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BIOSYNTHESIS OF SILVER NANOPARTICLES USING *FICUS RACEMOSA* LATEX AND ITS ANTIMICROBIAL ACTIVITY

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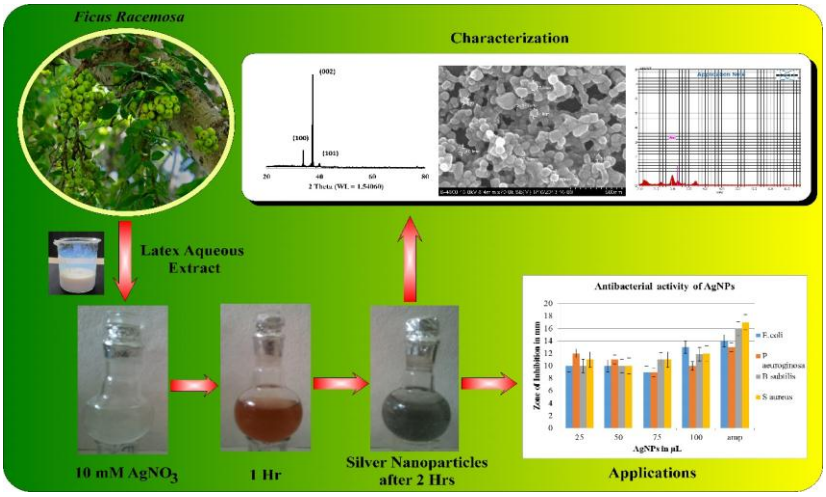
ABSTRACT

Herein, biosynthesis of silver nanoparticles (SNPs) and its antimicrobial activity was investigated. SNPs were synthesized using latex of *Ficus racemosa* and rapid formation of nanoparticles was observed within 10 min through change of colourless solution of silver ions to black colour solution of SNPs. SNPs were characterized using different techniques. The size of the silver nanoparticles was measured in the range of 50-120 nm. Further, the antimicrobial activity of biosynthesized SNPs was assessed by agar well diffusion method against bacterial strain such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus.*

KEYWORD: *Ficus racemosa*; Biosynthesis; Silver nanoparticles; Antimicrobial activity.

Graphical Abstract



INTRODUCTION

Noble metal nanoparticles are gaining significance for past few years due to their applicability in the field of medicine, biology, material science, physics and chemistry.[1] Among the several noble metal nanoparticles, silver metal nanoparticles (SNPs) have attained special focus in view of their distinctive properties, like good electrical conductivity, chemical stability, catalytic and antimicrobial activity.[2] Due to antimicrobial activity, SNPs have also been used in the food industry,[3] and cosmetics products.[4] In addition, SNPs have found applications in various fields including electronic devices, chemical/ biological sensing, and surface-enhanced Raman spectroscopy, drug delivery, gene delivery, catalyst, antimicrobial agents, conductive coating, and sensors.[5-7] Metallic nanoparticles have attracted the attention of the scientific community and technologists due to their ever-emerging, numerous, and fascinating applications in various fields, including biomedical sciences and engineering.[8-10] For instance, the unique optoelectronic and physicochemical properties of metal nanoparticles have already been successfully exploited for the purpose of drug delivery,[11] tissue/tumour imaging,[12] bio-sensing,[13] catalysis,[14] and surface-enhanced Raman scattering-based sensors.[15] The commonly employed methods for the synthesis of metallic nanoparticles involve toxic chemicals, hazardous conditions, and costly apparatus. In comparison, the green synthesis of metallic nanoparticles involves biocompatible ingredients under physiological conditions of temperature and pressure. Moreover, the biologically active molecules involved in the green synthesis of nanoparticles act as functionalizing ligands, making these nanoparticles more suitable for biomedical applications.[16] Recently, physiologically stable and bio-compatible SNPs have been synthesized by mixing the silver ion solution with leaf extract of *Azadirachta indica* without using any surfactant or external energy.[17] Moreover, SNPs were synthesized using hot water *olive* leaf extracts as both reducing and stabilizing agent, and evaluated for antibacterial activity against drug-resistant bacterial isolates.[18] Another, aqueous extract of *Lakshmi tulasi* (*Ocimum sanctum*) leaf as a reducing and stabilizing agent for synthesis of SNPs has been reported.[19] The synthesis and characterization of silver nanoparticles using *Iresine herbstii* and evaluation of their antibacterial, antioxidant, and cytotoxic activity have been reported.[20] The biological method for the synthesis of SNPs using *Annona squamosa* leaf extract and its cytotoxicity against MCF-7 cells has been reported.[21] SNPs with an average size of 26 nm have been also synthesized by exposing aqueous silver ions to *Coriandrum sativum* leaf extract as reducing agent.[22] Herein, Table 1 summarizes some recent works related to biosynthesis of SNPs.

