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Green Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles from the Extract of *Lagerstroemia speciosa*

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ABSTRACT

Lagerstroemia speciosa is commonly known as Banaba or Jarul which is used to get rid of various ailments such as fever, urinary infection, decongestion, diarrhoea, mouth ulcers, astringent, diabetes mellitus, kidney diseases, abdominal pains etc. The present work, describes the green synthesis of silver nanoparticles from ethanolic extract of fruits of *L. speciosa* (Ls-Ag NPs) and their analysis for antimicrobial activities. The characterisation of so obtained nanoparticles have been carried out with help of Field emission scanning electron microscopy (FESEM) and High-resolution transmission electron microscopy (HRTEM). Further, antimicrobial activities of ethanolic extract of fruits of *L. speciosa* (Ee-Ls), silver oxide (Ag_2O) and Ls-Ag NPs have been examined by using well-diffusion method against two bacterial strains: *Staphylococcus aureus* (*Gram-positive*) and *Escherichia coli* (*Gram-negative*) and one fungal strain: *Aspergillus niger*. It has been observed that the biosynthesized Ls-Ag NPs possess much effective antimicrobial activity against selected strains as compared to Ee-Ls and Ag_2O.

Keywords: *Lagerstroemia speciosa* fruits, Silver nanoparticles, Green synthesis, Antimicrobial activity.

INTRODUCTION

Advances in synthesis of nanoparticles from various substances and their applications have led no research area to be remained untouched towards new verdicts day by day. Nanoparticles of different materials have shown broad applications in the field of synthesis, chemicals, pharmaceuticals, health care, optics, environmental, food, mechanical, manufacturing, materials industries and many more[1-4]. The shape and size of nanoparticles depends upon working strategies and methods of their synthesis (chemical, physical and biological)[5-8]. The chemical and physical methods are not environment friendly due to the use of toxic organic solvents, reducing/ stabilizing agents; production of hazardous

by-products and high energy consumption[9-11]. Green synthesis has been identified as one of the alternate to replace these methods because of its large quantity capability, eco-friendly, cost-effective, simple work procedure[12-14].

Many researchers have proved that plants have effective potential towards green synthesis approach of metal nanoparticles[15-17] because of secondary metabolites. The secondary metabolites include alkaloids, essential oils, flavonoids, phenols, terpenoids etc.[18-20] and these act as reducing and capping agent for the formation of metallic nanoparticles[21,22]. The plant extract and its nanoparticles with metals (silver, gold, platinum, titanium, zinc, cerium, iron and thallium) have shown several biological activities such as anti-arthritic, antidiabetic, anti-inflammatory, antimicrobial, anti-nociceptive, anti-obesity, anti-oxidant, cytotoxicity and so on[23-26]. However, metal nanoparticle modification of crude extract has resulted into enhanced activity as compared to crude alone[27-30].

Lagerstroemia speciosa is commonly known as Banaba, Jarul, Queen of flowers, Crepe myrtle, Pride of India, belongs to Lythraceae family having more than 50 species[31-33]. Various parts of *L. speciosa* viz., fruits, leaves, bark, roots have been used as a traditional medicine to treat several diseases. The synthesis of nanoparticle from the fruits of this plant and study of their antimicrobial activities have not been reported till date. However, synthesis of silver[34,35] and zirconium oxide nanoparticle[36,37] from aqueous extract of *L. speciosa* leaves and analysis their antimicrobial, biofilm, photocatalytic and cytotoxicity activities[34-37] have been carried out so far. In the present study, we have synthesized the silver nanoparticles from ethanolic extract of fruits of *L. speciosa* (contains reducing/ stabilizing agent) through green synthesis method and have compared the activities of Ee-Ls, Ag_2O, and Ls-Ag NPs. Ag NPs are found to be better biologically active to that of Ee-Ls and Ag_2O.

MATERIALS AND METHODS

All the chemicals (ethanol, silver oxide and silver nitrate) have been purchased from Sigma-Aldrich. The Agar-agar powder, Nutrient broth, and Potato dextrose agar (Hi-media) were used for microbial culture. The standard drugs, Ciprofloxacin (for antibacterial) and Fluconazole (for antifungal) had purchased from Local Retail Pharmacy Shop.

