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ORIGINAL RESEARCH PAPER

Green Synthesis of Silver Nanoparticles using *Litsea glutinosa* L. Leaves and Stem Extracts and their Antibacterial Efficacy

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ABSTRACT

The present study explores the green approach for the preparation of silver nanoparticles (AgNPs) through the reduction of silver nitrate by the cell-free stem and leaf aqueous extracts of *Litsea glutinosa* (*L.glutinosa*) and its potential antibacterial activity. The analytical instruments include scanning electron microscopy, Fourier transforms infrared spectroscopy, UV-visible spectroscopy, and X-ray diffraction spectroscopy confirmed the synthesis of smaller, uniformly spherical AgNPs (10-40 nm). The average crystalline size of prepared AgNPs produced by *L. glutinosa* leaf extract was found to be 19 mm. From UV-visible spectral analysis, the maximum absorbance peak appeared at 444 nm for leaf extract AgNPs different from stem extract AgNPs (422 nm), which are found to be specific for AgNPs. The *L.glutinosa* stem extract-assisted AgNPs have shown significant antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) in comparison to Gentamycin. Hence, the AgNPs obtained by green synthesis can be therapeutically explored against bacterial infections.

Keywords: *Green synthesis; L.glutinosa; leaf extract; AgNPs; antibacterial activity.*

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INTRODUCTION

Nanotechnology is an emerging branch in the scientific field that deals with the synthesis of variable sizes, shapes, and compositions of nanomaterials, which are having a diverse range of controllable dispersity [1]. Inorganic nanoparticles (NPs) have raised much attention for their elevated surface area to volume ratio, continued discharge, and specificity [2; 3]. The preparation of NPs *via* green synthesis regarded as environmentally gracious progression and cost-effective is proven to be a better method owing to properties such as kinetics with low pace, growth of crystals in a controlled way, convenient handling, and stability [4]. Various reports have shown antimicrobial [5], antioxidant [6], and cytotoxic [7] properties of green synthesized nanoparticles. Silver nanoparticles (Ag NPs) demonstrate prospective bactericidal doings in opposition to gram-positive and negative bacteria along with strains about

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antibiotic resistance strains, owing to the high surface-to-volume ratio and are thus extensively put to use in water purification systems, medical devices, and cosmetics [8].

L. glutinosa is an aromatic evergreen medium-sized tree belonging to the family Lauraceae and found to be a very rare medicinal plant in the Western Ghats. The conventional practitioners inhabiting the environs of the Western Ghats are utilizing the leaf and bark of stem extracts as a demulcent and placid astringent for dysentery wherein the bark paste is applied in wound curative progression [9]. Phytochemical screening of the stem bark revealed the presence of β-sitosterol, norboldine, laurotetanine, tannin, actinodaphnine Boldine, n-methylactinodaphnine, n-methyllaurotetanine, sebiferine, litseferine and quercetin [10] and litseglutine A and B are aporphine alkaloids [11]. The present study is focused on the preparation of AgNPs through green synthesis using aqueous leaf and stem extracts of *L. glutinosa* and further, assessing their antibacterial activity against *B. subtilis* (gram-positive) and *E. coli* (gram-negative). The study has given an outcome of green synthesis of smaller-sized AgNPs is apt for the formulation of innovative sorts of bactericidal resources.

MATERIALS AND METHODS

Plant Material

Fresh leaves and stems of *L. glutinosa* are collected and their taxonomic detection is confirmed by matching up to that of the known identity accessible in the facility available in the department of Botany, Andhra University (India).

Preparation of Leaf and Stem Extract

After meticulous outside dirt-freeing, the explants are powdered with mortar and pestle. At 70 to 80°C the leaf and stem powders dissolved separately in distilled water (1%) and boiled for 30 min, cooled to room temperature, and then filtered through Whatman No. 1 filter paper. For the green synthesis of AgNPs, the collected filtrates are used further as reducing and stabilizing agents.

Synthesis of Silver Nanoparticles (AgNPs)

80 ml of 1mM silver nitrate solution is incubated (around 5 h) with 20 ml of leaf and stem extracts, separately under a light. After the color changes to dark brown from light yellow, the reaction is clogged by centrifugation for 8 min at the rate of 10,000 rotations per min. Then the collected pellet is ished three times with deionized water and dried for 3 h at 60°C, which is characterized further. To conduct studies on anticancer and antibacterial doings the AgNPs are re-dispersed in DMSO without drying.

