IJLSR (2019), Vol. 5, Issue 8 **Research Article**

IJLSR INTERNATIONAL JOURNAL OF LIFE SCIENCES AND REVIEW

Received on 20 June 2019; received in revised form, 24 August 2019; accepted, 29 August 2019; published 31 August 2019

SYNERGISTIC EFFECT OF BIO-CONJUGATE SILVER NANOPARTICLES WITH ANTIBIOTIC: ANTIBACTERIAL AGENT AGAINST MULTI-DRUG RESISTANCE BACTERIA

Richa Kothari * and Nagraj

Department of Chemistry, School of Sciences, ITM University, Gwalior - 474001, Madhya Pradesh, India.

ABSTRACT: Green synthesis of silver nanoparticles from naturally occurring biomaterials provides an alternative, eco-friendly and cost-effective means of obtaining nanoparticles. It is an important thrust area for all scientists and has gained importance because it prevents the environment from pollution. Multi-drug resistance is a growing problem in the treatment of infectious diseases and the widespread use of broad-spectrum antibiotics has produced antibiotic resistance for many human bacterial pathogens. Advancement in nanotechnology has opened a new field in nanomedicine, allowing the synthesis of nanoparticles and evaluation of their synergistic effect with pre-existing antibiotics that can be assembled into a complex structure. Novel studies and technologies are developed to understanding the mechanisms of disease for the design of new drugs, but unfortunately, infectious diseases continue to be a major health problem worldwide. Since ancient times, silver was known for its anti-bacterial effects and for centuries it has been used for prevention and control of disparate infections. Now-a-days vancomycin resistance in gram-positive *Cocci* is a growing global health problem. In the early 1950s, vancomycin was used as the primary antibacterial agent for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Progress in the development of new drugs to use vancomycin has been improved by using nanomedicines. Silver nanoparticles are attracting much interest because of their potent antibacterial activity. Many studies have also shown an important activity of silver nanoparticles against bacterial infections. This paper explains the new ideas to overcome current challenges and solutions in the treatment of infectious diseases, particularly the use of nanosilver particles as antimicrobials. The main objective of this work is to prepare silver nanoparticles from lichen, then prepare nanoconjugates with vancomycin antibiotics and explore their antibacterial properties. In the present study, we report a simple method of synthesis of bioconjugates of silver nanoparticles from ethanolic extract of lichen and their characterization by Ultraviolet-Visible (UV-vis), Fourier Transform Infrared (FTIR) spectroscopy, PL, XRD and TEM) analyses. Nanoparticles thus obtained were tested for antimicrobial activity against gram-positive bacteria and gram-negative bacteria.

Keywords: Biosynthesis, Lichen, Silver nanoparticles, Bioconjugates, Antimicrobial activity, XRD, TEM, MDRB

Correspondence to Author:
Richa Kothari

Department of Chemistry, School of Sciences, ITM University, Gwalior - 474001, Madhya Pradesh, India.

E-mail: richakothari70@gmail.com

INTRODUCTION: Metal nanoparticles (NPs) have attracted much attention during recent years owing to their excellent properties which are different from bulk material.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJLSR.5(8).117-24

The article can be accessed online on www.ijlsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJLSR.5(8).117-24

These particles gained importance during recent years owing to their broad-spectrum application in a number of processes such as agriculture, cosmetics, healthcare, drug or gene delivery, medical devices, biosensor and catalysis [1, 2, 3, 4, 5, 6, 7, 8, 9] beside their antimicrobial properties [10, 11]. Many metal NPs are essential nutrients to the living system while some are toxic [12]. Their efficiency depends on their shape and size. Among the coinage metals silver has highest thermal and electrical conductivity.

They may have a multidimensional structure such as nano-tubes and nano-wires. The green method of NP synthesis using plant extracts, bacteria, actinomycetes, fungi and enzymes are therefore, frequently used because of their environment-friendly nature and biocompatibility [13, 14, 15, 16]. The lichen, belonging to the Usneaceae family, grows like moss on trees in a temperate climate. They are slowest growing plants living in symbiosis with algae, fungi and perennial trees. Different genera of lichens are used in curing dyspepsia, amenorrhea and vomiting. Lichens produce secondary metabolites that are used as crude drugs. Silver nanoparticles have become a focus of interest because they play a significant role in biological systems, living organisms, and medicine [17, 18].

