Competitive rapid microwave-assisted biogenic synthesis of spherical silver nanoparticles using an aqueous leaf extract of *Lepidagathis cristata*: Characterization and antimicrobial studies

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**ABSTRACT**
In the present study, microwave assisted biogenic synthesis of silver nanoparticles (Ag NPs) was carried out by using aqueous leaf extract of *Lepidagathis cristata* (*L. cristata*) which can act itself as a reducing and capping agent. This method was very precious due to their economic and eco-friendly benefits. The microwave assisted biogenic synthesized Ag NPs were characterized and analyzed by various instruments such as ultraviolet-visible spectroscopy (UV-vis), fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (XRD), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) coupled with X-ray energy dispersive spectroscopy (EDS). The UV-vis absorption spectrum peak at 430 nm, corresponds to the characteristic peak of Ag NPs. The functional biomolecules present in the *L. cristata* were identified by FT-IR spectroscopy, the XRD analysis explored that the synthesized Ag NPs have high crystallinity, face-centered cubic structure, and spherical shape. Ag NPs properties like size and stability (Zeta potential) of the NPs were calculated by Nano Particle Size Analyzer (NPSA). Further, we evaluated the antibacterial activity of the Ag NPs. The microwave assisted biogenic synthesized Ag NPs to show an excellent antibacterial activity against bacteria’s like *Escherichia coli*, *Pseudomonas aeruginosa* (Gram −Ve bacteria), *Streptococcus pneumonia* (gram +Ve bacteria) and the zone of inhibition range was determined.

**INTRODUCTION**

Nanochemistry was one of the mainly optimistic research areas in the modern science and technology. Nanoparticles (NPs) synthesis can exhibit new and improved properties based on morphology and sizes of particles. The properties of NPs were strongly depended on the size, shape and surface nature (*Xu et al.*, 2013; *Jose et al.*, 2016; *Vidya et al.*, 2016). Synthesized NPs have extensive properties in catalysis (*Muthu et al.*, 2017), optical device (*Galletto et al.*, 1999), electrochemical analysis (*Welch et al.*, 2006), electronic (*Daniel et al.*, 2004), photonics (*Maier et al.*, 2001), information storage (*Sun et al.*, 2000), biosensing (*Han et al.*, 2001; *West et al.*, 2003), biolabeling (*Nicewarner-pena et al.*, 2001), and biomedical materials (*Mirkin et al.*, 1996). Among the various noble metals AgNPs have been widely studied due to broad application in the fields of physics, chemistry, materials science, optoelectronics, renewable energies, environmental remediation, biomedical, high antimicrobial and catalytical nature (*Ahmed et al.*, 2015; *Lopez-Esparza et al.*, 2016; *Ruiz-Baltazar et al.*, 2017; *Saha et al.*, 2017). There are several approaches in synthesis of the silver at nanoscale level such as electrochemical (*Huya et al.*, 2017; *Gulnaz et al.*, 2015), polyl process (*Fereshteh et al.*, 2016; *Han et al.*, 2017; *Ammosova et al.*, 2017), sonochemical (*Victor et al.*, 2017; *Hao et al.*, 2017; *Suchomel et al.*, 2016), chemical vapor deposition method (*Brunon et al.*, 2017), photochemical (*Kim et al.*, 2015; *Gabriel et al.*, 2017), physical and chemical approaches (*Abou El-Nour et al.*, 2010), microorganisms (*Otari et al.*, 2014) and plant extract. The physical method involves the use of costly equipment, operates at high temperatures and occupies huge space for its set up of equipment. The chemical method involves the use of toxic chemicals which are harmful to the environment, and also to the person handling it. Biogenic synthesis in recent years by the use of plant aqueous extracts for the manufacture of NPs was an easy...
and expedient substitute to chemical and physical methods. Hence the biogenic synthesis was more suitable than other methods. In recent years the use of plant extracts (leaf, root, seed, root, flowers, bark, fruit peel, callus, and stem) for the preparation of NPs an easy, short time, inexpensive, stable, low temperature, low risk of contamination, readily available in natural sources and intensive labor. So it is convenient to other alternative methods. Plant extracts have polyphenols, alkaloids, flavonoids, proteins, enzymes, and sugars. The biomolecules act as reducing and stabilizing agents during NPs synthesis. However, the usage of plant extracts was more advantageous than other biological processes as it prevents the risk as well as the complicated process of maintaining cell cultures and the reaction time decreases from days to hours (Shankar et al., 2004; Kumar et al., 2009). domestic microwave assisted biogenic synthesis was superior merits, then traditional methods, it was controlled temperature very quickly, a very rapid method with high yields, and enhanced the stability, shape, and morphology of NPs. Major research studies reported that microwave assisted biogenic synthesis was very advanced over another alternative method for the synthesis of NPs (Nadagouda et al., 2011; Jiang et al., 2006). Although NPs synthesis using plant extracts has previously been reported in various plants such as Pongamia pinnata (Rajeshkumar, 2016), Capsicum frutescens (Sankar et al., 2017), Polygonum hydropiper (Bonnia et al., 2016), Heterotheca inuloides (Morales-Luckie et al., 2016), Candida albicans (Bhat et al., 2015), Seshania grandiflora (Ajitha et al., 2016), Carica papaya (Banala et al., 2015), Moringa oleifera (Prasad et al., 2017), Psium sativum peels (Prasad et al., 2017), pomegranate leaves (Prasad et al., 2017), Bottle gourds (Prasad et al., 2017), Murraya koenigii (Prasad et al., 2017), there was still a lot of attention paid to this field because of the variety and the high probable of plants in producing NPs with different shape and size. Silver nanoparticles (Ag NPs) have fascinated the awareness of numerous researchers compelling in the field due to its broad applications.