Characterization of Silver Nanoparticles (AgNps)

The characterization of biosynthesized AgNPs is done by various instrumental analyses. The optimization for AgNP production is scrutinized with a UV–Vis spectrophotometer (UV-2450, Shimadzu, Japan). The mean particle size and morphology of the AgNPs are resolute by SEM analysis (JSM-6610LV, Jeol Asia PTE Ltd, Japan). By Energy Dispersive Spectroscopy (EDS), the presence of an elemental silver signal is also confirmed. Additionally, the crystalline arrangement of AgNPs is observed by (PanAnalytical, X-Pert pro, Netherland) X-ray diffractometer, using Cu Kαradiation (λ = 0.1546 nm). To settle on the diminution of Ag^+ to Ag^- ions, FTIR analysis (FT-IR Prestige21, Shimadzu, Japan) is conceded out with the spectral range of 400-4000 cm^{-1}. Briefly, the reaction pellet is grounded with KBr and is analyzed beneath FTIR.

Antibacterial Assay

The bactericidal effect of AgNPs produced from *L. glutinosa* leaf and stem aqueous extracts is determined by performing the disc diffusion method for test organisms i.e. gram-positive bacteria, *B.subtilis* (MTCC211) and a gram-negative bacteria, *E. coli* (ATCC 35218). From the 24 h grown broth cultures, by spread plate technique, bacteria are inoculated into Muller Hinton agar plates and permitted to nurture for about a day (24 h). Sterilized standard discs (6.4 mm) saturated with 50 µl of AgNPs dissolved in DMSO are applied to the grown cultures. As a positive control, gentamicin (10 µg) is used. The compounds and antibiotic dispersal is allowed for 1 h and then, the plates are incubated at 37°C for 24 h. Later, plates are scrutinized for recording the diameter of the zone of bacterial development reserve to express the antimicrobial action.

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

When the aqueous solution of silver nitrate is mixed with leaf and stem extracts of **L. glutinosa,** the solution color is changed to brown from green, due to the silver ion reduction. It is acknowledged

that when the outside plasmon ambiance in silver AgNPs is energized, the AgNPs in an aqueous solution show yellowish-brown color [12]. The presence of AgNPs is long-established by attaining a UV-Visible spectrum between 200 nm and 800 nm range of wavelengths (Fig.1). From this analysis, the absorbance peak is found at 444 nm for *L. glutinosa* leaf-extracted AgNPs and 422 nm to that of stem extracted AgNPs, which are found to be specific for AgNPs [13]. The acquired UV-Vis spectra also indicate an attentiveness-dependent addition to the sharpness of the incorporation peak, which conforms with the earlier. The obtainable molecules that are active biologically in the leaf and stem extracts might be accountable for the Ag metal ion diminution process.

X-ray Diffraction

In the present investigation, the X-ray diffraction method is utilized to recognize the phase, crystalline size and orientation of the synthesized NPs and has clearly shown the distinctive diffraction peaks connected with the crystalline state of Ag (Fig.2). The peaks of diffraction at 2θ degrees are seen at 78.15, 64.89, 44.57 and 38.27 to (311), (220), (200) and (111) miller indices for AgNPs produced by the *L. glutinosa* leaf and plant extracts, respectively.

By the Powder diffraction standards joint committee database bearing file #04-0783, the obtained results matched, thus corroborating the face-centered cubic crystalline elemental Ag. The unallocated peaks might be a result of the phase crystallization of the phases that are stuck to the

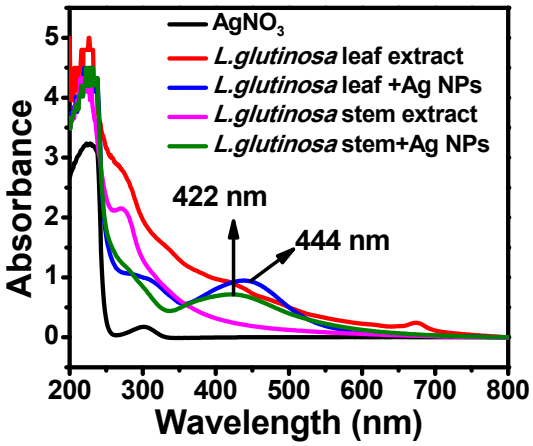


Fig.1. UV–Vis spectra of AgNPs synthesized from leaf and stem extracts of *L. glutinosa*