Plant materials

The fruits of *L. speciosa* were collected from campus garden of Maharishi Markandeshwar University, Sadopur (Ambala) in October, 2018. The authentication of the plant has been confirmed from Department of Natural Products in National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India. A voucher specimen number NIP-H-275 has been preserved for further verification.

Preparation of the extract

The air-dried fruits (1 kg) have been extracted with ethanol (60-70°C) by using a soxhlet apparatus for 72 h as per standard procedure[38]. After the completion of extraction, liquid was concentrated at 40-60°C by rotary evaporator till the saturated mixture obtained and then evaporated on a water bath at 40-50°C to gotten crude extract. The obtained crude extract (46 g) was kept in the refrigerator at 4°C in glass vials for further analysis.

Secondary metabolites analysis of the Ee-Ls

The secondary metabolites analysis of crude extract has been done by their specific confirmatory tests for alkaloids, carbohydrates, fat-oils, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins, and terpenoids[39-41]. The presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, steroids, tannins and terpenoids were shown in Table 1.

Preparation of the Ls-Ag NPs

Green synthesis method has been adopted to the preparation of Ls-Ag NPs. The reaction mixture was prepared by mixing 20 mL of ethanol extract with 180 ml of $AgNO_3$ (1.0 mM) solution in a 250 mL of the conical flask for reduction of Ag^+ to Ag^0 ions. The mixture has been stirred continuously on the hot plate magnetic stirrer at 60-70°C 1 hour. The colour of solution changed from pale yellow to dark brown, indicate the nanoparticles have been prepared[34, 37].

Purification of nanoparticles

To separate the non-metal components from the Ls-Ag NPs, coloured suspension was centrifuged at 6000 rpm for 20 minute. The ethanol layer of prepared Ls-Ag NPs was collected carefully

and dispersed with ethanol. This process was repeated thrice to separate the entities from metal nanoparticles. The centrifugation process was done by laboratory centrifuge (REMI India). To prevent the agglomeration of the ions, the purified solution of Ls-Ag NPs was kept for sonication after treating with ethanol for 10 minute[37].

Characterization of the Ls-Ag NPs

High-resolution transmission electron microscopy analysis

The size and shape of the prepared Ls-Ag NPs have been studied using High-resolution transmission electron microscopy. The sample has been carried out on an HRTEM, JEOL-2100 plus microscope, working at an acceleration voltage of 200 kV. It prepared by prior dispersion in ethanol at the appropriate concentration and placing a small drop of solution on a carbon-coated copper grid. After 2 min of deposition of the film on a TEM grid, the excess solution has been removed using a blotting paper and the grid was allowed to dry for overnight at room temperature to measurement.

Field emission scanning electron microscope analysis

The surface texture and morphology of the synthesis Ls-Ag NPs have been characterized by using Field emission scanning electron microscope, Hitachi FE 8010. A thin film of the sample has been prepared on a platinum-coated carbon tape by dropping a small amount of the sample on a grid, an extra sample has been removed, and then the film on the SEM grid was allowed to dry under a mercury lamp for 5 minute.

Antimicrobial assay

The antimicrobial analysis of the Ee-Ls, Ag_2O and Ls-Ag NPs have been confirmed by well-diffusion method against selected *Gram-positive* (*Staphylococcus aureus*) and *Gram-negative* (*Escherichia coli*) bacteria as well as pathogenic fungus *Aspergillus niger*. For the culturing of bacterial and fungal strains, Nutrient broth (NA) and Potato dextrose agar (PDA) were used as media. Loops full of all the bacterial and fungus cultures were inoculated in nutrient at 37°C for 24-48 h and in potato dextrose at 27°C for 48-72 hours[43-45].