A survey of literature has shown that no report on the biogenic synthesis of Ag NPs using aqueous leaf extract of L. cristata. In the present work, we report for the first time the synthesis of Ag NPs by via aqueous leaf extract of L. cristata via biogenic synthesis. L. cristata as a common perennial herb which was located in tirumala and Talakona hills at A.P., India. L. cristata family: Acanthaceae) local name in Telugu language was Mullalabanti, the whole plant body has been used as a traditional medicine. It was used in the treatment of fever, inflammations, skin itch, burns, wounds and antifungal. Leaf extract was also used in cleaning the cattle in rainy season. Recent research on L. cristata extracts showed the presence of alkaloids, glycosides, oleic acid which supports to the therapeutic effects believed in traditional medicine (Reddy et al., 2013; Ravikanth et al., 2001; Abudacker et al., 2014; Purma et al., 2013; Teeguarden et al., 2007). Leaf extract of L. cristata having bio molecules can act as reducing and capping agent and are responsible for the synthesis of Ag NPs. The synthesized Ag NPs was conformed by UV-vis, FT-IR, TEM, SEM, XRD and NPSA analysis and also studied the antibacterial activity.

MATERIALS AND METHODS

Reagents and apparatus

Silver nitrate (AgNO₃) was purchased from Sigma Aldrich. Aqueous leaf extract of L. cristata was collected from Talakona forest located in A.P., India. Bacterial culture, the standard drug was acquired from S.D Fine chemicals and domestic microwave (KOR-616T). The UV-vis absorption spectra of the aqueous colloid solution were tested by UV-VIS Thermo scientific Nanodrop 8000 spectrophotometer at a resolution of 1 nm in 220-750 nm wavelength range, the phase purities of synthesized compounds were checked by XRD technique on a Seifert 3003 TT X-ray diffractometer with Cu Kα radiation with a wavelength of 1.52 A°. The FT-IR spectra of Ag NPs and L. cristata leaf extract was inspecting with Thermo Nicolet FT-IR200. The morphological and particle range was determined by TEM which was carried out with a JEOL-JEM-3010 Instrument. SEM, the quantitative elemental study of the NPs were tested by on Oxford instruments Inca Penta FET × 3 EDS, zeta potential, and particle size range was measured by using Horbia Scientific Nano Particle Size Analyzer SZ-100.

