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By

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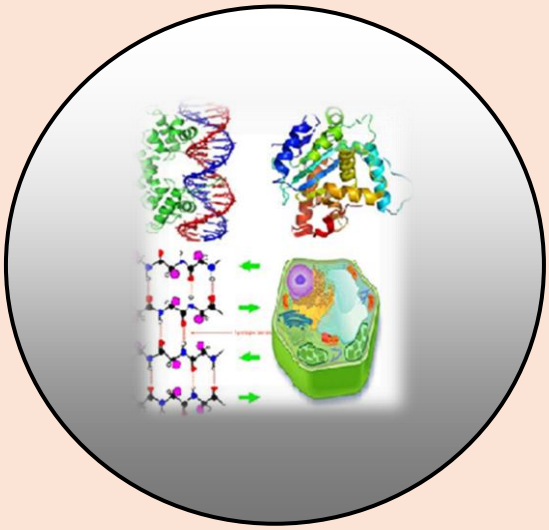

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Plant Mediated Synthesis of Silver Nanoparticles from *Loeseneriella arnottiana* Wight and its Antimicrobial Activity

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ABSTRACT

Nanotechnology is one of the rapidly developing areas in recent days due to its wide range of applications in various field, among different methods of nanoparticles synthesis, plant mediated synthesis is considered as reliable method. In the present study sliver nanoparticles were synthesized from the aqueous extract of the *Loeseneriella arnottiana*. Initially the presence of sliver nanoparticles was confirmed by the color change occurred in solution. Later UV-Visible spectroscopy and Scanning Electron Microscopy was performed. Maximum absorption at 235 nm was observed in UV-Visible spectroscopy and the size of the synthesized sliver nanoparticles was ranges of 12.40-33.55`nm as determined by Scanning Electron Microscopy (SEM). A Function group like alkenes and alkynes group, aromatic, nitriles, imines and ether moiety was detected via Fourier Transform Infrared Spectroscopy FTIR). The synthesized sliver nanoparticles were tested for antimicrobial activity against *Klebsiella pneumoniae, Proteus vulgaris* and *Streptococcus mutans.*

Keywords: *Loeseneriella arnottiana*, Bark aqueous extract, Silver nanoparticles, Antimicrobial activity, Scanning Electron Microscopy.

INTRODUCTION

Plant mediated synthesis of nanoparticles is one of the wildest rising field in the current world. Whereas chemical and mechanical methods of nanoparticles synthesis are non-ecofriendly techniques. Biosynthesis method includes plants and microorganisms mediated techniques which are ecofriendly and cost effective process and also these methods are free from use of stabilizers and hazardous wastes (Ram Prasad et al., 2014). Sliver nanoparticles are able to act against the several types of microbes effectively (Sharma et al., 2007). Previous studies have revealed that silver nanoparticles (AgNPs) can be used as potential antimicrobial agent against several bacteria.

It also shows anti-nematodes (Mahmoud *et al.,* 2016), anti-viral (Galdiero et al., 2011), anti-cancer (Oves et al., 2018; Aziz et al*.,* 2019) and anti-inflammatory properties (Manikandan et al., 2017).
Loseneriella arnottiana, a medicinal plant belongs to Celastraceae family. It is a lianas plant with greenish-brown bark commonly found in the Western Ghats regions of Karnataka, Tamil Nadu and Kerala. *L. arnottiana* used as folklore medicine for the treatment of the diabetes mellitus (Prajna et al., 2016). Phytochemical analysis of *L. arnottiana* root reported the presence of tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. In view of these aspects the present study aimed to synthesize and characterize the silver nanoparticles from *L. arnottiana* bark and to test its antimicrobial activity.

MATERIALS AND METHODS

Material collection

Loeseneriella arnottiana bark was collected from forests of Sullia, Dakshina Kannada district of Karnataka. It was washed in tap water followed by distilled water to remove dust and dirt. The material was chopped into small pieces which were completely dried under the shade for twenty days. Later it was finely powdered by powdering mills. The powdered sample was stored at room temperature in clean and dry container for further use.

