Synthesis, Characterization and Antibacterial Properties of Silver Nanoparticles Fabricated Using the Fruit Extract of *Lagerstroemia Speciosa*

Ramesh Vinayagam[a], Raja Selvaraj[a] and Thivaharan Varadavenkatesan[b]*

[a]*Department of Chemical Engineering, Manipal Institute of Technology (MIT), Manipal Academy of Higher Education (MAHE), Manipal, Karnataka- 576104, India*
[b]*Department of Biotechnology, Manipal Institute of Technology (MIT), Manipal Academy of Higher Education (MAHE), Manipal, Karnataka- 576104, India, E-mail:* thivaharan.v@manipal.edu

Abstract

Nanoparticle synthetic protocols have witnessed a sea change of approaches involving physical, chemical and more recently, the eco-friendly "green" synthesis methods. Owing to cost-effective and mechanistically simple reasons, plant-derived sources are a major route for the synthesis of nanoparticles. Silver has a long-standing history of anti-microbial properties and in the current study, silver nanoparticles were fabricated using the aqueous extract of *Lagerstroemia speciosa* fruits. During the synthesis, a visible change of colour of the fruit extract to golden brown underlined the formation of silver nanoparticles, that showed a unique peak at 423 nm when analyzed using a UV–vis spectrophotometer. At a magnification of 35 kX, scanning electron microscopy revealed spherical silver nanoparticles of sizes less than 100 nm. Spectral analysis using electron diffraction underscored the presence of zero-valent silver in the sample. The crystalline nature of the nanoparticles was verified from the X-ray diffraction patterns that depicted a discrete peak at 38.79°, that corresponded to the (1 1 1) crystal plane, with a crystallite size of 17.38 nm. The average hydrodynamic diameter of the silver nanoparticles was recorded at 61.12 nm and was proven to be monodisperse as the polydispersity index was 0.225. The negative zeta potential value of –11 mV accounted for maintaining the stability of the colloidal silver nanoparticles in solution. Analysis using Fourier Transform infrared spectroscopy corroborated the dual roles of reduction and stabilization, pertaining to the phenolic compounds arising from the fruit extract. The silver nanoparticles portrayed antibacterial activity against a test Gram-positive microbe. The study thus serves as yet another source for the derivation of silver nanoparticles using a novel plant source, with antibacterial potential.

Keywords: Lagerstroemia speciosa; Silver nanoparticles; Green synthesis; Antibacterial Activity

1. Introduction

The impact of nanobiotechnology in different areas has risen due to the multitude of advantages, that the nanoparticles extend. Synthesis of nanoparticles employing ecofriendly techniques are necessary, as the Earth struggles with the barrage of pollutants, in all the spheres of life. Under this backdrop, naturally-sourced plant samples have huge potential to act as both stabilizing and reducing agents, when metal nanoparticles are synthesized (Kumar, Sharon & Choudhary, 2010)

Silver is relatively cheaper when compared against the remaining noble elements. Thus, silver nanoparticles (SiNPs) constitute the most researched metal atom and has found its way in varied areas like cosmetic (Borase *et al*, 2013) and food-based (Chaudhry & Castle, 2011) industries. The body of literature surrounding the green synthesis of SiNPs using plant-based extracts, like, *T. purpurea* (Ajitha, Reddy & Reddy, 2014) and *D. denudatum* (Suresh *et al*, 2014) underline the said fact.

To fabricate nanoparticles, many physical, chemical and biological methods are usually employed. Nowadays, an eco-friendly approach called "green synthesis" is fast emerging due to its inherent characteristics like the use of natural resources, simpler protocols, rapidity, lower costs, stability, less toxicity of reagents etc., The extracts from different plant parts have gained mileage due to the fact plant biomolecules have the dual potential of forming and stabilizing the nanoparticles.

The plant, *Lagerstroemia speciosa*, regionally known as "*Banaba*" fits in the *Lythraceae* family of flowering plants. It is made use of to eradicate hyperglycaemic conditions in the Philippines, also sometimes doubling up as decorative ornamental flora (Gardea-Torresdey *et al*, 2003). The plant has a whole range of bioactive compounds like alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and many vitamins (Vidhu & Philip, 2014). The plant was chosen for this study owing to its multi-functional clinical features like anti-obesity (Sai *et al*, 2017), and anti-arthritic (Liu *et al*, 2001) activities. The extract from the fruits borne by the plant are utilized for the transformation of monovalent silver ions to zerovalent SiNPs.