Table 1. Some reported biosynthesis of SNPs.

Sr. No.	Material used for biosynthesis	Applications	Ref.
1.	Exopolysaccharides (EPS) of lactic acid bacteria (LAB)	Antimicrobial activity	[23]
2.	*Penicillium chrysogenum*	Antimicrobial activity and its anticancer effect in human liver cancer and fibroblast cells	[24]
3.	*Bacillus thuringiensis* SSV1 culture supernatant	Anticancer Activity	[25]
4.	proteinaceous pigment phycocyanin extracted from *Nostoc linckia*	In vitro anticancer activity against breast cancer cell line and in vivo cytotxicity	[26]
5.	Encapsulated biomass beads of *Phoma exigua* var. exigua	Antibacterial activity	[27]
6.	Black cardamom (B.C.) extract	Antibacterial activities	[28]
7.	*Solanum tuberosum* (potato) extract	Interaction with human serum albumin	[29]
8.	*Pseudomonas spp*. isolated from effluent of an electroplating industry	--	[30]
9.	Freshwater microalgae strains	Activity against 14 bacterial strains, the fungal strain *Candida albicans*, *hepatocellular carcinoma* (HepG2) and breast cancer (MCF7) cell lines, and Newcastle Disease Virus (NDV) on Huh7-infected cells	[31]
10.	*Cyanobacterium Synechococcus* elongatus	Bacteriocidal and algicidal efficacy	[32]
11.	leafy green extract of Belgian endive (*Cichorium intybus* L. var. sativus)	Antibacterial activity	[33]
12.	*Sugarcane leaf* (Saccharum officinarum) extract	Antifungal activity	[34]
13.	*Nigella arvensis* Seed Extract	Antimicrobial and Cytotoxic Effects	[35]
14.	*Myristica fragrans* seed (nutmeg) extract	Antibacterial activity	[36]
15.	Extract of *Comamonas acidovorans*	Antibacterial activity	[37]
16.	Using *Arachis hypogaea* (Ground Nut) Root Extract	Antibacterial and Clinical Applications	[38]
17.	beta-Caryophyllene Isolated from *Murraya koenigii*	Antimalarial (Plasmodium falciparum 3D7) and Anticancer Activity (A549 and HeLa Cell Lines)	[39]
18.	*Abutilon indicum* (Link)	Anti-inflammatory and Antioxidant Potential against Carrageen Induced Paw Edema in Rats	[40]

The green approach to the synthesis of nanoparticles using plant materials, such as reducing and capping agents, could be considered attractive in nano-biotechnology. This technology is safe, simple, non-toxic, efficient, environmental friendly, and also provides efficacious single-pot synthesis reactions without the need for additional surfactants or capping agents [41] In our previous studies, we have bio-synthesised silver and gold nanoparticles, and investigated the nanoparticle application in pH dependent binding study with different amino acids and in photocatalytic activity in dyes reduction.[42,43] This study focuses on the green synthesis of SNPs using latex of *Ficus racemosa* and detail characterization of SNPs using various instruments. Further, the green synthesized SNPs were tested for antimicrobial activity against a different microorganism to review its biological potent nature.

EXPERIMENTAL

MATERIALS AND METHODS

The UV-visible absorption spectra were recorded for the samples in the range of 300-800 nm wavelength by UV/Vis spectrophotometer (Shimadzu 2450 Japan) using 1.0 cm quartz cell. X-ray diffraction (XRD) patterns of samples were recorded on Rigaku Rotalflex RU-200B diffractometer using a Cu Kα (λ = 1.5418 Å) radiation and operated over the scanning angle between 20° to 80°. Surface morphology of the samples were characterized by using field emission scanning electron microscopy (FESEM) instrument (S-4800, Hitachi, Japan) operated at 10 kV and subsequent elemental analysis was performed by energy dispersive spectroscopy (EDS) coupled with FESEM. Thermal gravimetric analysis (TGA) of the samples were carried out using Perkin Elmer TGA 4000 instrument under purified nitrogen gas flow with 10 °C/min heating rate. Particle size analysis is carried out using dynamic light scattering technique by Zeta sizer instrument (Malvern Instrument Ltd., Germany). Photoluminescence (PL) spectra of SNPs samples were recorded on FluoroMax®4 spectrometer (Horiba, Japan). The bio-synthesized silver nanoparticles were separated from the liquid phase by REMI R4C centrifuge machine at 3000 rpm for 1 h.