Synthesis of silver nanoparticles from the aqueous extract of *Loeseneriella arnottiana* bark

Five gram of bark powder was mixed thoroughly with 100ml of distilled water in a conical flask and kept in water bath at 80^{o}C for 30 min. Filter the solution using Whatman No. 1 filter paper and store at 4^{o}C for further work. The bark aqueous extract of 10 ml was mixed with 90 ml of one millimolar (mM) silver nitrate solution and kept in water bath at 70-75^{o}C until the color changes.

Characterization Synthesized Nanoparticles

UV-Vis spectroscopy analysis (Tuzun et al., 2017)

UV-Vis spectroscopy analysis is to investigate the reduction of Ag+ ions which was revealed by measuring the UV-Vis spectrum of the reaction medium of 6 hours after diluting the small amount in distilled water. UV-Vis spectroscopy analysis was performed by using UV-Vis spectroscopy, AgNPs are known to exhibit maximum absorption in the range of 200-500nm.

Scanning Electron Microscopy (Rajendran et al., 2012)

Scanning Electron Microscopy analysis was performed whereas thin film of the sample was prepared carbon coated grid by dropping very small amount of sample, excess solution was removed, and then film in the SEM grid was allowed to dry under a mercury lamp. The size and shape of the nanoparticles was observed.

Fourier Transform Infrared Spectroscopy (Oliveira et al., 2019)

To perform FTIR analysis, approximately 1mg of lyophilized samples was mixed with 100mg of potassium bromide. For the formation of the pellets, experimental samples were submitted to hydraulic press under vacuum and controlled pressure. Wave band ranging between 400 and 4000 cm^{-1} to obtain the spectra in the transmission percentage mode. This was used to determined stability of biomolecules and functional groups present in the synthesized silver particles.

Antimicrobial Activity (Rautel et al., 2019)

Well diffusion method was followed to check the antimicrobial activity of the synthesized nanoparticles against *Klebsiella pneumoniae, Proteus vulgaris* and *Streptococcus mutans*. Muller Hinton agar plates were prepared. Test organisms were spread over the surface of media using sterile swab stick; 7mm diameter well was done using sterile metallic cork borer. Different concentration of 100 mg/mL and 200 mg/mL of nanoparticles were dispensed into the well. Pencillin G of 10 µg was taken as positive control and silver nitrate as negative control. Then the plates were incubated at 37^{o}C for 24 h. The zone of inhibition was measured.

RESULTS

Synthesis of Silver Nanoparticles

Silver nanoparticles synthesis was initiates once the aqueous extract introduced into 1mM silver nitrate solution. Gradually color changes from golden yellow to reddish brown which indicates the formation of silver nanoparticles (Plate 1). The color change is due to Surface Plasmon Vibration, optical property which is unique to the noble metals. Nanoparticles formation was further confirmed by UV-Visible spectroscopy, Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy.

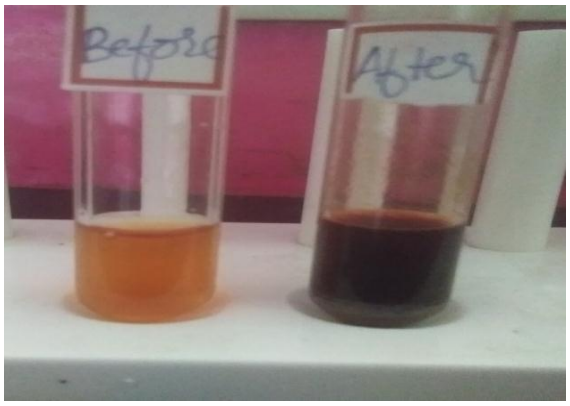

Plate 1. Color changes in the extracts after addition of silver nitrate solution.

UV-Visible Spectroscopy Analysis

It is a crucial tool that provides information of synthesized silver nanoparticles at the initial synthesis stage. The characteristic depending upon the shape, size and distribution of AgNPs due to Surface Plasmon Resonance. UV-Visible absorption peak at 235 nm (Fig. 1) which indicates the presence of the silver nanoparticles.