Collection of plant materials and preparation of aqueous leaf extract

L. cristata was confirmed by Department of Botany, Sri Venkateswara University, Tirupati, A.P., India. The fresh, young leaves of L. cristata were collected from Talakona forest near Tirupati, A.P., India. Leaves were washed to remove impurities with tap water and then double distilled water for 3 to 4 times and were dried at room temperature in dust and moisture free condition for 2 days. The fully dried leaves were powdered with sterile electric blender and 10 g of the fine dry leaves powder was dispersed in 100 mL of milli-Q-water in 250 mL round bottom flask and stirred for 45 min at 60°C. Then, the solution was carefully filtered by using Whatman no.1 filter paper and stored at 4°C for NPs synthesis.

Biogenic synthesis of Ag NPs

In order to synthesize Ag NPs, 20 mL of the aqueous leaf extract was mixed with 80 mL of 1 mM AgNO₃ solution taken in 250 mL beaker and shake well. Then the reaction mixture was kept under domestic microwave (KOR-616T) and exposed to microwave radiation for 5 min at a power of 800 W with a fixed frequency of 2450 MHz. After microwave radiation treatment, the resultant solution was cooled to room temperature and stored in dark condition. The change in color wine red to dark brown designates the evolution of colloidal Ag NPs, the colloidal NPs solution was centrifuged at 3500 rpm for 20 min and the progress of the reaction was monitored with the help of UV-vis absorption spectrophotometer. The formation of Ag NPs was shown in Figure 1.

Antibacterial activity

The antibacterial activity of the biogenic Ag NPs was tested against Common human pathogenic strains of Escherichia coli, Pseudomonas aeruginosa (Gram −Ve bacteria) and Streptococcus pneumonia (Gram +Ve bacteria) by the agar well diffusion method. The Sterile nutrient agar was poured uniformly on Petri plates and the selected bacterial cultures were spread on the nutrient agar medium by using L-rods. Wells were prepared in the agar plates followed by different concentrations of colloidal Ag NPs 12.5 µg/mL (D1), 25 µg/mL (D2) and 50 µg/mL (D3)
were added into each one of the wells and Streptomycin (50 µg/mL) was used as a standard. The Petri plates were incubated at 37°C for overnight (24 hours) in aerobic condition. After the incubation period, the zone of inhibition was measured.

RESULTS AND DISCUSSION

UV-vis analysis

UV-vis spectroscopy was one of the outstanding techniques to characterize the noble metal NPs. *L. cristata* leaves extract having biomolecules which can effectively act as reducing agent, actively involved in the synthesis of Ag NPs. The gradual color change from wine red (Leaf extract) to dark brown (colloidal Ag NPs) due to the reduction of silver ion (Ag⁺) and formation of Ag NPs (Ag⁰). *L. cristata* leaves extract and biogenic Ag NPs were investigated by UV-vis spectroscopy, the leaf extract did not show any characteristic bands in the visible region while the Ag NPs shows the maximum absorbance band at approximately 430 nm (Figure 2), which was due to the excitation of surface plasmon resonance (SPR) of Ag NPs. According to Mie’s theory, only a single SPR band was expected in the absorption spectra of spherical NPs. UV-vis spectra results show a clear SPR band was observed, which lead to predicting that the Ag NPs are a spherical shape.