Preparation of latex extract

Methods for *F. racemosa* latex extract preparation and nanoparticle synthesis has been followed according to our earlier report.[42] Briefly, about 0.5 mL of fresh latex of *F. racemosa* (collected from university campus) was dissolved in 5 mL of double-distilled water followed by vacuum filter through 0.45µ filter to remove any suspended particles. The

collected filtrate was diluted to 10 mL to obtained a 5% aqueous latex solution which was used as reducing agent for SNPs synthesis.

Synthesis of silver nanoparticles (SNPs)

For SNPs synthesis, 40 mL of 10 mM silver nitrate ($AgNO_3$) solutions were diluted in 360 mL double-distilled water in 1000 mL beaker. Subsequently, 1 mL of the above prepared aqueous latex extract was added to silver nitrate solution and thoroughly mixed using magnetic stirrer. The mixture (silver solution + aqueous latex) solution was kept in the dark for an appropriate period. At equilibrium, the colourless solution of silver nitrate changes to black colour solution indicates formation of SNPs (Fig.1). The SNPs nanoparticles obtained were separated from colloidal suspension by centrifugation at 3000 rpm for 1 h. The SNPs was then purified by washing with 10 mL methanol to remove any residual latex impurities. This washing step with methanol was repeated for 3-4 times to obtained pure SNPs, and finally stored in methanol for characterization. Please note that for antibacterial studies, stored SNPs were separated from solvent by centrifugation at 3000 rpm for 1 h. Then separated SNPs were dried, and appropriate quantity was re-suspended in double distilled water to prepare a fresh suspension of SNPs solution having concentration of 100 mg/L.

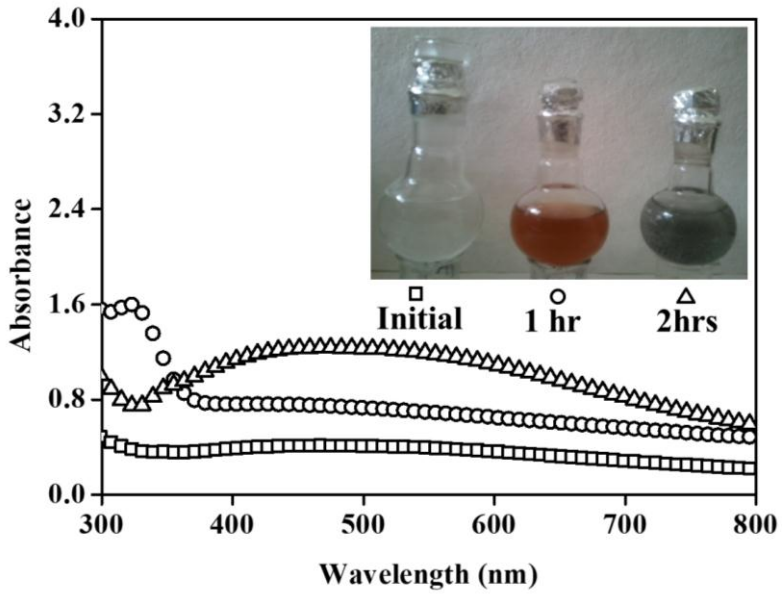


Fig. 1. UV-Visible absorption spectra of SNPs at different stages during synthesis of silver nanoparticles (SNPs) from *Ficus racemosa* latex (Inset photograph shows colour changes at each stage).

Antibacterial studies

The antibacterial activity of synthesized silver nanoparticles was studied against gram negative bacteria *Escherichia coli* (ATCC 8739), *Pseudomonas aueroginosa* (ATCC 9027), and gram positive bacteria *Bacillus subtilis subsp. spizizenii* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538). The antibacterial activity study was performed by Well diffusion method and the method detail are given below.