In the present medical industry, silver NPs are very useful in preventing infection in burns and open wounds [19]. Silver nanoparticles have also been reported to possess antifungal [20] antiviral [21], and anti-platelet activity [22]. They are also beneficial as antimicrobials and therapeutic agents [23, 24] and the development of Ag NPs as an antibacterial agent is underway. Silver nanoparticles (AgNPs) have found plenty of applications as antibacterial agents because of their biological activities and safety. Their synthesis is generally carried out through the reduction of Ag^{3+} using inorganic agents or through biogenic approaches.

Numerous functional bioconjugate NPs such as the single-strand DNA [25], anti-bodies, ciprofloxacin [26] phospholipids, and various proteins capped NPs, have emerged in the past decade for the development of surface modification techniques on chemical structure, allowing useful application in the fields of drug design, drug delivery, biosensors, and so on [27]. In our study vancomycin was conjugated to the biosynthesized silver nanoparticles, starch was used as a bridge linker and then its antimicrobial activities against vancomycin-resistant MDR pathogens were determined.

EXPERIMENTAL:

Materials and Methods: $AgNO_3$ (Merck, India Ltd.), ethanol (AR grade) and double distilled water were used. An aqueous solution of starch was used as a bridge linker. Lichen was procured from the medicinal garden of ITM University, Gwalior, India. UV-Vis spectral measurements were done with Perkin Elmer Spectrophotometer between 200 and 800 nm. FTIR spectra were recorded with Perkin-Elmer Spectrometer, FTIR spectrum one, in 4000-400 cm^{-1} region as KBr disc. TEM Images of Ag NPs were obtained using JEOL, JEM 2100 transmission electron microscope at 190 KV.

Samples were prepared using a drop of colloidal solution of Ag NPs on a carbon-coated copper grid and to allow the above sample to completely dry in a vacuum desiccator. The sediment particles obtained were used for scanning FTIR spectra. TEM images were obtained with a TEM electron microscope. Doubly distilled and deionized water was used as the solvent for preparing the stock solutions of all reagents. Buffer and all other chemicals were prepared according to standard laboratory procedures and the manufacturer's guidelines.

Preparation of Plant Extract: Lichens plants were collected from the medicinal garden of ITM University, Gwalior, Madhya Pradesh. Lichens were then washed thoroughly with distilled water and 20% alcohol then air-dried, homogenized. The sample is then filtered and dried by using a rotary evaporator. The dried sample was collected and stored at 4 °C for further use.

Phytochemical Screening of the Plant Extract: The extract was then subjected to qualitative phytochemical screening, which was done to understand the phytochemical constituents of the plant extract chosen for the study. For this, some specific tests were performed to evaluate the presence of particular phytochemicals. The tests performed for checking the availability of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins[28].

Synthesis of Silver Nanoparticles: Aqueous solution of silver nitrate 0.01M and thiosemicarbazide 0.01 M were prepared in separate beakers by adding 10 ml distal water and vigorous magnetic stirring for about 10 min. An ethanolic solution of lichen was added in silver nitrate solution and stirred for about 1 h. The solution of thiosemicarbazide is now added slowly dropwise and further stirred for another 30 min at

60 °C. The solution was then centrifuged at 4000 rpm for 20 min. The obtained NPs were washed several times using ethanol and dried at room temperature for 36 h in atmospheric condition was collected and allowed to centrifuge at 12000 rpm. Pellets were collected and then washed thrice by means of ethanol and then kept for drying on the dry bath. The sample is then collected and allowed for characterization.

Preparation of Bioconjugate of Silver Nano-particles: Conjugates were prepared to disperse Ag-NPs in 25 ml phosphate buffer (50mM, pH 6.5) in the presence of starch as a binding agent. After 30 min, 20 mg of vancomycin was added to the mixture and stirred at room temperature for 24 h under vigorous mixing. Excess vancomycin and other chemicals were eliminated by removing the supernatant after centrifugation at 12000 rpm for 20 min, and resuspended in deionized water and stored at 4 °C.