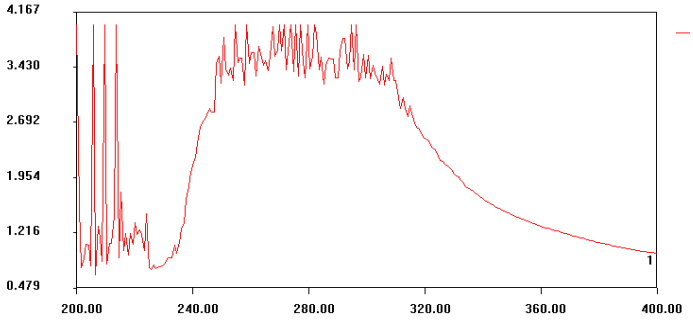

Figure 1. UV-Visible spectroscopy analysis for absorptions peak.

Scanning Electron Microscopy (SEM)

SEM a microscopic technique uses the electron beam to examine the nano dimension of material. Microscopes produce highly focused beams of electrons that are scanned sequentially across the specimen in vacuum chamber. In the present study, size of the synthesized silver nanoparticles ranges between 12.40- 33.55 nm (Plate 2).

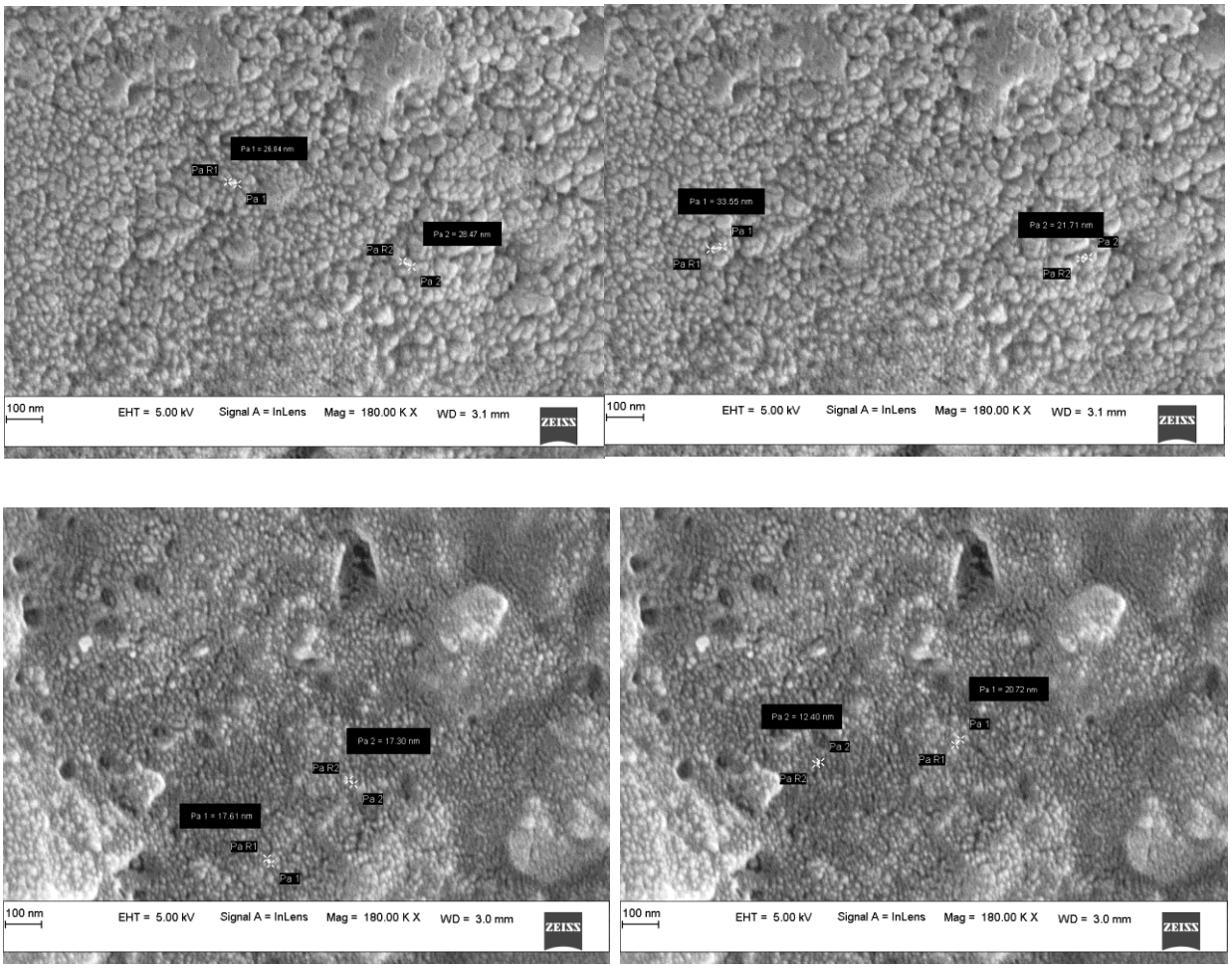

Plate 2. Size and shape of the synthesized silver nanoparticles from *L. arnottina* bark.

Fourier Transforms Infrared Spectroscopy (FTIR)

Nanoparticles sample were analyzed in Fourier Transforms Infrared Spectroscopy to identify the possible biomolecules responsible for the reduction reaction. The obtained spectrum was ranges