The Well diffusion method was performed in nutrient agar (NA) medium at 35±2 °C for 24 h. The active bacterial culture was prepared one day prior to the experiment. To get all the bacterial suspensions, a single colony was picked from the master plates and carefully transferred to nutrient broth (NB) medium each in the separated 100 mL flask, incubated at 37±2 °C for 24 h. For the experiment, all the cultures were diluted with sterile NB medium to get equal organism population in all experimental sets. Four wells of 6 mm diameter per plate were made using gel puncture. Using sterile pipette, wells were filled with 25 µL, 50 µL, 75 µL and 100 µL of SNPs of 100 mg/L and a standard antibiotic Ampicillin as control, followed by incubation at 35±2°C for 24 h. After completion of incubation, clearly visualized zone of inhibition was seen and measured around each poured well of various volumes of 100 mg/L SNPs.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles using latex of *Ficus racemosa*

The nanoparticle synthesis reaction begin after the extracted latex was introduced into 10 mM $AgNO_3$ solution. After 2h of reaction time in the dark room condition, the colourless reaction mixture was turned into black colour solution indicates the synthesis of SNPs (Fig. 1). An intermediate colour change to brown colour solution after 1h of reaction time suggest initiation silver ion reduction (Fig. 1). To confirm the colour change from colourless to black was primarily due to SNPs synthesis, a parallel experiment was performed by adding extracted latex to double distilled water without $AgNO_3$, incubated for 2h in dark, and found no colour change, further support our SNPs synthesis. The colour change of the solution was ascribed to the active molecules present in the extracted latex, which reduced the silver ions into SNPs. The intensity of the colour change was increased as in direct proportion to the incubation period of nanoparticle synthesis.

Characterization of silver nanoparticles

The primary information of SNPs synthesis was obtained by recording UV–visible spectra for the latex extract of *F. racemosa* added 10 mM $AgNO_3$ solution after 2h of incubation in the dark room. The silver nitrate latex extract reaction mixture exhibits the broad peak with maximum absorbance at 435 nm (Fig. 1). It may be due to the excitation of surface plasmon resonance for the synthesized SNPs. Silver nanoparticles exhibit unique and tunable optical properties on account of their surface plasmon resonance, dependent on the shape and size distribution of the nanoparticles. Nakkala et al. (2014) reported similar UV-visible spectrum of silver nanoparticles synthesized using aqueous rhizome extract of *Acorus calamus*.[44] The spectrum shows a single broad peak having wavelength maximum at 421 nm, and also the peak broadening in spectrum was suggested to be due to poly dispersed nature of SNPs. Another bio-synthesis methods of SNPs involving biological extract from Lemon and *Viburnum lantana* leaves also shown similar UV-visible spectra of SNPs with peak maximum in the range of 400-450 nm.[45,46]

The typical powder XRD patterns of the prepared SNPs are shown in (Fig. 2). The data show diffraction peaks at 2θ = 35.4°, 37.5°, and 40.2° which can be indexed to (100), (002), and (101) planes for silver and are consistent with the values reported in standard JCPDS record (PDF card no. 01-071-5025) which corresponds to the hexagonal crystal structure. Using Debye–Scherrer's formula, the average size of SNPs was calculated to be 95.49 nm which was further confirmed using particle size analyser results explained later. From both results, it confirmed the formation of SNPs by green chemistry approach at room temperature.