Characterization of Silver Nanoparticles: UV-Vis spectral measurements were done with a Perkin Elmer Spectrophotometer between 200 and 800 nm. FTIR spectra were recorded with Perkin-Elmer Spectrometer, in 4000-400 cm^{-1} region as KBr disc. TEM Images of NPs were obtained using JEOL, JEM 2100 transmission electron microscope at 190 KV. Samples were prepared using a drop of colloidal solution of Ag NPs on a carbon-coated copper grid and to allow the above sample to completely dry in a vacuum desiccator. The sediment particles obtained were used for scanning FTIR spectra.

Bacterial Strains and Antimicrobial Testing Conditions: Bacterial strains and their relevant attributes are mentioned in **Table 1**. Strains were received from PGI Chandigarh, India and maintained in sterile broth medium.

TABLE 1: BACTERIAL STRAINS USED IN OUR STUDY

S. no.	Bacterial strains	Strain no.
1	*Escherichia coli*	MTCC-1563
2	*Stayphylococcus aureus*	MTCC- 3160
3	*Enterococcus faecalis*	MTCC-439

To determine the minimum inhibitory concentration (MIC) of silver nanoparticles and vancomycin conjugate AgNPs strains were grown in Muller Hinton Broth with aeration at 37 °C for 24h. The effects of nanoparticles, pure vancomycin, and vancomycin capped AgNps on individual bacterial isolates were determined according to the following method [29]. The minimum inhibitory concentration (MIC) determined by the microdilution method with serially diluted samples. The samples were diluted to get a series of concentrations from 0.19 mg/ml to 100 mg/ml in sterile nutrient broth.

The bacterial inoculums were prepared by adjusting the turbidity to 0.5Mc Farland (1.5×10^8 CFU/ml) standard. The microorganism suspension of 50 microlitres was added to the broth dilutions. These were incubated for 18 h at 37°C. Then 10 microlitre of each suspension was added to the solution to obtain a final concentration. The lowest concentration of antibiotic preventing the appearance of turbidity is considered to be UV spectro-photometer at 660 nm. Similarly, this protocol was performed for plant extract, AgNPs, pure vancomycin and bioconjugate of AgNPs. The results were then compared to [30].

RESULTS AND DISCUSSION:

Qualitative Analysis of Phytochemicals Present in Plant Extract: The plant extract chosen for the study showed the presence of the following phytochemicals, discussed in **Table 2**.

TABLE 2: QUALITATIVE ANALYSIS OF PHYTO-CHEMICALS PRESENT IN PLANT EXTRACT

S. no.	Phytochemicals	Ethanolic extract of lichen
1	Steroids	Present
2	Alkaloids	Present
3	Flavonoids	Absent
4	Terpenoids	Absent
5	Tannins	Present
6	Saponins	Present
7	Phenol	Present
8	Quinone	Absent
9	Coumarin	Present
10	Protein	Present
11	Sugar	Present
12	Gum	Present

Visible Analysis: Silver nitrate solution has turned dark brown in color, which indicated the formation of AgNPs **Fig. 1**. This color change is due to the property of quantum confinement which is a size-dependent property of nanoparticles that affects the optical property of the nanoparticles. The color change in silver nitrate solution treated with Plant leaf extract.

FIG. 1: SYNTHESIZED SILVER NANOPARTICLES FROM PLANT EXTRACT

UV Spectra: It is known that when nanoparticles are formed, the colour of the solution containing both the NPs and plant extract turns dark brown depending on the presence of organic molecules in the extract. In our study, $AgNO_3$ was mixed with ethanolic extract of lichens incubated at room temperature; its color turned dark brown after 24h. The UV-Vis spectrum of this colloidal solution was run from 200 to 800 nm at room temperature which displayed peaks at 350 and 400 nm. The highest peak at 385 nm has been attributed to the excitation of surface plasmon resonance (SPR) of AgNPs.

