (Fig. 2). All the phytochemical constituents of leaf extract are not only cogently reducing silver salt but also exhibit outstanding tenacity against agglomeration (Prabu and Johnson, 2015).

Fig. 1: Color change of *C. pareira* L. leaf extract (indicates the formation of AgNPs).

Green synthesis provides advancement over physical and chemical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis. There is no need to use high pressure, energy, temperature and toxic chemicals (Sathyavathi *et al.*, 2010).

FTIR Analysis:

The formation or synthesis of silver nanoparticles could be due to the presence of active functional groups in the selected medicinal plant which also acts as a reducing and capping agent and was confirmed by FT-IR spectra (Figs. 3a,b). The FT-IR spectrum (Fig. 3b) of silver nanoparticles showed peaks at 3200(Alkenes C=C-H), 1587(Nitro Groups N=O Stretch), 1391 (Amides N-HStretch) and 577(alkyl halides) cm⁻¹. This represents the different functional groups of biomolecules on the surface of nanoparticles. It indicates the influence of organic moieties on the formation of silver

Fig. 2: UV-Vis spectrum of green synthesized silver nanoparticles, AgNO₃ and *C. pareira* extract.

(A) Aqueous extract of *C. pareira*

(B) Green Synthesized Nanoparticles of *C. pareira*

Fig. 3 (A and B): FT-IR spectrum synthesized silver nanoparticles of *C. pareira*.

nanoparticles and its stabilization. The observed peaks could be attributed due to flavanoids and terpenoids excessively present in plants extract (Banerjee *et al.*, 2014; Prathna *et al.*, 2011; Rastogi and Arunachalam, 2011). The FT-IR results suggested that the reasonable mechanism of AgNPs formation may be due to the reduction of Ag⁺ ions that takes place together with oxidation of

flavonoids, phenolic components of polyols (Klaus *et al.*, 1999; Vijayaraghavana *et al.*, 2012; Garibo *et al.*, 2020).

SEM with EDAX analysis:

The morphology of the Silver nanoparticles were examined using SEM (Fig. 4). Well defined spherical structures were noticed i.e. in some

places, agglomeration of AgNPs were evidenced from the acquired images. The average particle size within the selected area of SEM images were predicted at 20 nm. Same trend of results were recorded by Vijayakumar *et al.* (2013) with the *C. pareira* L. mediated silver nanoparticles.

Fig. 4: SEM images of Biosynthesized Silver nanoparticles of *C. pareira* L.

The EDAX pattern clearly shows that silver nanoparticles were crystalline in nature by the reduction of silver ions from leaves of *C. pareira* L. The EDAX spectrum of the solution containing silver nanoparticles observed at 3KeV, which was typical for the absorption of metallic AgNPs. Signals obtained in the present study also confirmed the presence of elemental Silver, Oxygen and Carbon (Fig. 5).

Antibacterial activity:

The zone of inhibition was observed in the discs of positive control (Ampicillin), aqueous extract, 1 mM AgNO₃ solution, Negative control (Water), and synthesized AgNPs. The prominent zone of inhibition was measured in diameter and represented in the Table1 and Figures 6 and 7. Green synthesized AgNPs was found to have high antibacterial activity against all the pathogens used in the present study-- *E. coli* (12.08±0.10), *P. mirabilis* (13.3±0.33), *S. typhii* (12.2±0.22) and *S. aureus* (11.1±0.22) when compared with AgNO₃ and Aqueous extract. In general all the strains along with known standard showed high significant values ($p < 0.001$). Among the Gram^{-ve} bacterial strains *P. mirabilis* showed higher zone of

inhibition followed by *E. coli* and *S. typhii* as compared to gram^{+ve} bacteria *S. aureus*. Gram^{-ve} pathogen showed maximum zone of inhibition (Oves *et al.*, 2018).

Fig. 5: EDAX image of Biosynthesized Silver Nanoparticles showing peaks (signals) indicating the presence of elemental silver.

There are many more mechanisms proposed in the literature for antibacterial and fungal activity of AgNPs. Patil *et al.* (2012) claimed that the cell death arising out of exposure to AgNPs might be due to the cytoplasmic membrane disorganization and consequent leakage of various biomolecules such as amino acids, protein and carbohydrates. Moreover, they indicated that the cell death could be because of inhibition of various essential enzymes. The change in membrane permeability caused by the action of silver nanoparticles as a function of conductivity was studied by Krishnaraj *et al.* (2010); Ajitha *et al.* (2014) and Ramya *et al.* (2017). The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death (Sondi and Salopek-Sondi, 2004; Morones *et al.*, 2005; Vanaja *et al.*, 2013; Skandalis *et al.*, 2017; Oves *et al.*, 2018; Seetharaman *et al.*, 2018). The mechanism of the bacterial activity of AgNPs has been reported by binding of the membrane of microorganism through the electrostatic interaction, the cell wall disruption and affecting the intracellular processes such as DNA, RNA and protein synthesis (Umashankari *et al.*,

Fig. 6: Antibacterial activity of *C. papeira* L. leaf extract and silver nanoparticles. W-negative control; A.e-Aqueous extrat; AgNO₃; and NPs-AgNPs.

Table 1: Antibacterial activity of *C. papeira* L. leaf extract, Silver nanoparticles, AgNO₃ (1 mM)

Organisms	Zone of Inhibition (mm)			
	Standard (5 µg/ml)	Aqueous extract (20 µg/ml)	AgNO ₃ (20 µg/ml)	AgNPs (20 µg/ml)
<i>E. coli</i>	14.3±0.21*	2.5±0.10*	9.9±0.1*	12.08±0.10*
<i>S. typhii</i>	13±0.35*	2.4±0.54*	7.78±0.21*	12.2±0.22*
<i>P. mirabilis</i>	15±0.34*	3.7±0.65*	7.5±0.48*	13.3±0.33*
<i>S. aureus</i>	11.3±0.44*	2.4±0.54*	7.8±0.273*	11.1±0.22*

Values are mean ± Standard Deviation of six replicates; and * indicates significant values (p<0.001)

Fig. 7: Antibacterial activity of green synthesized silver nanoparticles of *C. papeira* L.

2012; Yudha *et al.*, 2013). According to Ahmed *et al.* (2016); Nikparast and Saliani (2018); Seetharaman *et al.* (2016); Araujo *et al.* (2019); Bastos *et al.* (2020) and Singh *et al.* (2021) the nanoparticles disrupt the polymer subunits of cell membrane and bacterial protein synthesis of the cell.

Conclusion

The biosynthesis of silver nanoparticles has been produced by *C. papeira* extract, which is an economical, efficient and eco-friendly process. UV-Vis spectrophotometer, FT-IR, SEM with EDAX techniques have confirmed the reduction of silver nitrate to silver nanoparticles. The zones of inhibition were well formed in the antimicrobial sensitivity assay indicates, that the AgNPs has the efficient antibacterial activity against bacterial strains of *E. coli*, *S. aureus*, *P. mirabilis* and *S. typhii*. The biologically synthesized silver nanoparticles were proved to be powerful weapons of antibacterial activity.

Acknowledgements

The authors wish to express their gratitude to DST-FIST Instrumentation Lab for providing FTIR-

ATR (Bruker) facility at Presidency College, Chennai 600005.

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