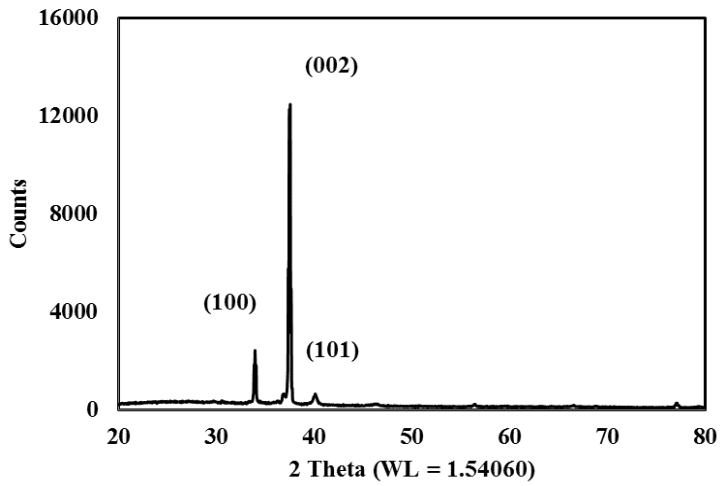
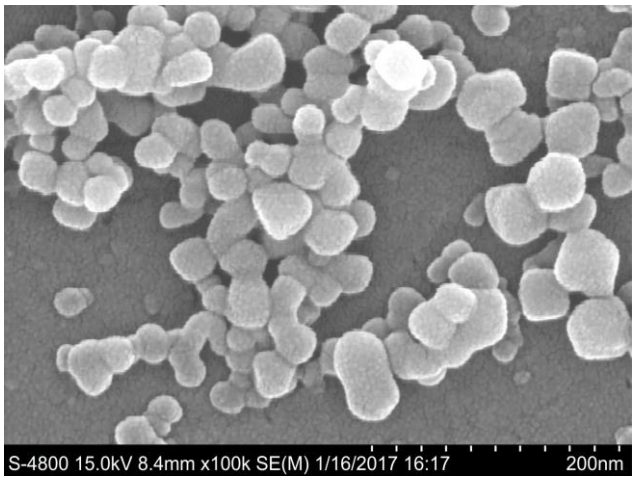
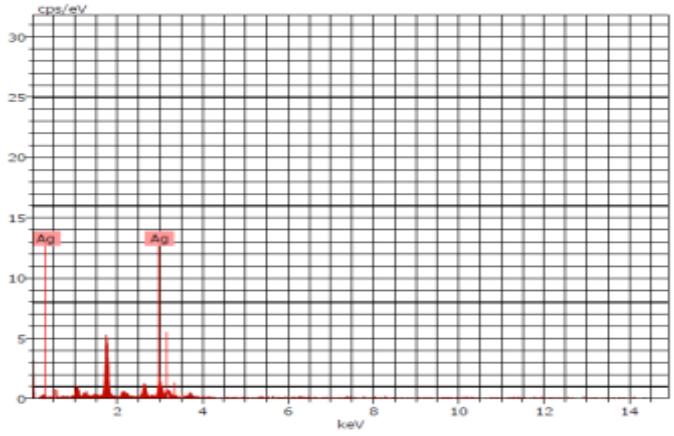


Fig. 2. XRD spectrum of SNPs.

Surface morphology and elemental composition of SNPs where presented by means of FESEM micrograph and EDS spectrum which are shown in Fig. 3. For analysis, a clean glass slide was coated with synthesized SNPs and dried in hot air oven. In micrograph, it is observed that the SNPs grains are in the size ranges of 50 to 120 nm which is an agglomeration of small crystallites observed as tiny dark and bright spots (Fig. 3a). A EDS spectrum of synthesized SNPs (Fig. 3b) shows a peak for silver which further reveals the presence of SNPs.



(a)



(b)

Fig. 3: FE-SEM micrograph (a) and EDS spectrum (b) of SNPs onto glass substrates.

A photoluminescence (PL) technique is an effective tool to identify the sensing ability of a material. With this aim, PL spectra for bio-synthesised SNPs has been recorded by using 200 and 250 nm excitation wavelength at 5 nm slit width and the obtained results are shown in Fig. 4. It was observed that SNPs shows a sharp peak at 330 nm and a broad beak between 500-700 nm. Zhang et al. (2008) also showed similar peaks at 330 nm and 500-700 nm for

SNPs synthesized using sodium citrate and suggest that a peak at 330 nm is basically due to Ag-Ag interaction.[47] This result suggest that the bio-synthesised SNPs are luminescent active and can be utilised as sensors for biological studies.

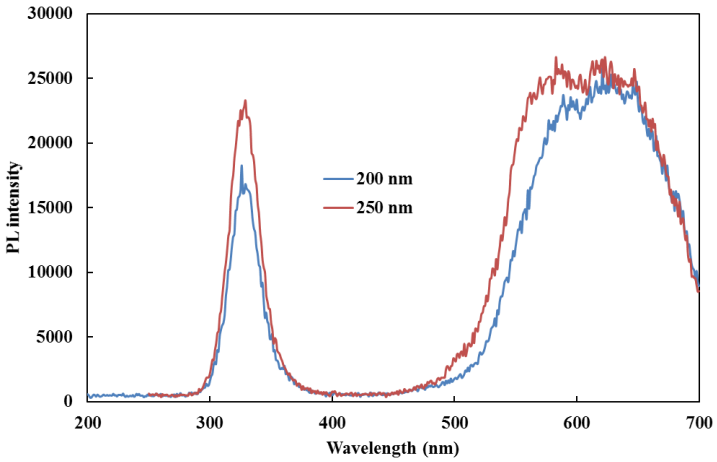


Fig. 4: Photoluminescence spectra for SNPs.