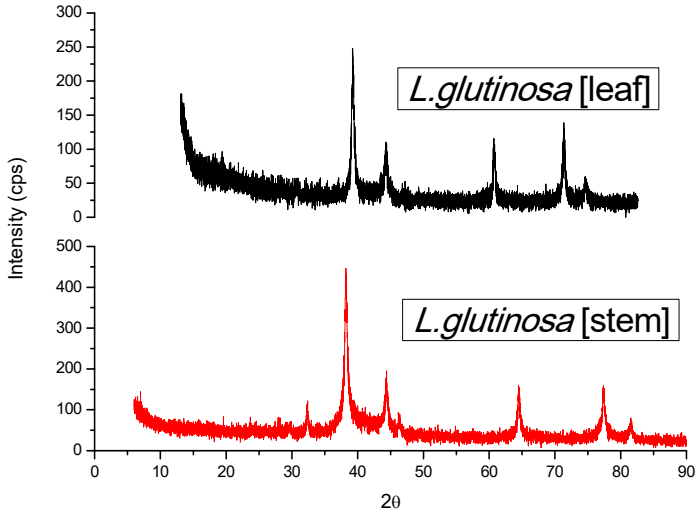


Fig. 2. X-ray diffraction analysis of *L. glutinosa* leaf and stem AgNPs.

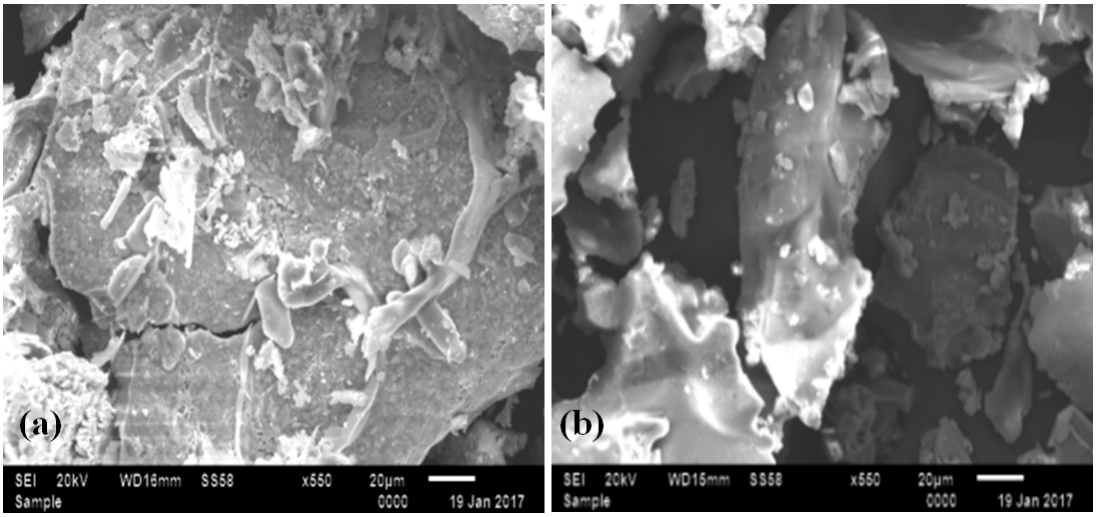


Fig.3. SEM images of AgNPs prepared with *L.glutinosa* (a). Leaf and (b). Stem extract