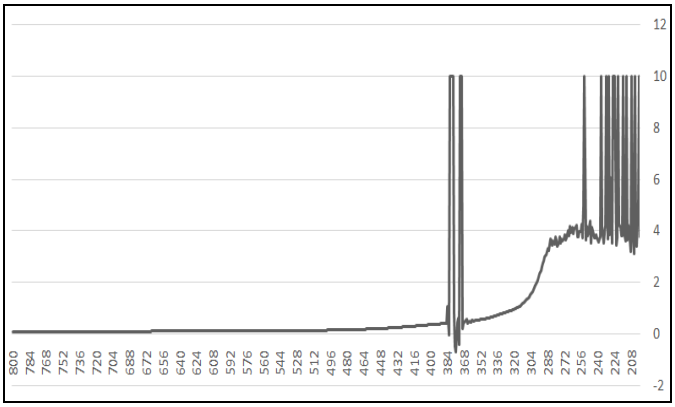

FIG. 2: ULTRAVIOLET-VISIBLE ABSORPTION SPECTRUM OF SYNTHESIZED SILVER NANO-PARTICLES USING ALCOHOLIC EXTRACT OF LICHEN

Photo-oxidation of chemical constituents present in the extract may also have occurred [29]. Profile of the UV-Vis spectrum depends on the concentration of substrate, silver ions). Colour of these NPs remained unchanged even after several weeks [30]. It has been observed that when the colloidal solution containing Ag NP is slowly heated up to 60 °C the color intensity increases with increasing temperature and NPs are quickly formed. It demonstrates the effect of temperature on the biosynthesis of NPs. Absorption peaks in the UV-Vis spectrum are related to the shape of NPs. According to the criterion of Zhang and Nogues [31], the peaks at 385 nm correspond to cubical Ag NPs. Since we have observed a major peak at 385 nm AgNPs are supposed to be mainly spherical though the presence of a small amount of other types of NPs cannot be ignored.

FT-IR Spectral Analysis: FT-IR spectrum was run to identify the involvement of biomolecules present in lichen for the reduction of $AgNO_3$ to AgNPs. It is known to contain phenol, amines, aldehydes, and ketones besides many other compounds in traces. Since, all these compounds are excellent reducing agents, they undergo changes in stretching frequencies of their functional groups as a consequence of the reduction of $AgNO_3$ to AgNPs. IR spectrum is very complicated because of the overlap of frequencies in the same region.

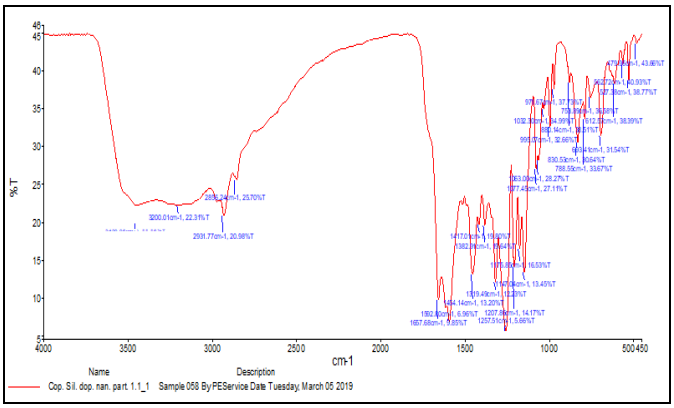

FIG. 3: FT-IR SPECTRA OF SILVER NANOPARTICLES

However, we have attempted to identify the shifts in stretching vibrations after the formation of Ag NPs. Primary amines exhibit two N-H stretching frequencies in 3500-3300 cm^{-1} region which has been found to appear at 3410 and 3455 cm^{-1} in the NPs containing lichen extract. The band is 1600-1500 cm^{-1} is due to CO stretching but amide bands also appear in the same region of the spectrum.

We have observed amide I and amide II bands at 1658 and 1540 cm^{-1}. A band at 1560 cm^{-1} has been assigned to (C=O) stretching frequency. The COO^- group generally appears above 1600 cm^{-1} but overlaps with amide II band [32]. These spectral results indicate the involvement of organic molecules in the reduction of $AgNO_3$ leading to the formation of Ag NPs.

