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RESEARCH ARTICLE

Evaluation of Biologically Synthesized Silver Nanoparticles from Gum Extract of *Boswellia ovalifoliolata* Bal. & Henry – An Endemic Endangered Medicinal Plant of Tirumala Hill Range of Andhra Pradesh, India

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ABSTRACT

Boswellia ovalifoliolata Bal. and Henry (Burseraceae) is a potential medicinal tree used traditionally in the treatment of various diseases. Metal nanoparticles have been using as an ingredients in the preparation of complementary medicines to cure different diseases is an age old medicinal practices. The present study aimed to synthesize the silver nanoparticles from gum extract of *Boswellia ovalifoliolata* and characterized by UV-Vis spectra, Scanning Electronic Microscope (SEM), Energy Dispersive X-ray Analysis (EDAX) and Atomic Force Microscope (AFM). The results revealed that the spherical shaped silver nanoparticles with average size of 24.34 nm were able to synthesize and these silver nanoparticles were tested for their antimicrobial activity by measuring the inhibitory zone, showed highest toxicity to *Klebsiella* followed by *Bacillus, E. coli* and *Proteus* and lowest toxicity towards to *Pseudomonas* in bacterial species. The results indicate that the gum of *B. ovalifoliolata* had the potential to synthesis the nanoparticles of size 24 nm and these silver nanoparticles are having capability to mitigate the microbial cell proliferation which can prove the way to synthesis of naval therapeutic compounds in place of conventional medicines against microbes.

KEYWORDS

Boswellia ovalifoliolata, gum, silver nanoparticles, endemic plant, Tirumala hills

INTRODUCTION

Nanotechnology provides the tools and technology platform for the investigation of biological systems and biological offers inspiration models for bio-assembled components to nanotechnology. Nanobiotechnology is defined as a field that applies the nanoscale principle and techniques to understand and transform biosystems and which uses biological principles and materials to create new devices and systems integrated from the nanoscale. Nanotechnology is the study of assembling, controlling and manipulating matter on molecular at atomic size, it has attracted a great interest in recent years due to its expected impact to many areas such as agricultural and food technology, energy, electronics and medicine.[1] Particles with a size upto 100 nm are usually referred as nanoparticles and they exhibit completely new properties based on their size, distribution and morphology.[2]

Silver nanoparticles find use in many fields, and the major applications include their use as catalysts, as optical sensors of Zeptomole (10^{-21}) concentration, in textile engineering, in electronics, in optics and most importantly in the medical field as a bactericidal and as a therapeutic agent. Silver ions are used in the formulation of dental resin composites, in

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coatings of medical devices as a bactericidal coating in water filters, as an antimicrobial agent in air sanitizer sprays, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpastes, washing machines and many wound dressing.[3] Silver has some medicinal uses going back for centuries. The Phoenicians are said to have stored water, wine and vinegar in silver bottles to prevent spoiling. In the early 1990's silver gained regulatory approval as an anti-microbial agent[4] and people would put silver coins in milk bottles to prolong the milks freshness.[5] Hippocrates, the father of medicine wrote that silver had beneficial healing and antimicrobial properties. In recent days, a number of living organisms are already well known to elaborate nanostructured composites such as cynobacteria, bacteria, fungi, actinomycetes, biomolecules and various plant materials such as *Svensonia hydrobadensis*[6], *Boswellia ovalifoliolata* and *Shorea tumbuggaia*[7], *Shorea tumbuggaia*[8] and *Thespesia populnea*.[9]