between 1000-3000 cm^{-1}. The peaks observed in 3352.52-3141.75 cm^{-1} represent the presence of alkyls and alkenes , 3084.35 cm^{-1} assigned with aromatic moiety, 2655.08-2355.65 cm^{-1} assigned with nitriles, 1412.49 cm^{-1} assigned with imines and 1079.10 cm^{-1} assigned with ether moiety (Fig. 2).

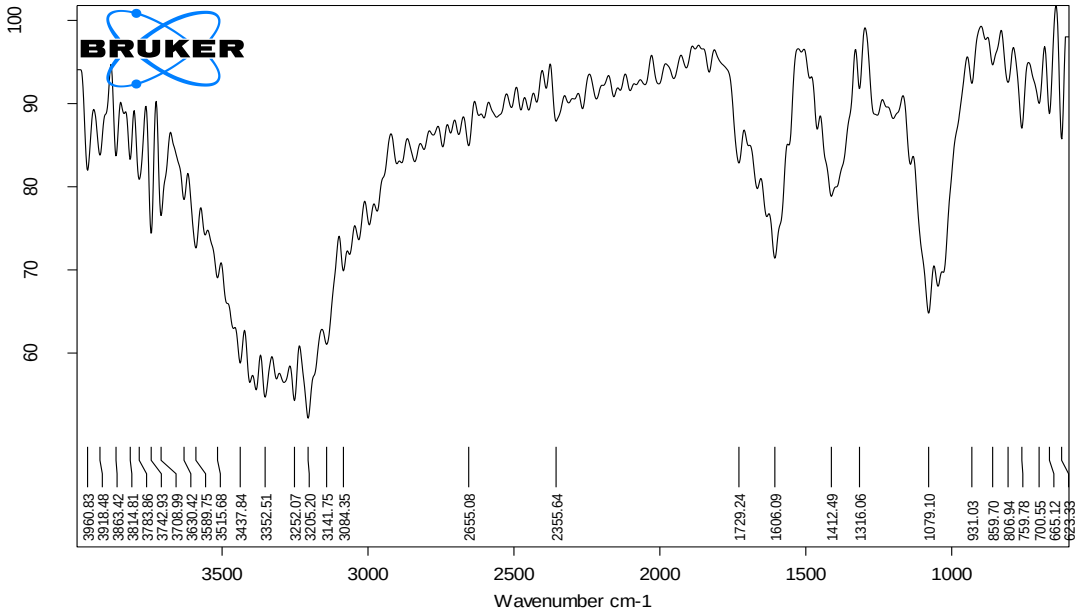

Figure 2. Fourier Transforms Infrared Spectroscopy.

Antimicrobial Activity

The synthesized nanoparticles showed inhibition zone against *Klebsiella pneumoniae, Proteus vulgaris* and *Streptococcus mutans* in different concentrations i.e., 100 mg/mL and 200 mg/mL (Plate 3). The synthesized silver nanoparticles were shown higher significance against *Proteus vulgaris* and *Streptococcus mutans* in both the concentrations and lower significance against *Klebsiella pneumoniae* (Table 1).