TGA has been recorded to assess the thermal properties of synthesised SNPs. Thermogram (Fig. 5) has shown that ~18% weight loss was observed in the temperature range 200 to 600^{o}C which suggests good stability of SNPs at a higher temperature. Vivek Vishnu et al., (2014) reported similar TGA curve for SNPs synthesized by root extract of *Desmodium gangeticum.*[48] The finding also suggest SNPs with high thermal stability might be used in biomedicine as drug delivery agent.

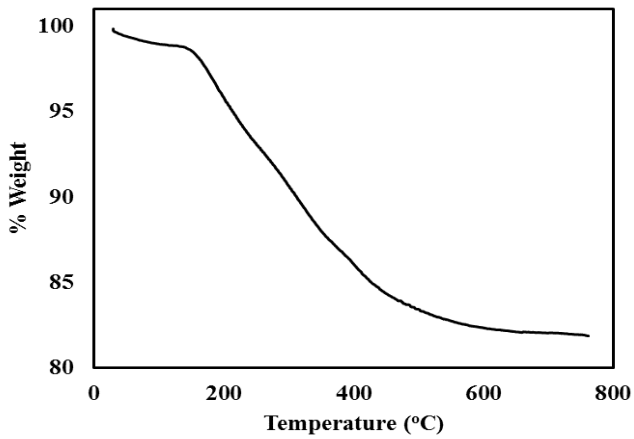


Fig. 5. TGA spectra for SNPs.

The particle size of synthesised SNPs was measured using particle size analysis and result are shown in Fig. 6. Compared to FESEM analysis, increased particle size (115.8 nm) as was

observed due to the aggregation of nanoparticles in suspension form which might be due to repetitive washing of SNPs with water and methanol to remove the plant extract residue after synthesis. This also suggest that in absence of plant extract as capping or stabilizing agent greatly affect the stability of SNPs.

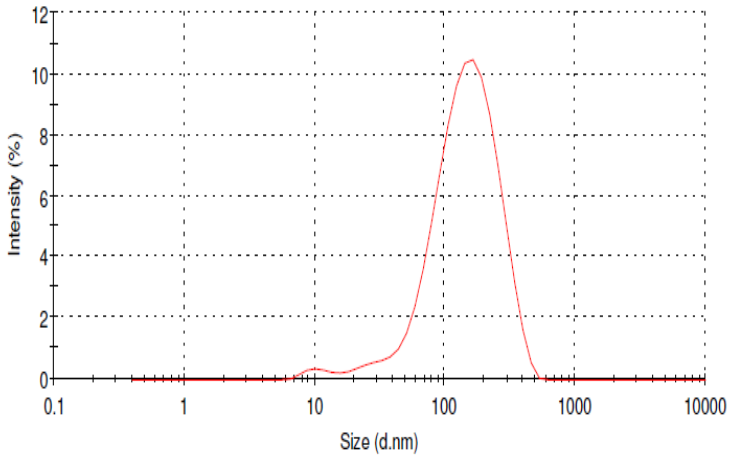


Fig. 6: Particle size analysis for SNPs.

Biological activity

It is well reported in the literature that bacteria have different cell wall structure and their ability of capsule formation, due to which their susceptibility towards antibacterial agents differs. Reports suggested that antimicrobial activity of SNPs is associated with the structure of bacterial cell wall and thickness of peptidoglycan layer of bacterial cell wall, while there are several other reports that attributed the inhibitory effect of SNPs to their particle size which enables them to easily get attached to bacterial cell membrane and reach nuclear content of the bacterial cell, resulting in a disturbance in their structure and rendering them permeable to a larger extent, that leads to the leaking of ions and other cell contents. This kind of disturbance in the enzymatic activity inside the bacterial cell results in the death of the bacterial cell. Bio-synthesized SNPs were evaluated for their antimicrobial activity against different microorganisms. The zone of inhibition was recorded to evaluate the antimicrobial activity. The results obtained displayed good variation in the antimicrobial activity of SNPs.