Boswellia ovalifoliolata Bal. and Henry is an endemic, endangered, globally threatened medicinal taxon belongs to the family Burseraceae.[10] This deciduous medium sized tree occurs at an altitudinal range of 250-600 m on Seshachalam hill range of Eastern Ghats of India.[11] The gum obtained from the trunk which is highly medicated, this gum is sold in the local market by the native tribals as Konda sambrani in Telugu language. Small lumps of fresh light yellow coloured liquid oozes out from the stem and hardens on exposure.[12] Amyrins are the chief constituents of the gum together with resin acids and volatile acids. Shade dried gum is powdered dissolved in water and mixed with curd and given orally to cure amoebic dysentery.[13] Gum powder of *Boswellia ovalifoliolata* and *Boswellia serrata* and fruit powder of *Pedalium murex* mixed in equal parts and made into paste and apply externally on the affected part of the testicle to cure hydrocoel. Gum powder mixed with white precipitate of pounded stem of *Tinospora cardifolia* and honey given orally in small quantities (10 ml) two times a day to cure hydrocoel.[14] Equal mixture of gum and stem bark in one tea spoonful given daily with sour milk on empty stomach for a month to cure stomach ulcers.[15] Tribals (Nakkala, Sugali and Chenchu) and local healers of surrounding villages making deep incisions on the main trunk to extract the gum but unknowingly causes damage to immature plants leading to depletion of this species in its natural habitat. Herbal medicines are crude plant drugs used by tribals and rural folk and has also been studied for biological synthesis of silver nanoparticles and antimicrobial activity[7,16,17], phytochemical screening[11], quantification of phytochemicals[18], antiulcer activity[19] and antihyperlipidermic activity[20].

MATERIAL AND METHOD

Plant Material and Preparation of the Extract

Gum was collected from Tirumala hills of Chittoor District, Andhra Pradesh, India during the year 2012. Then the gum was shade dried and ground to make a fine powder. 5 g of powder were taken into 250 ml conical flask and added 100 ml of sterile distilled water and boiled for 10 minutes at 100^0C. Then the gum extract was collected in separate conical flask by standard filtration method.

Synthesis of Silver Nanoparticles

1 mM $AgNO_3$ solution was prepared and stored in amber colour bottle. The gum extract was added to 1 mM $AgNO_3$ solution. The colour change of the solution from yellow to brown indicated the silver nanoparticles were synthesized from the gum for the characterization of silver nanoparticles and antimicrobial activity.

UV-Vis Spectra Analysis

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV- 2450 (Shimadzu).

SEM Analysis of Silver Nanoparticles

Scanning Electron Microscope (SEM) analysis was carried out by using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry.

EDAX Measurements

In order to carry out EDAX analysis, the drop of gum extract with reduced silver nanoparticles was dried on coated with carbon film and performed on Hitachi S-3400 N SEM instrument equipped with thermo EDAX attachments.

AFM Measurements

The silver nanoparticles extracted through above protocol were visualized with an atomic force microscope. A thin film of the sample was prepared on a glass slide by dropping 100 micro liters of the sample on the slide were allowed to dry for 5 min, the slides were the scanned with the AFM (Nano Surf ® AG, Switzerland, Product: BTO2089, BRO).

Antimicrobial Activity

Microorganisms

Pure culture of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris* and *Klebsiella pneumoneae* species of bacteria and *Fusarium oxysporum, Curvularia lunata, Rhizopus arrhizus, Aspergillus niger* and *Aspergillus flavus* species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Science (SVIMS). The experiments of antimicrobial activity were carried out in the Department of Microbiology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Antibacterial Activity

The antibacterial activity of SNPs was carried out by disc diffusion method.[21] Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10 µg/ml) and placed in the nutrient agar plate and kept for incubation at 37^0C for 24 h. Zones of inhibition for control, SNPs and silver nitrate were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

Antifungal Activity

Potato dextrose agar plates were prepared, sterilized and solidified, after solidification fungal cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10µg/ml) and placed in the agar plate and kept for incubation for 7 days. After 7 days zone of inhibition was measured.

RESULTS AND DISCUSSION

In the present study silver nanoparticles (SNPs) were synthesized by using aqueous gum extract of *B. ovalifoliolata* within 10 to 20 min of incubation period yellowish brown colour was developed rapidly by addition of Ag $(NO_3)_2$ to the extract. Change of colour has been ranging from light yellow to thick brown within 15 to 20 min in *B. ovalifoliolata* (Fig-1). The signatory brown colour was obtained which resulted due to the excitation of the Surface Plasmon Resonance vibrations of the silver nanoparticles formed, similar results were observed in various plants studied by Savithramma *et al*.[22]; Lingarao and Savithramma[23] and Sasikala and Savithramma[24].