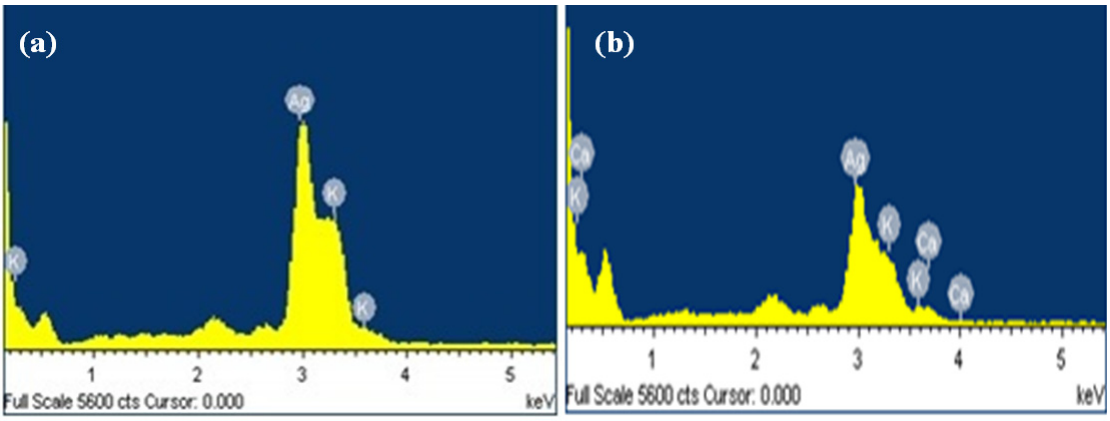


Fig. 4. Energy Dispersive X-ray Spectroscopy images of synthesized AgNPs for L.glutinosa. a) Leaf and b) Stem extract.

prepared nano-particle surfaces [14, 15]. The intended lattice stricture is a = 4.0569Å with lattice size 66.7788 (Å)^3. Further, the sourness of the peak (111) identifies the nano-formulations that are long-established by using Debye-Scherer's equation D = ($k\lambda/\beta\cos\theta$), where 'D' indicates mean crystalline particle size, 'λ' represents X-ray wavelength i.e. 0.154 nm, 'θ' is the Bragg angle, 'β' is FWHM and 'k =*0.9*' denotes the shape factor [16] which gave the 19 mm mean size for the AgNPs produced by *L. glutinosa* leaf and plant extracts respectively.

SEM Analysis

For the colloidal AgNP preparations, the SEM images of the samples arranged at 80°C, substantiate the survival of small and uniformly spherical NPs. From the SEM images, larger particles of AgNPs formed due to the aggregation of NPs can be observed resulting from the solvent fading. SEM images also show AgNPs with a particle size ranging from 10 to 40 nm with well-disseminated spherical shapes as in (Fig.3). The EDS images also reveal the presence of elemental Ag gesture established in the sample by different X-ray emission peaks (Fig. 4). The AgNPs-crystallites present an optical assimilation band showing a peak at 3 keV which is emblematic of the combination of metallic AgNP-crystallites. Earlier similar explanations are reported [17, 18].

FT-IR Spectroscopy

By FT-IR spectroscopy, the chemical composition of the AgNPs surface is characterized. As can be seen in Fig.5, the peaks at 3416 cm^{-1} and