Fluorescence Analysis: The emission spectra of biosynthesized silver nanoparticles have been measured in the ethanolic solution at room temperature in a very dilute solution (10^{-3} to 10^{-5} mol). The fluorescence spectra of AgNPs are shown in **Fig. 4**. The AgNPs showed an emission band at 395 nm, 450 nm upon photoexcitation at 384 and a weak band at 695 nm.

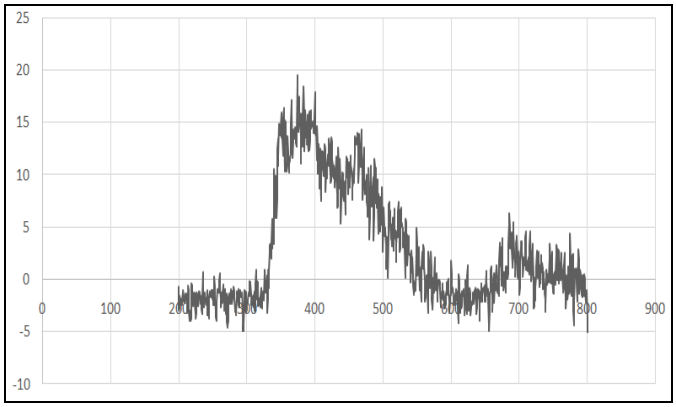

FIG. 4: FLUORESCENCE SPECTRA OF SILVER NANOPARTICLES

XRD Analysis: The synthesized AgNPs were further characterized using X-ray diffractometry **Fig. 5**. The X-ray diffraction pattern showed four intense peaks (38.2°, 44.3°, 64.5°, 77.5°) in the whole spectrum of 2θ values ranging from 20° to 80°, which correspond to the (111), (200), (220), and (311) crystallographic planes of face-centered cubic silver, respectively. The crystallite domain size was calculated using the Debye-Scherrer formula: D =0.94 λ/β cos θ, where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the wavelength of $Cu_{k\alpha}$, β is the full width at half maximum, and θ is the Bragg diffraction angle. The average crystallite size of the synthesized AgNPs was 0.54 nm, derived from the full width at half maximum of the peak corresponding to the (111) plane, which is in a good agreement with the particle size determined by TEM analysis[31].

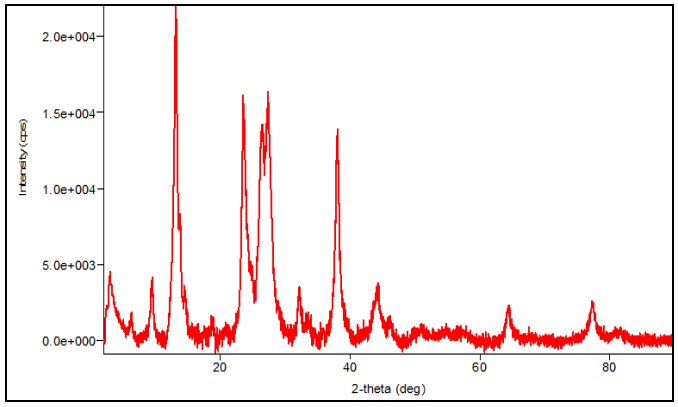

FIG. 5: XRD SPECTRA OF SILVER NANO-PARTICLES

TABLE 3: DETERMINATION OF CRYSTALLINE SIZE OF BIOSYNTHESIZED AgNPs BY USING DEBYE-SCHERRER'S EQUATION

S. no.	2-theta (deg.)	d (angle)	FWHM(deg.)	Particle size
1.	38.2	2.36	0.652	0.300nm
2.	44.3	2.054	1.40	0.21 nm
3.	64.5	1.44	0.84	1.2 nm
4.	77.5	1.234	0.91	0.46nm

Size and Morphology of Biosynthesized Silver Nano Particles & Its Bioconjugate: The shape and size morphology of biosynthesized Ag- nano-particles and its bioconjugate were characterized using Transmission Electron Microscopic (TEM) study as shown in **Fig. 6A-B** which demonstrated the formation of slightly spherical AgNPs and irregularly shaped its bioconjugate. The size of the AgNPs & its bioconjugate is 50 nm 200 nm.