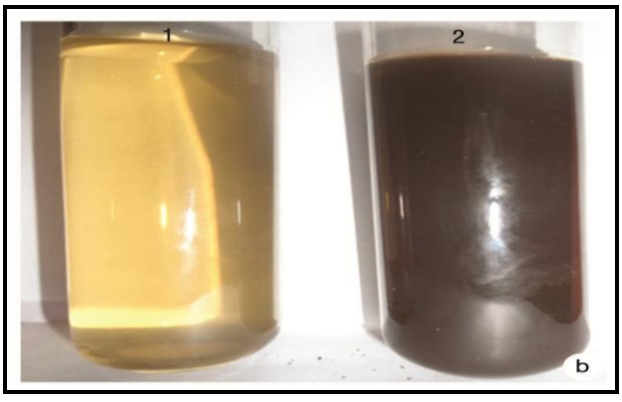

Figure1: Synthesis of SNPs: (1. Plant extract, 2. Plant extract with silver nitrate (SNPs)) the color change of gum extract of B. ovalifoliolata

The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of SNPs synthesized from aqueous gum extract of *B. ovalifoliolata* has the characteristic absorbance peaks ranging from 400 to 450 nm (Fig-2). The broadening of peak indicated that the particles are poly-dispersed. The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions same results were observed in *Cadaba*.[25] The reaction could easily be tracked by the change in color and reconfirmed by UV-VIS spectroscopy. An absorption band at 270 nm is attributed to the aromatic amino acids of proteins. It is well known that the absorption band at 270 nm arises due to electronic excitations in tryptophan and tyrosine residues in the proteins this observation indicates the release of proteins into solution by *B. ovalifoliolata* and suggests a possible mechanism for the reduction of the metal ions present in the solution.

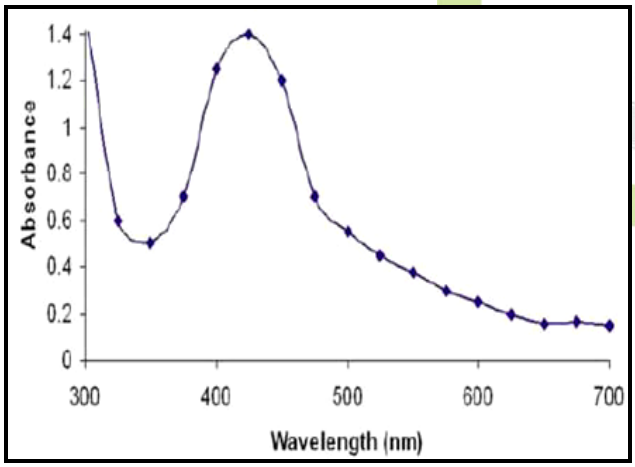

Figure 2: UV-Visible spectroscopy of synthesized silver nanoparticles of B. ovalifoliolata gum

SEM images of SNPs derived from the aqueous gum extracts of *B. ovalifoliolata* showed the particles in spherical shape and size ranged from 20.6 to 26.3 nm (Fig-3). The morphology of the SNPs was predominantly spherical and they appear to be monodisperse. Further analysis of the silver particles by Energy Dispersive Spectroscopy confirmed the presence of the signal characteristic of silver (Fig-4). All the peaks of Ag are observed and are assigned. Peaks of Ag are from the grid used and the peaks of S, P and N correspond to the protein capping over the AgNPs. EDAX information has given the various elements along with SNPs of gum extracts of *B. ovalifoliolata* was identified elements like C, O, Mg, Si and Ag with different percentages.

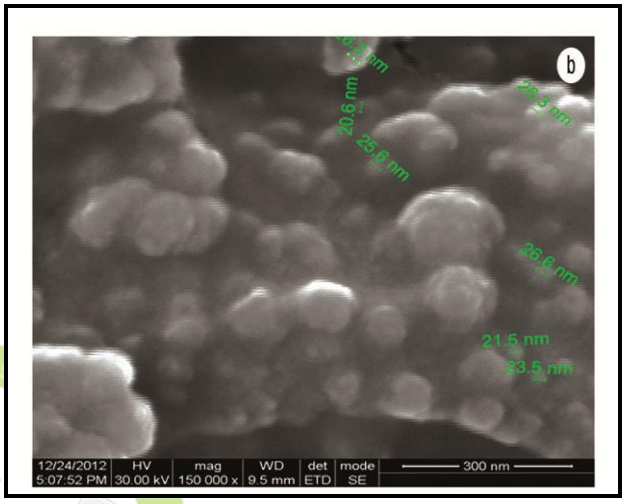

Figure 3: SEM images of synthesized silver nanoparticles from *B. ovalifoliolata* gum