Table 1. Antimicrobial activity of silver nanoparticles synthesized from bark extract.

Test organisms	Zone of inhibition of sliver nanoparticles in different concentrations measured in millimeter(mm)				
	100 mg/mL	200 mg/mL	Standard	Positive control	Negative control
Streptococcus mutans	0.5	0.8	0.3	0.4	0.1
Klebsiella pneumoniae	0.2	0.3	0.2	0.3	0.1
Protease vulgaris	0.3	0.5	0.2	0.3	0.1

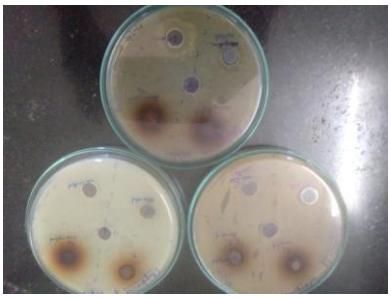

Plate 3. Antimicrobial Activity.

DISCUSSION

In the field of material science, plant mediated synthesis gained attention via reliable, nontoxic and ecofriendly protocol for synthesis. In the present study initially color change indicates the presence of sliver nanoparticles. Further characterization was done in which UV-Vis spectroscopy had maximum absorption peak at 235 nm. Similar type of work was performed by Nadeem et al. (2017), Pirtarighat et al. (2019) and Rautela et al. (2019) with the maximum absorption at 450 nm, 555 nm and 432 nm respectively. In the same works the size of silver nanoparticles was found to be 19-125, 10-33 and 4-11 nm characterized by Scanning Electron Microscopy. In the present study size of the synthesized nanoparticles were in the range of 12.40-33.55 nm. Sudha et al. (2013) worked on algal mediated synthesis of nanoparticles, the size of the synthesized nanoparticles ranges between 44-79 nm. Fourier Transforms Infrared Spectroscopy analysis revealed the presence of alkyls and alkenes, aromatic moiety, nitriles, imines and ether moieties. Presence of functional groups like alcohol, aromatic, alkyl halides, alkene and single aldehyde, aldehydic, C-H stretching, carbonyl stretch in proteins, ethylene and C-N stretching and hydroxyl, amine, carboxylate, aldehyde, ester and carboxylic acid groups was reported by some earlier workers (Devaraj et al., 2013; Elamawi et al., 2018; Rama Bhat et al., 2019; Shameli et al., 2020). Similarly they were also reported the inhibition zone produced by *B. subtilis*, *B. vallismortis*, *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* against AgNPs. In the present study the synthesized silver nanoparticles was shown higher resistance against *Proteus vulgaris* and *Streptococcus mutans* and lower activity against *Klebsiella pneumoniae*. In another study conducted by Jayachitra et al. (2015) and Parajuli *et al*. (2021) in which they observed the higher inhibition zone against *B. subtilis*, *Staphylococcus aureus, Escherichia coli* and *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Salmonella typhi and Klebsiella pneumoniae* respectively. Nanoparticles from various metals and plants synthesized have wide applications in environmental remediation (Singh et al., 2018; Jodaun et al., 2021).

CONCLUSION

Sliver nanoparticles were synthesized from the aqueous extract of the *Loeseneriella arnottiana* bark in the present study. This was initially confirmed by the color change was in occurred in the solution. Synthesized nanoparticles were further characterized by the UV-Vis spectroscopy and Scanning Electron Microscopy whereas the maximum absorption at 235 nm was reported and size of synthesized nanoparticles was 12.40-33.55 nm. Fourier Transforms Infrared Spectroscopy analysis revealed the presence of alkyls and alkenes, aromatic moiety, nitriles, imines and ether moiety. A synthesized silver nanoparticle was tested for antimicrobial activity against *Klebsiella pneumoniae, Proteus vulgaris* and *Streptococcus mutans* exhibited higher activity compared to *Klebsiella pneumoniae.*

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