Through serial dilution method, different concentrations (100, 200, 400 ppm) of samples Ee-Ls, Ag_2O and Ls-Ag NPs have been prepared in DMSO and compared with standard antibiotic Ciprofloxacin (100 ppm) for bacterial and Fluconazole (100 ppm) for fungal assay. The respective solvent (sterile DMSO) has been used as a negative control. The freshly prepared inoculums (108 CFU/mL) of each test bacterium spread on the sterile petri plates. The plates were allowed to dry, then four wells were bored having 7 mm diameter using sterile cork-borer and were labelled properly[46]. Subsequently, 40 μL of each dilution were added in triplicate wells using microtiter-pipette and plates were allowed to stand at least 1 h for diffusion to take place. The plates were then incubated in an upright position at 37°C for 24 h for bacterial analysis and for fungal assay the plates have been incubated at 27°C for 72 hours. The results were evaluated by measuring the width of the zone of inhibition growth against the selected microorganisms in comparison with antibiotics (Ciprofloxacin and Fluconazole) and mean values were tabulated.

RESULTS

Secondary metabolites analysis of the Ee-Ls

In the present investigation, the secondary metabolites analysis has been done for Ee-Ls fruits by their specific confirmatory tests[39-41]. The presence of secondary metabolites are shown in Table 1.

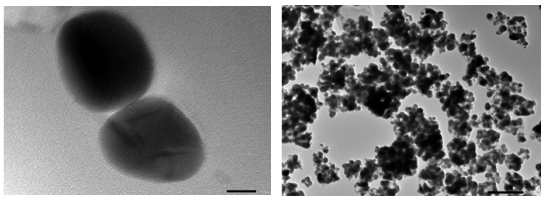
Table 1: Secondary metabolites analysis of the Ee-Ls

Phytochemical Constituents	Ee-Ls
Alkaloids	-
Carbohydrates	+
Fats/oils	-
Flavonoids	-
Glycosides	-
Phenols	+
Proteins	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	-

'+' indicates presence, '-' indicates an absence

High-resolution transmission electron microscopy

High-resolution transmission electron microscopy performed to acquire the size and shape of the synthesized Ls-Ag NPs, shown in Fig. 1 (1a and 1b). The average particle size of Ls-Ag NPs was calculated around 50-60 nm. The eclipsed shape of Ls-Ag NPs was appeared in the characterized TEM images.



1a 1b

Fig. 1. HRTEM images of Ls-Ag NPs from Ee-Ls (a) scale 20 nm, (b) scale 500 nm

Field emission scanning electron microscope

The FESEM analysis explain the surface texture and morphology of the synthesis Ls-Ag NPs shown in Fig. 2. The microscope image prove that Ls-Ag NPs are in nanoscale range and have uniform distribution.