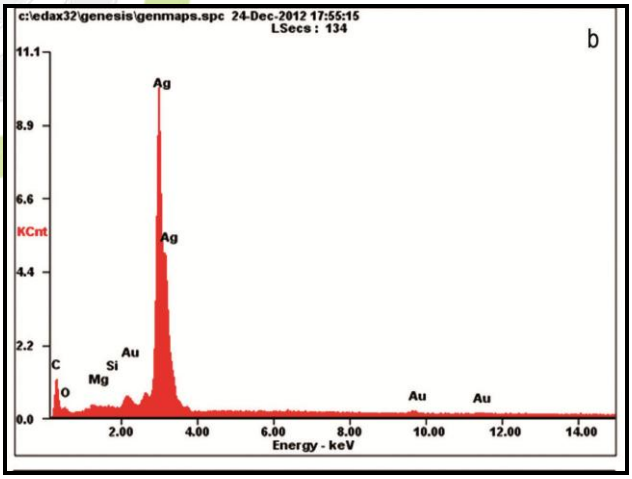

Figure 4: EDAX information of synthesized silver nanoparticles from B. ovalifoliolata gum

AFM analysis the SNPs were clearly distinguishable owing to their size difference. AFM images have given average sizes of SNPs of *B. ovalifoliolata* is 38.41 nm with three dimensional structures. SNPs attached with one another and look like a cluster in an area of 15 µm with rod shape in 3D view (Fig-5). The

physicochemical properties of nanoparticles differ dramatically from fine particles of the same composition.[26]

Antimicrobial Activity

In the present study the antimicrobial activity of silver nanoparticles was carried out against various pathogenic microbes such as gram negative and gram positive bacteria of *E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa* and *Bacillus subtilis,* fungal species of *Aspergillus niger, Aspergillus flavus, Fusarium, Curvularia* and *Rhizopus by* using disc diffusion method. The extraction without silver nanoparticles served as control. Gum aqueous extract of *B. ovalifoliolata* showed broad spectrum of antimicrobial activity. The diameter of inhibition zone around each disc with SNPs is represented and each disc contains of 10 µl of SNPs solution (Table-1). The SNPs of gum extract of *B. ovalifoliolata* showed highest antibacterial activity against *Klebsiealla* followed by *Bacillus, E. coli* and *Proteus* and lowest activity towards *Pseudomonas* and maximum inhibition zone was observed against fungal species of *A. flavus* followed by *Curvularia, Fusarium* and *Aspergillus niger* and minimum inhibition zone was observed against *Rhizopus* (Fig-6).

At low concentrations SNPs could prolonged the lag phase unit the concentration of SNPs was upto 40 µg/ml.[27] The inhibitory effect of silver is probably the sum of distinct mechanisms of action. Some studies reported that silver ions react with SH groups of proteins and play an essential role in bacterial inactivation. The uncouple respiratory electron transport from oxidative phosphorylation which inhibits respiratory chain enzymes or interferes with membrane permeability to protons and phosphate. The presence of silver ions and sulphur in the electron dense granules observed after silver ion treatment in the cytoplasm of bacterial cells suggests an interaction with nucleic acids that probably results in the impairment of DNA replication.[28] Thus, it is reasonable that the biosynthesized nanosilver can be used to manage the disease caused by *X. canpestris* pv. *malvacearum* in cotton plant.[27] Li *et al*.[29] reported that the antibacterial mechanism

Table 1: Antimicrobial activity of synthesized SNPs from gum of *B. ovalifoliolata*

S. No.	Name of the Pathogen	Standards	$Ag(NO_3)_2$	Plant extract	SNPs
1.	*Escherichia coli*	24.23±0.73	10.30±1.05	8.01±1.06	14.1±1.05
2.	*Pseduomonas aeruginosa*	25.54±2.04	12.22±1.10	8.12±0.14	10.14±2.01
3.	*Klebsiella pneumoneae*	27.61±1.50	13.62± 0.02	10.0±0.576	15.25±1.68
4.	*Bacillus subtillis*	25.10±1.02	11.25±0.05	8±0.577	14.59±1.12
5.	*Proteus vulgaris*	27.56±2.08	11.20± 0.23	10±1.000	13.14±0.03
	Fungal species	**Nystatin**			
6.	*Aspergillus flavus*	13.2±0.25	6.01±0.34	6.5±0.577	10.65±1.000
7.	*Aspergillus niger*	23.3±1.00	8.22±2.03	6.2±1.026	9.65±0.25
8.	*Fusarium oxysporum*	21.13±2.35	7.35±1.23	6.15±2.13	9.78±2.05
9.	*Rhizopus arrhizus*	15.56±0.55	8.14±0.21	6.5±0.577	8.63±0.71
10.	*Curvularia lunata*	14.5±0.58	6.63±1.52	7±1.000	10±2.000