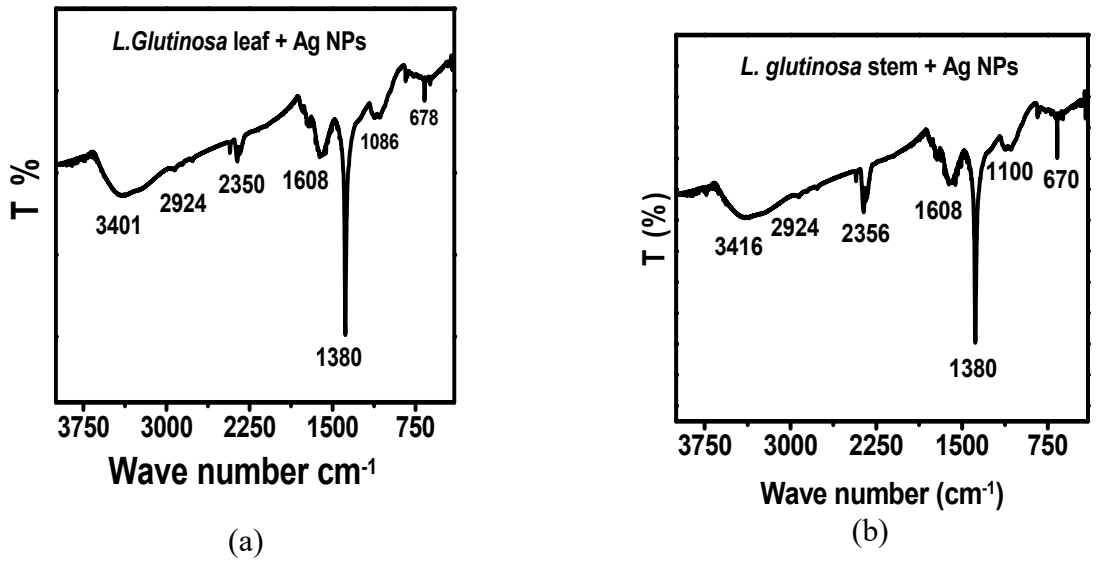


Fig.5. FT-IR spectral analysis of *L.glutinosa.* a) Leaf and b) Stem AgNPs

3401 cm^{-1} point toward the incidence of N-H and O-H groups of plant extract and water components. The peaks at 2924 cm^{-1} can be accredited to the stretching pulsation of the C-H group [19]. The bands from 2350 cm^{-1} to 2356 cm^{-1} are due to the presence of aldehydic C-H stretching and 1608 cm^{-1} keeps up a correspondence to the carbonyl group's precise assimilation. The band at 1380 cm^{-1} corresponds to C-N elongating ambiance of aromatic amine and the C-O-C groups bending vibrations relevant peaks are observed at 1086 cm^{-1}.

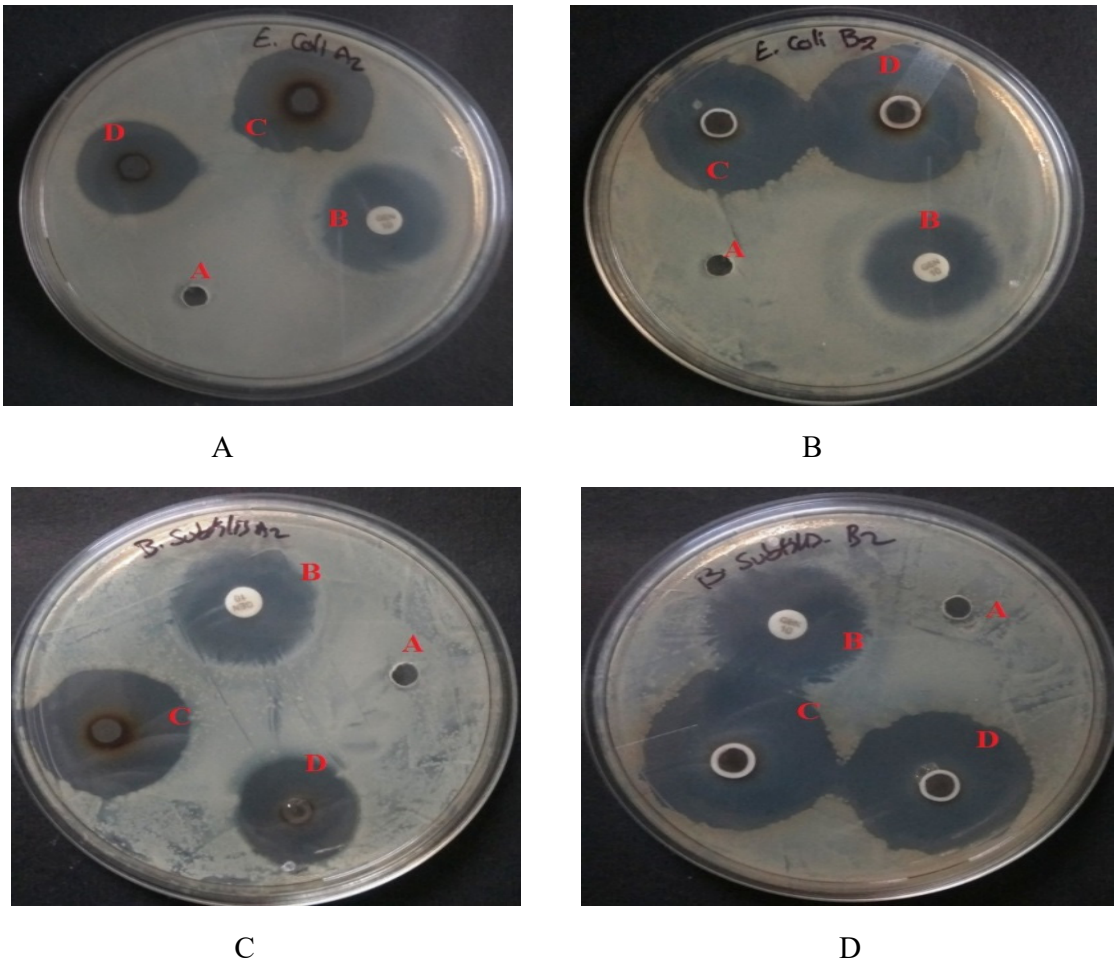
With this, the peaks at 1086 cm^{-1}, and 1100 cm^{-1} are related to -C-O-C and C-OH stretching [20]. With the outcomes long-established the adsorption of glycosides, flavonoids, and carbohydrates, of plant extract on the exterior of AgNPs, implies their vital role in the Ag^{+} reduction reaction. The earlier phytochemical analysis also identified similar components in the extracts of *L.glutinosa* [21].