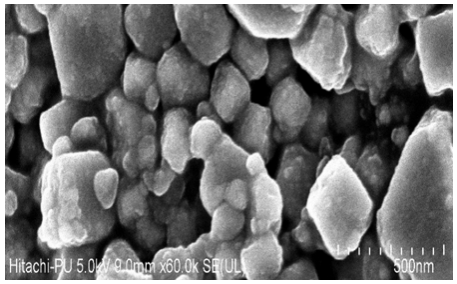


Fig. 2. FESEM image of Ls-Ag NPs from Ee-Ls; scale 500 nm

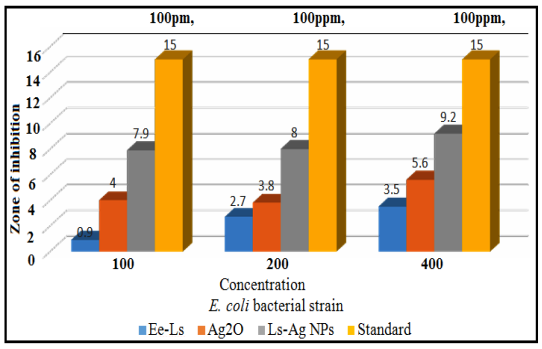
Antimicrobial activity

In the presence study, the antimicrobial activity of synthesis Ls-Ag NPs compared with Ag_2O and Ee-Ls at concentration (100, 200, 400 ppm) by well-diffusion method against *Staphylococcus aureus* (*Gram-positive*) and *Escherichia coli* (*Gram-negative*) bacterial strains and one fungal strain viz. *Aspergillus niger*. The Ee-Ls, Ag_2O, and Ls-Ag NPs inhibited the growth of all tested microorganisms with a zone of inhibition range from 0.9±0.1 to 5.7±0.2mm, 3.62±0.2 to 7.3±0.2mm and 7.5±0.2 to 10±0.2mm for bacterial strains and fungal strain the range of zone of inhibition was 0.5±0.1 to 3.4±0.2mm, 1.5±0.2 to 4.3±0.2mm and 4±0.1 to 6.1±0.1mm. The Ls-Ag NPs have been shown effective results against selected bacterial (*E. coli* and *S. aureus*) and fungal strain (*A. niger*) shown in Table 2 and Fig. 3. The Ls-Ag NPs have been exhibited the maximum zone of inhibition against *Gram-positive* (*S. aureus*; 10±0.2 mm) bacterial strains compared with *Gram-negative* (*E. coli*; 9.2±0.2 mm) bacterial strains at 400 ppm concentration. For fungal strain, Ls-Ag NPs have been observed maximum zone of inhibition (*A. niger*; 6.1±0.2 mm) at 400 ppm concentration.

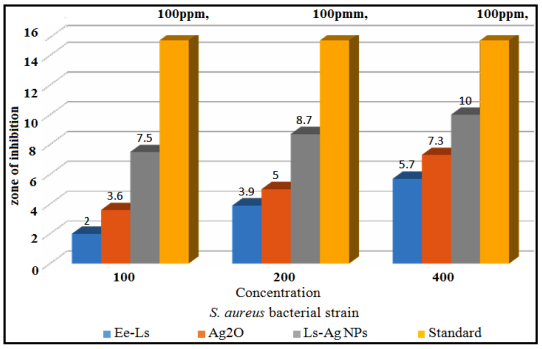
Table 2: Antimicrobial activity of Ee-Ls, Ag_2O, and Ls-Ag NPs against different antimicrobial organisms

Test organis ms	Different concentra tions (ppm)	Ee-Ls (mm)	Ag_2O (mm)	Ls-Ag NPs (mm)	Ciprofl oxacin	Flucon azole (100 ppm)	DMSO (100 ppm)
				Zone of inhibition			
Bacterial strains							
E. coli	100	0.9±0.1	4±0.2	7.9±0.2	15±0.3	----	NO
	200	2.7±0.2	3.8±0.2	8±0.2			
	400	3.5±0.2	5.6±0.2	9.2±0.2			
S. aureus	100	2±0.2	3.6±0.2	7.5±0.2	15±0.3	----	NO
	200	3.9±0.2	5±0.2	8.7±0.2			
	400	5.7±0.2	7.3±0.2	10±0.2			
Fungal strains							
A. niger	100	0.5±0.1	1.5±0.2	4±0.1	----	9.5±0.2	NO
	200	2.5±0.1	3.5±0.1	5.7±0.1			
	400	3.4±0.2	4.3±0.2	6.1±0.2			

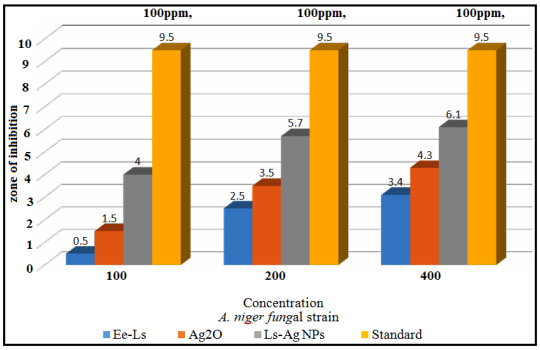
'NO'-not observed



3a



3b



3c

Fig. 3. Histogram of Antimicrobial activity of Ee-Ls, Ag_2O, and Ls-Ag NPs against different antimicrobial organisms (3a) *E. coli*; (3b) *S. aureus*; (3c) *A. niger*