***In-vitro* Antibacterial Screening:** Bacteriological examination was performed in sterile nutrient for both liquid systems. The conjugates exhibited antibacterial activity against MDR bacterial strains. The MIS of plant extract, pure vancomycin and vancomycin capped silver nanoparticles for these bacteria was calculated as the lowest concentration at which bacterial growth was inhibited [32].

The MIC of pure vancomycin and AgNPs and vancomycin capped AgNPs for these bacteria was calculated as the lowest concentration at which bacterial growth was inhibited. The MIC of pure vancomycin for gram-negative strains was ≥3.2 µg/ml. When pure AgNPs were employed, the MIC was 0.186 µg/ml, 0.197 µg/ml & 0.051 µg/ml. Furthermore, when NPs are used as a capping agent in combination with vancomycin, MIC decreased considerably. In this condition, the MIC were 0.009 µg/ml, 0.053 µg/ml & 0.039 0 µg/ml. The MIC corresponded to the MBC in all bacterial species. The results are shown in **Table 4**.

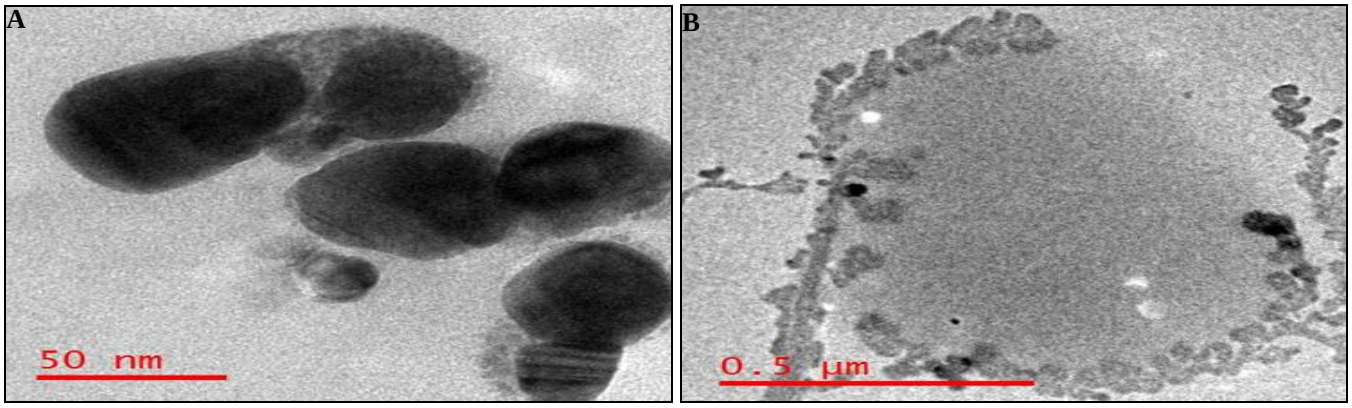

FIG. 6: TEM MICROGRAPH OF (A) BIOSYNTHESIZED Ag NANOPARTICLES (B) ANTIBIOTIC CAPPED AgNPs

TABLE 4: EFFECTS OF PLANT EXTRACT, BIOSYNTHESISED AgNPs, PURE VANCOMYCIN AND VANCOMYCIN CAPPED SILVER NANOPARTICLES

S. no.	Bacterial strains	Plant extract	Biosynthesised AgNPs	Pure vancomycin	Vancomycin capped AgNPs
1	*Escherichia coli* (MTCC -1563)	0.25	0.186	> 3.2	0.009
2	*Staphylococcus aureus* (MTCC-31620)	0.20	0.197	3.2	0.053
3	*Enterococcus faecalis* (MTCC- 439)	0.197	0.051	3.2	0.006

FIG. 7: *IN-VITRO* ANTIBACTERIAL ACTIVITY (A) PLANT EXTRACT (B) BIOSYNTHESIS SILVER NANOPARTICLES (C) VANCOMYCIN CAPPED SILVER NANOPARTICLES

The literature revealed that AgNPs with larger surface area provides better contact with microorganisms [33]. Thus, these particles may penetrate the bacterial cell membrane or attach to the bacterial surface and inhibit their replication [34, 35]. In our experiment, AgNPs have been found to be most effective against *Enterococcus faecalis*. It has been reported that antibacterial efficiency is increased by lowering the particle size [36]. Usually, NPs attach to the cell wall of bacteria and damage membrane and respiration system leading to cell death [37]. The toxicity of smaller NPs was greater than those of larger ones because the smaller ones can easily adhere to bacterial cell wall [38].