![Fig. 1: A colloidal solution of (A) *Lepidagathis cristata* Leaves Extract (Wine red color), (B) Silver Nitrate metal ion solution (colorless) and (C) Ag NPs (Dark brown color).](image)

![Fig. 2: UV-Visible absorption spectra for (a) *Lepidagathis cristata* Leaf extract and (b) synthesized Ag NPs.](image)

FT-IR spectrum analysis

Reports of the FT-IR spectrum of the aqueous leaf extract of *L. cristata* indicates the presence of strong absorption peaks at 3300 cm⁻¹ and 1630 cm⁻¹. The broad signal at 3300 cm⁻¹ corresponds to the presence of secondary amides (N-H) stretching frequency, a strong signal at 1630 cm⁻¹ corresponds to overlap of the carbonyl group (C=O) stretching and amide (–NH) bending (Figure 3). The data clearly indicates the presence of an amide
group which belongs to tryptophan derived alkaloid in *L. cristata* leaf extract. The amides present in the leaf extract dynamically involved in the reduction of silver ions to metallic Ag NPs. Earlier reports strongly supported that *L. cristata* aqueous leaf extract contains tryptophan derivative alkaloids.

![Image](51x326 to 286x463)

**Table 1**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5 µg/mL</td>
<td>D1: 12 ± 0.5, D2: 23 ± 0.5, D3: 25 ± 0.5</td>
</tr>
<tr>
<td>25 µg/mL</td>
<td>D1: 18 ± 0.5, D2: 25 ± 0.5, D3: 28 ± 0.5</td>
</tr>
<tr>
<td>50 µg/mL</td>
<td>D1: 22 ± 0.5, D2: 28 ± 0.5, D3: 31 ± 0.5</td>
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</table>

**Antibacterial studies**

The biogenic synthesized Ag NPs with different concentrations viz. 12.5 µg/mL (D1), 25 µg/mL (D2) and 50 µg/mL (D3) were tested for their antibacterial activity by using agar well diffusion method. Ag NPs showed a potential antibacterial activity against the test pathogens *Escherichia coli*, *Pseudomonas aeruginosa* (Gram −Ve bacteria) and *Streptococcus pneumonia* (Gram +Ve bacteria). Table 1 and Figure 8 show the zone of inhibition for three different concentrations of biogenic synthesized Ag NPs against the bacterial activity.

The results showed that biogenic synthesized Ag NPs at their lower concentrations inhibited the growth of test pathogens, as a result, clear inhibition zone was observed for all selected pathogens such as *E. coli* (9 ± 0.5 mm), *P. aeruginosa* (6 ± 0.5 mm) and *S. pneumonia* (7 ± 0.5 mm). This was clearly indicated that *E. coli* was more susceptible to Ag NPs than *P. aeruginosa* and *S. pneumonia*. Three different concentrations of Ag NPs such as D1, D2, and D3 showed clear various zone of inhibition against the selected bacterial pathogens, these results are compared with that of relative antibacterial activity of standard antibiotic i.e. Streptomycin (which showed the Max. inhibition zone 23 ± 0.5 mm, 22 ± 0.5 mm and 26 ± 0.5 mm for *P. aeruginosa, E. coli*, and *S. pneumonia* respectively at the concentration of 50 µg/mL).

The results clearly demonstrated that the zone of bacterial inhibition increases with increase in the concentration...
of biogenic synthesized NPs. D1 and D3 concentrations showed more inhibition zone on *E. coli* and *Streptococcus pneumonia* similarly *Pseudomonas aerogenosa* was also reasonably inhibited. D2 concentration also possesses good inhibition zone on all three bacterial pathogens.

Observations of the present investigation obviously stated that three different concentrations of biogenic synthesized Ag NPs exhibited fine antibacterial activity against selected pathogens in Agar well diffusion method.

![Transmission electron microscope (TEM) image of synthesized Ag NPs.](image1)

![Scanning electron microscope (SEM). Energy dispersive X-ray spectroscopy (EDX) images of synthesized Ag NPs.](image2)

![Particle Size Analyzer (PSA). Zeta potential (ZP) images of synthesized Ag NPs.](image3)