DISCUSSION

In the present study, the prelims phytochemicals i.e., carbohydrates, phenols, proteins, saponins, and tannins have been observed in Ee-Ls while alkaloids, Fats/oils, flavonoids, glycosides, steroids, terpenoids have been found to be absent[39-41]. The secondary metabolites quantified in this analysis have a great deal of biological effects and pharmacological properties[49]. These prelims phytochemicals have been observed from the different parts (fruits, leaves and roots) of *L. speciosa*[53-55]. Therefore, the carbohydrates, phenols, proteins, saponins and tannins present in Ee-Ls are the most effective biomolecule in synthesis of Ls-Ag NPs[50, 51].

The morphology of Ls-Ag NPs has been observed by HRTEM analysis. The Ls-Ag NPs have been detected the eclipse in shape with 50-60 nm of average particle size. The prelims constituent's viz. carbohydrates, phenols, proteins, saponins, and tannins have been reported as potential reducing/ stabilizing agent in the synthesis of Ls-Ag NPs[47,48]. According to Saraswathi and Santhakumar (2017), the presence of tannins will help to cap the zirconium oxide nanoparticles and shown photocatalytic activity[37]. Likewise, the carbohydrates content reflects the capping properties of the extract[52]. Saraswathi *et al.,*(2017) have been reported that the formation of silver nanoparticles by using secondary metabolites (tannins, phenols and flavonoids) which act as a reducing agent during synthesis and shown biofilm activity against selected *Pseudomonas aeruginosa* clinical strains[35].

The surface texture and morphology of synthesis Ls-Ag NPs have been explained by using the FESEM analysis. The SEM images have been gathered on to the surface due to the hydrogen bond and electrostatic interactions between the bio-organic capping molecules bound to the Ls-Ag NPs[56]. Similar spectacle has been reported, where the SEM morphology shows crystalline spherical Ag NPs[34].

The antimicrobial activity of synthesis Ls-Ag NPs compared with Ag_2O (uncapped Ee-Ls) and Ee-Ls against selected two bacterial strains i.e., *Staphylococcus aureus* (*Gram-positive*) and *Escherichia coli* (*Gram-negative*) and one fungal strain viz. *Aspergillus niger*. Result shows that the synthesis Ls-Ag NPs have been enhanced antimicrobial activity than Ag_2O and Ee-Ls, similar result has been observed by Elumalai and Velmurugan (2015)[57]. The possible reason due to the activity depends on the particle size, morphology, specific surface area and presence of phytochemicals components. In green synthesized Ls-Ag NPs, Ee-Ls fruits act as capping agent surround around the Ls-Ag NPs to reduce the size of particles and enriches the antimicrobial properties of particles[58]. Sundararajan and kumara (2014) have been reported that the synthesized Ag NPs showed more effective antimicrobial results than that of the *L. speciosa* leaves extract [34]. Hence, we can say that the synthesis Ls-Ag NPs are the useful and effective agent against bacterial and fungal pathogens, which will be more specific and cost-effective.

CONCLUSION

In summary, the silver nanoparticles have been synthesised by using the green method. The analysis of secondary metabolites has revealed the presence of carbohydrates, phenols, proteins, saponins and tannins in ethanolic extract of fruits of *L. speciosa* (Ee-Ls). These metabolites act as reducing agents, thereby these reduce the metal ions into nanoparticles (Ls-Ag NPs) in environment friendly manner while Ee-Ls stabilises the nanoparticles and hence belongs to green synthesis method. Their morphological studies have

revealed particles size of 50-60 nm with eclipsed shape. The biosynthesized Ls-Ag NPs possess much effective antimicrobial activities against two bacterial strains: *Staphylococcus aureus* (*Gram-positive*) and *Escherichia coli* (*Gram-negative*) and one fungal strain: *Aspergillus niger* as compared to Ee-Ls and Ag_2O. These findings seem to be imperative from environmental, pharmaceutical and therapeutic point of view.

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Conflict of interest

The authors declare that they have read policy and guidelines of the journal and there is no conflict of interest.

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