'+' indicates standard error, '-' indicates no effect

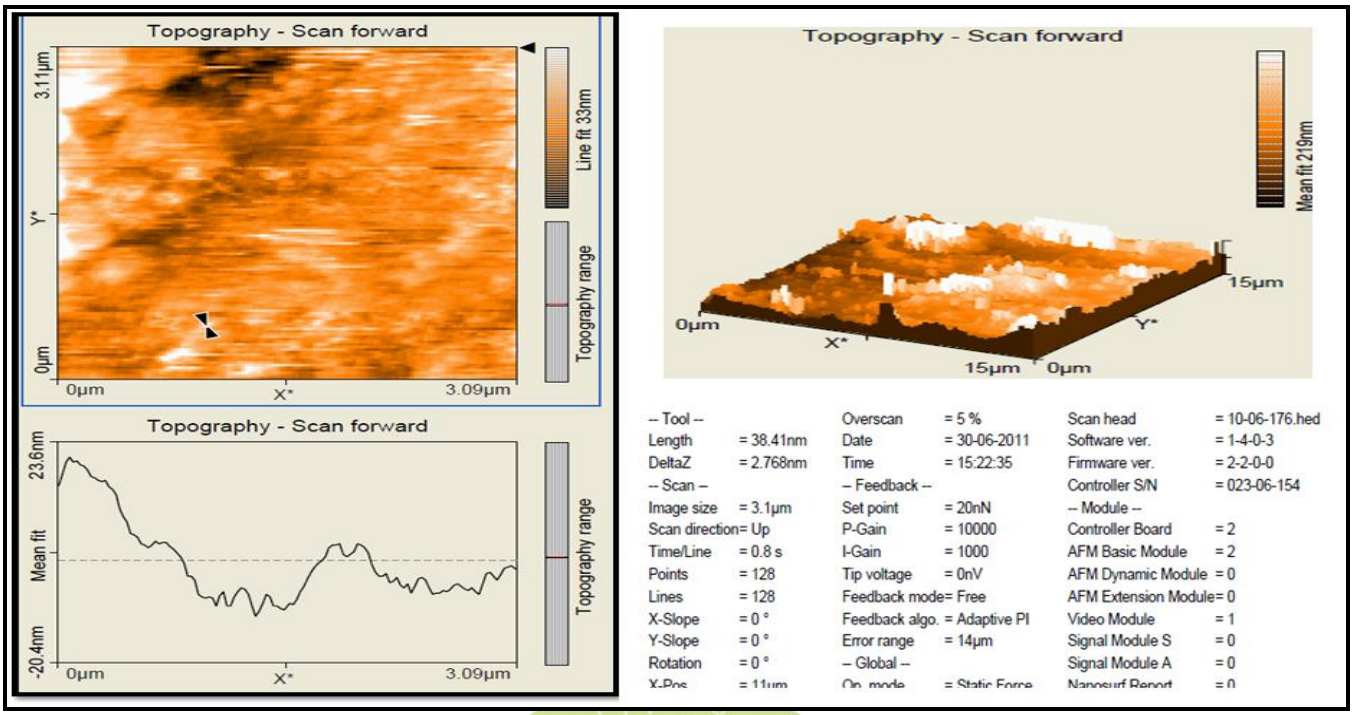

Figure 5: AFM data of synthesized SNPs from gum of *B. ovalifoliolata* a) size of the SNPs, b) three dimensional structure and c) plane of arrangement of SNPs.