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>S.P. Area Ratio</th>
<th>Mean</th>
<th>S.D.</th>
<th>Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>29.0 nm</td>
<td>0.1 nm</td>
<td>29.0 nm</td>
</tr>
<tr>
<td>2</td>
<td>---</td>
<td>--- nm</td>
<td>--- nm</td>
<td>--- nm</td>
</tr>
<tr>
<td>3</td>
<td>---</td>
<td>--- nm</td>
<td>--- nm</td>
<td>--- nm</td>
</tr>
<tr>
<td>Total</td>
<td>1.00</td>
<td>29.0 nm</td>
<td>0.1 nm</td>
<td>29.0 nm</td>
</tr>
</tbody>
</table>

Cumulant Operations
Z-Average: 3755.2 nm
PI: 0.342 (Polydispersity index)

![Particle Size Analyzer (PSA). Zeta potential (ZP) images of synthesized Ag NPs.](image4)

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Zeta Potential</th>
<th>Electrophoretic Mobility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-23.9 mV</td>
<td>-0.000186 cm2/Vs</td>
</tr>
<tr>
<td>2</td>
<td>--- mV</td>
<td>--- cm2/Vs</td>
</tr>
<tr>
<td>3</td>
<td>--- mV</td>
<td>--- cm2/Vs</td>
</tr>
</tbody>
</table>

*Fig. 5:* Transmission electron microscope (TEM) image of synthesized Ag NPs.

*Fig. 6:* (a) Scanning electron microscope (SEM). (b) Energy dispersive X-ray spectroscopy (EDX) images of synthesized Ag NPs.

*Fig. 7:* (a) Particle Size Analyzer (PSA). (b) Zeta potential (ZP) images of synthesized Ag NPs.
Table 1: The variation of the zone of inhibition measured by well diffusion method for different bacteria.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bacterial Strain</th>
<th>−Ve control</th>
<th>D1 (12.5 µg/mL) ± SD*</th>
<th>D2 (25 µg/mL) ± SD*</th>
<th>D3 (50 µg/mL) ± SD*</th>
<th>Streptomycin standard (50 µg/mL) ± SD*</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>0</td>
<td>9 ± 0.5</td>
<td>13 ± 0.5</td>
<td>16 ± 0.5</td>
<td>23 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas aeruginosa</td>
<td>0</td>
<td>6 ± 0.5</td>
<td>11 ± 0.5</td>
<td>15 ± 0.5</td>
<td>22 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>Streptococcus pneumoniae</td>
<td>0</td>
<td>7 ± 0.5</td>
<td>13 ± 0.5</td>
<td>16 ± 0.5</td>
<td>26 ± 0.5</td>
</tr>
</tbody>
</table>

*± SD: standard deviation.

Literature suggests that there are several methods by which Ag NPs could be killing the microorganisms, the synthesized Ag NPs was to achieve extensively high antimicrobial activity at low concentration levels and smaller Ag NPs shows good antimicrobial activity than bigger ones. Ag NPs have the capability to attach to sulfur containing proteins in the cell membrane, inside the cell and phosphorus in the nucleic acid (DNA) are likely to be the favorite sites for binding of Ag NPs and then penetrate it, thereby causing structural changes in the cell membrane like permeability of the cell membrane and injure of the cell membrane and death of the cell (Ocsoy et al., 2013; Del Lago et al., 2011; Zhao et al., 2010; McDonnell et al., 1999). The amount of DNA injury within the cell increases with increasing concentration of the Ag NPs this indicates that Ag NPs could damage the DNA effectively and to kill the relevant bacteria (Velusamy et al., 2015).

CONCLUSION

In summary, we have established that Ag NPs were synthesized from aqueous leaf extract of L. cristata in a quick method by using eco-friendly microwave assisted biogenic synthesis. The synthesized Ag NPs UV–vis spectroscopy results tell that NPs absorbance peak was in the visible region, FT-IR results L. cristata leaf extract having alkaloids which actively involved in reduction of silver ions to metallic Ag NPs, powder XRD analysis showed that Ag NPs was face-centered cubic structure, agglomerated spherical shape it was confirmed by TEM and the elemental analysis was investigated by EDS. Furthermore, the synthesized AgNPs have potential antimicrobial activity at room temperature.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


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