Mode of Action of Silver Ions: Silver ions penetrate into the cytoplasm; denature the ribosome leading to the suppression of enzymes and proteins which eventually arrest their metabolic function resulting in apoptosis of bacteria. Bactericidal activity is due to silver ions released from Ag NPs as a consequence of their interaction with microbes [11]. However, four possible mechanisms of antibacterial activity of AgNPs have been proposed (a) interference during cell wall synthesis (b) suppression during protein biosynthesis (c) disruption of the transcription process and (d) disruption of primary metabolic pathways [17].

Each mechanism involves structural changes, biochemical changes and charges on both the silver ions and biomolecules in the microbial cells. AgNPs also inhibit the proliferation of cancer cell lines by different modes of action [37]. They mediate and amplify the death signal by triggering the activation of Caspase-3 molecule. The DNA splits

into fragments by Caspase-3. Ag NPs may interfere with the proper functioning of cellular proteins and induce subsequent changes in cellular chemistry. Sometimes Ag NPs alter the function of mitochondria by inhibiting the catalytic activity of lactate dehydrogenase. AgNPs may also cause proliferation of cancer cells by generating ROS which ultimately leads to DNA damage [40].

CONCLUSION: The present study aims to employ the use of plants as a source of synthesizing silver nanoparticles with less hazards towards the environment, easily scaled up, stable and economically viable. The silver nanoparticles were synthesized by using lichen extract. The biosynthesized nanoparticles are then allowed to characterize by means of visible observation, UV Spectroscopy, XRD, & TEM analysis.

The present study provides new insight into the synthesis of stable silver metal nanoparticles with less hazards along with exploring its antibacterial activity in the development of a potential tool in biomedics. In our study, we obtained the UV-Vis absorption of the AgNPs around 385 nm that shows the plasmaon resonance peak. The shape and size of AgNPs and the context in which the assay is performed are important factors in its capacity to annihilate superbugs.

This work explained that vancomycin can be conjugated with AgNPs to enhance antibacterial activity against gram-positive bacteria, but not gram-negative bacteria which can be justified because it is associated with the outer membranes of the gram-negative bacteria. *In- vitro* bactericidal examinations showed that the vancomycin capped with AgNPs exhibited substantial activity against MDRB. According to these results, we can say that such a nano-conjugate system may provide a very important method for the development of a new generation of effective antibacterial agents.