Antibacterial Activity

By performing disk diffusion assay, the antibacterial potential of AgNPs is examined, which has shown improved well-defined zone of inhibition (diameter in mm) against *E.coli* (Fig. 6-A and B; Table-1) and *B.subtilis* (Fig. 6-C and D; Table-1). The individual *L. glutinosa* leaf and stem extracts did not show any zone of inhibition, indicating their insignificant pressure over the antibacterial effect. Whereas, the isolated AgNPs that are synthesized by *L. glutinosa* leaf and stem extracts showed idiosyncratic elevated zones of inhibition, indicating effective antibacterial activity. Against *B. subtilis*, the 5, 10 µg AgNPs prepared with the leaf and stem extracts of *L. glutinosa* and positive control (10 µg Gentamycin) showed significantly higher zone of inhibition values, in comparison to the untreated control ($P < 0.001$).

The stem-extracted AgNPs induced slightly advanced values of a zone of inhibition in comparison to that of both leaf-extracted ones and positive control. Against *E.coli* also, the 5, 10 µg AgNPs prepared using leaf and stem extracts of *L. glutinosa* and positive control (10 µg Gentamycin) showed significantly higher zones of inhibition in comparison to the untreated control ($P < 0.001$). Overall, the observations designate higher and probable antibacterial activity of stem-extracted AgNPs to that of leaf-extracted AgNPs and positive control. These explanations are in concord with previous reports that showed the antibacterial activity of AgNPs [22; 23].

The smaller size and higher surface-to-volume ratio of AgNPs produced from *L. glutinosa* leaf and stem aqueous extracts could attribute to their strong interaction with the microbial membrane. The inhibition of bacterial cell development by AgNPs involves the release of silver ions, which act together with the thiol groups of cellular enzymes [21]. The AgNPs' superior antibacterial action against gram-positive bacteria might be because of the presence of a thin, easily broken peptidoglycan layer thus making their penetration into the cell wall of bacteria easy [3].



A B C D

Fig. 6. Antibacterial activity of AgNPs. A& B. AgNPs prepared using leaf and stem extracts of *L. glutinosa* applied on *E. coli* culture plates. C & D. AgNPs prepared using leaf and stem extracts of *L. glutinosa* applied on *B. subtilis* culture plates.

Table 1. Zone of inhibition values for AgNPs prepared using leaf and stem extracts of *L. glutinosa*

Sample (in µg)		Zone of inhibition (mean ± SD) in mm	
		B.subtilis	*E.coli*
Untreated		-----	-----
AgNPs prepared with leaf extract of *S. anacardium*	5	27 ± 3*	24 ± 3*
	10	32 ± 3*	30 ± 3*
AgNPs prepared with stem extract of *S. anacardium*	5	32 ± 3.61*	32 ± 3*
	10	35 ± 2*	35 ± 3*
Gentamycin	10	21 ± 2*	26 ± 2.65*

Significant difference of treated samples versus untreated control determined by Dunnett's post test. * = P < 0.001.

CONCLUSIONS

A greener approach was employed in this study to obtain AgNPs using the leaf and stem aqueous extracts of *L.glutinosa* provided an advantage of smaller size and elevated surface area to volume ratio. Analytical sophisticated instruments were used in this study to attain optical, spectroscopic properties of prepared Ag NPs. They demonstrated impending bactericidal activity against *B.subtilis* and *E.coli.* The *L. glutinosa* stem extract synthesized AgNPs have shown significant antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) in comparison to Gentamycin. The application of green synthesized AgNPs possessing antibacterial action in medical therapies is beneficial over conventional schemes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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