The current experimental work aimed to fabricate the SiNPs using the fruit extract of *L. speciosa* and characterize them using a plethora of techniques. Finally, the synthesized SiNPs were checked and examined for potential antibacterial property.

2. Materials and Methods

2.1. Materials and Preparation of *Lagerstroemia Speciosa* Fruit Extract (LSFE)

Freshly-sourced fruits of *Lagerstroemia speciosa* were obtained from our University lawns. They were then cleaned properly with tap water and then with double-distilled water. The fruits were sliced and dried under shade. 10 g of the cut pieces were then added to 0.1 L of double-distilled water, taken in a beaker. The contents were heated in boiling water for a duration of 10 min. Post-cooling the water, a simple filtration step was performed to obtain *L. speciosa* fruit extract (LSFE). The extract was stored at a temperature of 4°C, before being used further.

2.2. Synthesis of SNPs (LS–SNPs)

To obtain *L. speciosa*-derived silver nanoparticles (LS-SNP), LSFE and $AgNO_3$ (1 mM) were mixed in 1:9 v/v ratio. The contents were heated to 90°C for 15 min. The change of colour indicated the realization of LS-SNP.

2.3. Characterization

UV-visible spectrophotometry helped in recording the absorption spectrum of LS-SNP. Morphological and size-based analysis of the LS-SNP was done with scanning electron microscopy (SEM). The elemental constitution was verified with energy dispersive X-ray (EDX) approach. To verify its purity and phase realization, X-ray diffraction (XRD) analysis of LS-SNP was carried out. Zeta potential and analysis of

SiNPs' size were derived by recording dynamic light scattering (DLS) measurements. The chemical moieties in the sample were recognized using Fourier Transform Infrared (FTIR) analysis.

2.4. Antibacterial Activity

The antibacterial nature of LS-SNP was evaluated by performing standard well-diffusion assays against *Staphylococcus aureus*, prepared in nutrient broth. Sterile nutrient agar plates were prepared, onto which circular wells were punctured. Samples of the test LS-SNP samples were loaded onto the wells and placed in an incubator at 37 °C for 12-18 h. Zone of inhibition, if any, was quantified.

3. Results and Discussions

The mixing of LSFE to the aqueous $AgNO_3$ and then heating led to color change from pale brown to dark brown-black colour (Fig. 1 inset) and this implied the manifestation of LS-SNP. Surface plasmon vibration pattern portray a peak in 425–460 nm range, when analyzed spectrophotometrically (Swamy *et al*, 2015). The observed band at 423 nm verified the realization of SiNPs (Fig. 1).

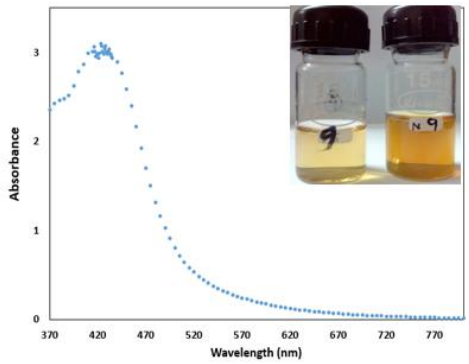

Fig.1 UV-vis spectrum of LS-SNP (Inset shows the color transformation of *Lagerstroemia speciosa fruit* extract to SiNPs).

Imagery obtained using SEM (Fig. 2A) showcased the nano-size of the LS-SNPs and displayed their spherical morphology. A strong 3.1 keV signal emanated, as seen from the EDX analysis (Fig. 2B). Such a strong peak in the range of 2.5-3.5 keV in a typical EDX spectrum is indicative of zerovalent silver (Chunfa *et al*, 2016).

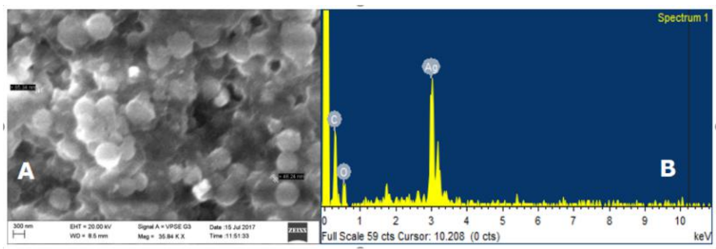

Fig. 2 (A) SEM Image and ((B) EDX Analysis of LS-SNP.