The antibacterial assay of SNPs was screened because of the great medicinal relevance of SNPs and their numerous applications. Diameters of the clear zone known as bacterial inhibition zones were measured and represented as the antimicrobial activity of the test

sample (Fig. 7). The result revealed that SNPs exhibited a potential antibacterial activity against all the tested bacterial strains and their activity was varied with bacterial strain at the different volumes (Fig. 8). The SNPs exhibited good inhibition zone for *E. coli* at 100 µL (13 mm) followed by *B. subtilis* and *S. aureus* at 100 µL (12 mm) and *P. aeuroginosa* at 25 µL (12 mm) while the least activity was observed for *E. coli* and *P. aeuroginosa* at 75 µL (9 mm).

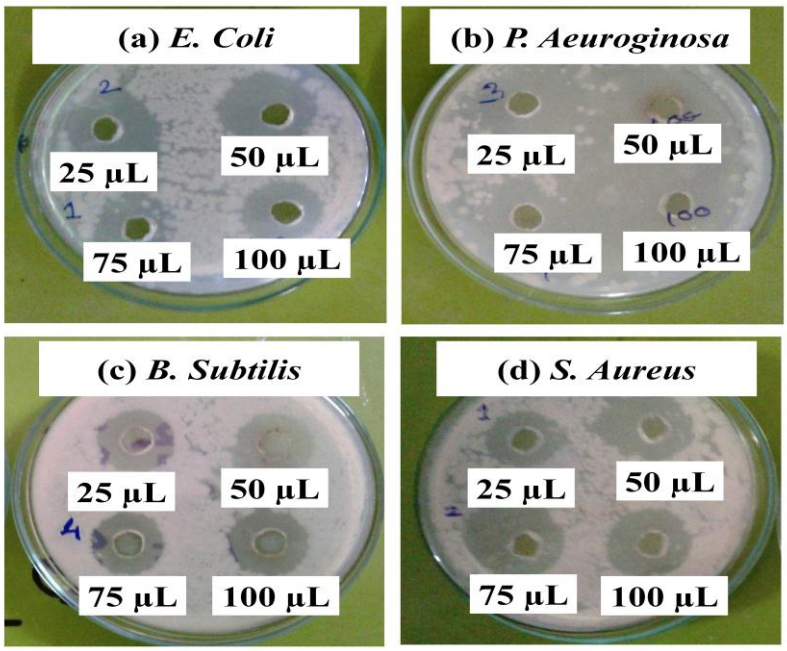


Fig. 7: Photograph showing zone of Inhibition for antibacterial study of SNPs.

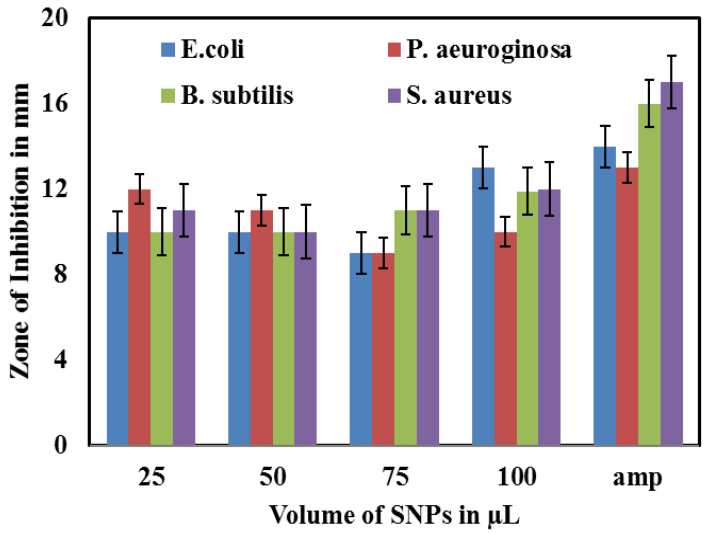


Fig. 8: Antibacterial activity of SNPs.

CONCLUSION

The biosynthesis of silver nanoparticles (SNPs) using latex of commonly available plant *Ficus racemosa* is achieved. The formation of nanoparticles was rapid with reaction time of 2 h. The instrumental characterization of biosynthesized SNPs suggest that particles size is in the range of 50-120 nm, crystalline, shows high thermal stability and luminescent active. Further, the biosynthesized SNPs showed anti-bacterial activity against both the gram-positive and gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus.* Thus, the plant extract mediated bio-synthesis of SNPs can be considered as alternative method to the conventional one which make use of expensive and toxic chemical, and also require some special experimental condition. Further, the biological activity of bio-synthesized SNPs uncovered its potential application in the field of drug delivery, a solution to drug-resistant bacterial strains, cancer therapy etc.

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