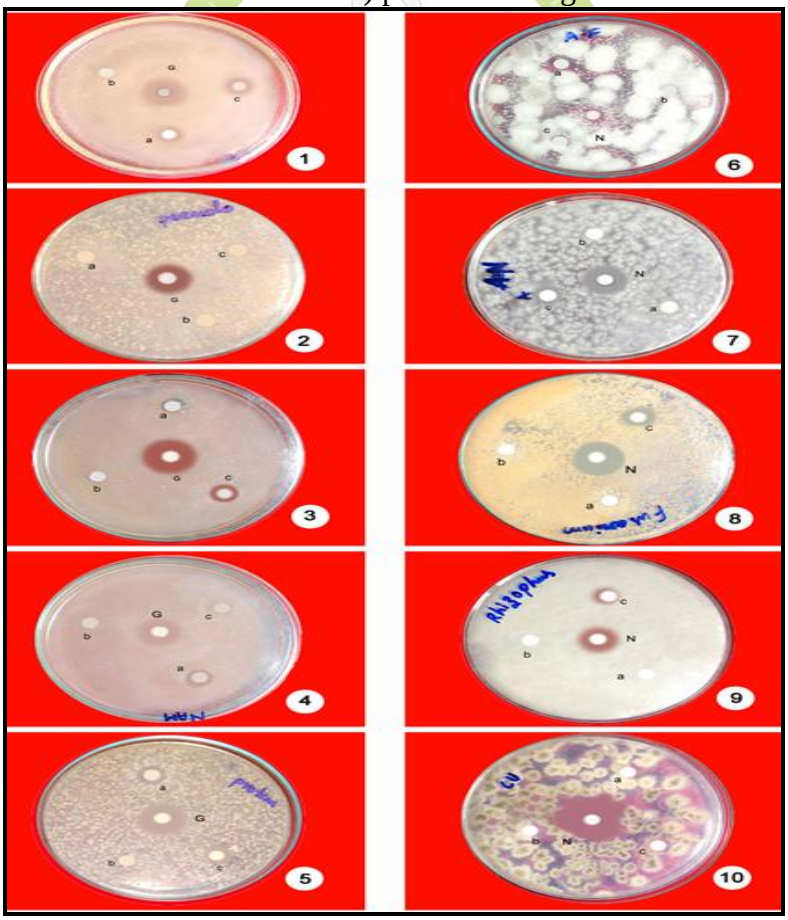

Figure 6: Antimicrobial activity of silver nanoparticles of gum of *B. ovalifoliolata;* 1. *E. coli*, 2. *Pseudomonas*, 3. *Klebsiella*, 4. *Bacillus*, 5. *Proteus*, 6. *A. flavus*, 7. *A. niger*, 8. *Fusarium*, 9. *Rhizopus*, 10. *Curvularia* G – Gentamycin, N – Nystatin 'a' – $Ag(NO_3)_2$, 'b' – Plant extract, 'c' – SNPs

of SNPs towards *E. coli* as a model organism and the leakage of proteins and reducing sugars at 2 h of exposure to 100 µg/ml of SNPs.

Recently, proteomics analysis revealed that even a short exposure of *E. coli* to nanosilver resulted in alterations in the expression of panel of envelope and Heat shock proteins (HSP).[30] Therefore, these particles can penetrate and can disrupt the membranes of bacteria. A massive loss of intracellular potassium was induced by nanosilver. Furthermore, the molecular targets for the nanosilver could be protein thiol groups (respiratory enzymes). The phospholipid portion of the bacterial membrane may also be the site of action for the nanosilver.[27]

The SNPs synthesized via green route are highly toxic towards bacterial strains when compared to fungal strains. Silver ions have been demonstrated to interact with the protein and possibly phospholipids associated with the proton pump of bacterial membranes.[22] These results in a collapse of membrane proton gradient causing a disruption of many of the mechanisms of cellular metabolisms and hence cell death.[31] Silver ions interact with a wide range of molecular processes within a microorganisms resulting in a range of effects from inhibition of growth and loss of ineffectiveness. The mechanism depends on both the concentration of silver ions present and the sensitivity of the microbial species to silver. The spectrum of activity is very wide and the development of resistance relatively low.[32]

The growth of microorganisms was inhibited by the green synthesized SNPs may be due to the presence of peptidoglycon, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This change may contribute to the sequestration of free silver ions.[22]

In the present scenario the bacterial and fungal strains are getting resistance to traditional and standardized drugs. It is inevitable to finding new drugs for curing ailments caused by microorganisms. The use of plant extracts is an effective against various microorganisms including plant pathogens. The silver nanoparticles of gum secreted from *B. ovalifoliolata* will definitely serve as novel drug for resistant microbial strains.

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