The crystalline property of the SiNPs was verified from XRD (Fig. 3-Left) that depicted a discrete peak at 38.79°, that corresponded to the (1 1 1) crystal plane, with a crystallite size of 17.38 nm. This is in agreement with JCPDS file No. 87-0719 Netala *et al*, 2016). The average hydrodynamic diameter of the LS-SNPs was recorded at 61.12 nm (Fig.4-Right) and was proven to be monodisperse as the polydispersity index was 0.225. The negative zeta potential (–11 mV) accounted for maintaining colloidal stability of SiNPs in solution. A strong electrostatic repulsive interaction between the nanoparticles led to elevated stability of the sample, thereby hampering the SiNPs from getting agglomerated in the medium (Gopalakrishnan & Raghu, 2014).

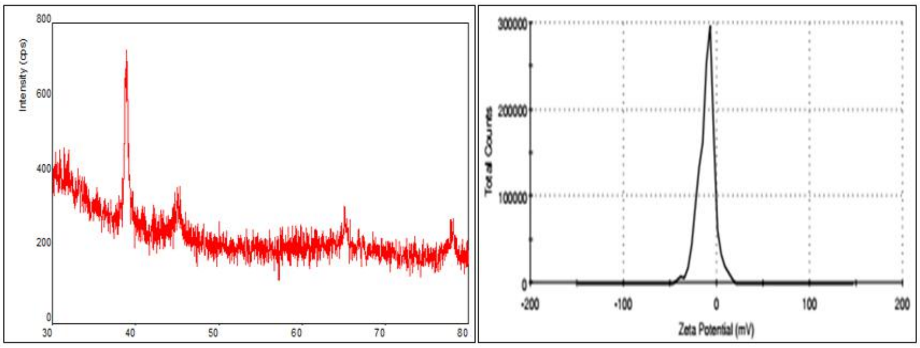

Fig. 3 Left: XRD Analysis of LS-SNP and Right: Zeta potential measurement of LS-SNP by DLS.

Analysis using Fourier Transform infrared spectroscopy corroborated the dual roles of reduction and stabilization, pertaining to the phenolic compounds arising from the fruit extract. Strong bands were seen at 3579, 1728, 1612, 1380, 1195, 1041, 825 and 671 cm^{-1} (Fig. 4). This corroborated the incidence of amino groups, amides, carboxyl and poly phenols, accountable for reduction mechanism as well as capping effect of the LS-SNPs (Pernia, Hakalaa & Prokopovichab, 2014)

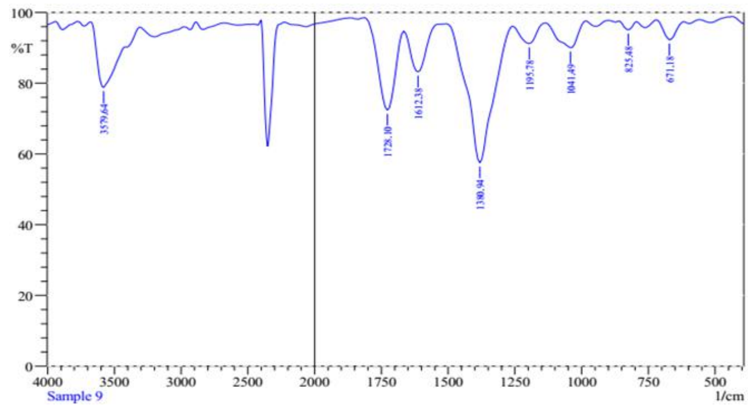

Fig. 4 FTIR analysis of LS-SNP.

The SiNPs portrayed antibacterial activity against the tested Gram-positive microbe (Fig. 5). Silver ions are known to be toxic to bacterial specimens. The antibacterial action of the LS-SNPs was put to test against *S. aureus* by employing well-diffusion protocol. The observed zone of inhibition of 16 mm proved the anti-bacterial potential of the LS-SNPs. The structural disturbances caused to the cell membrane and the resulting unregulated movement of species across the membrane is thought to kill the bacterial cells (Sondi & Sondi, 2004). The study thus serves as yet another source for the derivation of SiNPs using a novel plant source, with antibacterial potential.

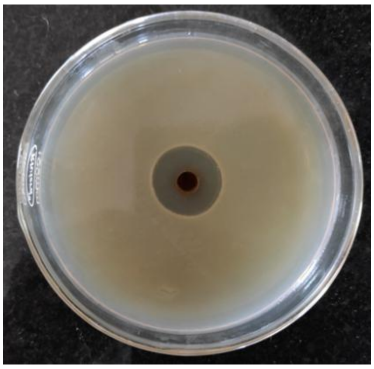

Fig. 5 Antibacterial Activity of LS-SNPs.

4. Conclusions

The study validated that the green synthesis SiNPs using aqueous extract of *Lagerstoemia speciosa* fruits. The synthesized LS-SNP were characterized by suitable analytical approaches. Further, the anti-bacterial property of LS-SNP showed good promise against. So, the work presents a rapid, cost-friendly and eco-balanced approach for the realization of SiNPs and their application as a good means to curb anti-bacterial attack.

5. References

Kumar, R., Sharon, M., and Choudhary, A.K. (2010) Nanotechnology in agricultural diseases and food safety, *J. Phytol.,* 2, 83–92.

Borase, H.P., Patil, C.D., Suryawanshi, R.K., and Patil, S.V. (2013) *Ficus carica* latex-mediated synthesis of silver nanoparticles and its application as a chemophotoprotective agent, *Appl. Biochem. Biotechnol.*, 171, 676–688.

Chaudhry, Q. and Castle, L. (2011) Food applications of nanotechnologies: An overview of opportunities and challenges for developing countries, *Trends Food Sci. Technol.* 22, 595–603.

Ajitha, B., Reddy, Y.A.K., and Reddy, P.S. (2014) Biogenic nano-scale silver particles by *Tephrosia purpurea* leaf extract and their inborn antimicrobial activity, *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc*., 121, 164–172.

Suresh, G., Gunasekar, P.H., Kokil, D., Prabhu, D., Dinesh, D., Ravichandran, N., Ramesh, B., Koodalingam, A. and Siva, G.V. (2014) Green synthesis of silver nanoparticles using *Delphinium denudatum* root extract exhibits antibacterial and mosquito larvicidal activities, *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.*, 127, 61–66.

Gardea-Torresdey, J.L., Gomez, E., Peralta-Videa, J., Parsons, J.G., Troiani, H.E. and Jose-Yacaman, M. (2003) Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles, *Langmuir*, 19, 1357–1361.

Vidhu, V.K. and Daizy Philip, (2014) Catalytic degradation of organic dyes using biosynthesized silver nanoparticles, *Micron*, 56, 54–62.

Sai Saraswathi, V., Tatsugi, J., Shin, P.K. and Santhakumar, K. (2017) Facile biosynthesis, characterization and solar assisted photocatalytic effect of ZnO nanoparticles mediated by leaves of *L. speciosa*, *J. Photochem. Photobiol. B*, 167, 89–98.
Liu, F., Kim, J.K., Li, Y., Liu, X, Li, J. and Chen, Z (2001) An extract of *Lagerstroemia speciosa* L. has insulin like glucose uptake- stimulatory and adipocyte differentiation inhibitory activities in 3T3 –L1 cells, *Am Soc. Nutr. Sci.*, 131, 2242–2247.
Swamy, M.K., Sudipta, K. M., Jayanta, K. and Balasubramanya, S. (2015) The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract, *Appl Nanosci,* 5, 73–81.
Chunfa, D., Xianglin, Z., Hao, C., and Chuanliang, C. (2016) Sodium alginate mediated route for the synthesis of monodisperse silver nanoparticles using glucose as reducing agents, *Rare Metal Mat Eng.*, 45, 261-266.
Netala, V.R., Kotakadi, V.S., Bobbu, P., Gaddam, S.A. and Tartte, V. (2016) Endophytic fungal isolate mediated biosynthesis of silver nanoparticles and their free radical scavenging activity and antimicrobial studies, *3 Bio*, 6, 132.
Gopalakrishnan, R., and Raghu, K. (2014) Biosynthesis and characterization of gold and silver nanoparticles using milk thistle (*Silybum marianum*) seed extract. *J Nanosci*, 2014, 1-8.
Pernia, S, Veera Hakalaa, V. and Prokopovichab, P. (2014) Biogenic synthesis of antimicrobial silver nanoparticles capped with L-cysteine. *Colloids Surf A: Physicochem Eng Aspects*, 460, 219-224.
Sondi, I and B.S. Sondi, J. (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Colloid Interface Sci*, 275, 177–182.