ACKNOWLEDGEMENT: The authors are thankful to the management of ITM University for providing the lab facilities and the Director, SAIF, Jiwaji University for recording XRD, TEM spectra. The authors also acknowledge the PBRI, Bhopal for providing the antibacterial activity studies.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Siddiqi KS, Husen A, Sohrab SS and Osman M: Recent status of nanomaterials fabrication and their potential applications in neurological disease management. Nano Res Lett 2018; 13: 231.
2. Husen A and Siddiqi KS: Phytosynthesis of nanoparticles: concept, controversy and application. Nano Res Lett. 2014; 9: 229.
3. Husen A and Siddiqi KS: Plants and microbes assisted selenium nanoparticles: characterization and application. J Nanobiotechnol 2014; 12: 28.
4. Husen A and Siddiqi KS: Carbon and fullerene nano-materials in plant system. J Nanobiotechnol 2014; 12: 16.
5. Siddiqi KS and Husen A: Fabrication of metal nano-particles from fungi and metal salts: scope and application. Nano Res Lett 2016; 11: 98.
6. Siddiqi KS and Husen A: Fabrication of metal and metal oxide nanoparticles by algae and their toxic effects. Nano Res Lett 2016; 11: 363.
7. Siddiqi and Husen A: Engineered gold nanoparticles and plant adaptation potential. Nano Res Lett 2016; 11: 400.
8. Siddiqi KS and Husen A: Green synthesis, characterization and uses of palladium/platinum nanoparticles. Nano Res Lett 2016; 11: 482.
9. Siddiqi KS, Rahman A and Tajuddin HA: Biogenic fabrication of iron/iron oxide nanoparticles and their application. Nano Res Lett 2016; 11: 498.
10. Siddiqi KS and Husen A: Recent advances in plant-mediated engineered gold nanoparticles and their application in biological system. J Trace Elements Med Biol 2017; 40: 10-23.
11. Siddiqi KS, Husen A and Rao RAK: A review on biosynthesis of silver nanoparticles and their biocidal properties. J Nanobiotechnol 2018; 16: 14.
12. Siddiqi KS, Rahman A and Tajuddin HA: Properties of zinc oxide nanoparticles and their activity against microbes. Nano Res Lett 2018; 13: 141.
13. Tagad CK, Dugasani SR, Aiyer R, Park S, Kulkarni A and Sabharwal S: Green synthesis of silver nanoparticles and their application for the development of optical fiber-based hydrogen peroxide sensor. Sensors Actuators B Chem 2013; 183: 144-49.
14. Venkateswarlu S, Kumar BN, Prathima B, Anitha K and Jyothi NVV: A novel green synthesis of Fe_3O_4–Ag core shell recyclable nanoparticles using *Vitis vinifera* stem extract and its enhanced antibacterial performance. Physica B 2015; 457: 30-35.
15. Rao Y, Kotakadi VS, Prasad TNVKV, Reddy AV and Sai Gopal DVR: Green synthesis and spectral characterization of silver nanoparticles from Lakshmi tulasi (*O. sanctum*) leaf extract. Spectrochim Acta A 2013; 103: 156-59.
16. Husen A: Gold nanoparticles from plant system: synthesis, characterization and their application. Nanoscience and plant–soil systems, Springer international publishing AG, Gewerbestrasse 11, 6330 Cham, Switzerland 2017; 48: 455-79.
17. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Deepak V, Pandian SRK and Muniyandi J: Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Surf B Biointerfaces 2009; 74: 328-35.
18. Parashar V, Parashar R, Sharma B and Pandey AC: Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Dig J Nanomater Biostruct 2009; 4: 45-50.

19. Ip M, Lui SL, Poon VKM, Lung I and Burd A: Antimicrobial activities of silver dressings: an *in-vitro* comparison. J Med Microbiol 2006; 55(Pt 1): 59-63.
20. Kim KJ, Sung W and Suh B: Antifungal activity and mode of action of silver nano-particles on *Candida albicans*. Biometals 2009; 22: 235-42.
21. Lara H, Ayala-Nunez N, Ixtepan-Turrent L and Rodriguez-Padilla C: Mode of antiviral action of silver nanoparticles against HIV-1. J Nanobiotechnology 2010; 8: 1.
22. Shrivastava S, Bera T, Singh SK, Singh G, Ramachandrarao P and Dash D: Characterization of anti-platelet properties of silver nanoparticles. ACS Nano 2009; 3: 1357-64.
23. Rai M, Yadav A and Gade A: Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 2009; 27: 76-83.
24. Kim JS, Kuk E and Kim JH: Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med 2007; 3: 95-101.
25. Thaxton Shad C, Haley Hill D, Georganopoulou DG, Stoeva SI and Mirkin CA: A bio-bar-code assay based upon dithiothreitol-induced oligonucleotide release. Anal Chem 2005; 77(24): 8174-78.
26. Banoee M, Seif S, Nazari ZE, Jafari-Fesharaki P, Shahverdi HR and Moballegh A: Zno nanoparticles enhanced antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. J Biomed Mater Res B Appl Biomater 2010; 93(2): 557-61.
27. Wang S, Mamedova N, Kotov NA, Chen W and Studer J: Antigen/antibody immunocomplex from CdTe nano-particle bioconjugates. Nano Lett 2002; 2(8): 817-22.
28. Krishnaiah D, Devi T, Bono A and Sarbatly R: Phytochemical screening and *in-vitro* antioxidant properties of the stem bark of *Trichilia tessemannii* (Harms) (Meliaceae). World Journal of Pharmacy and Pharmaceutical Sciences 2015; 4(3): 76-90.
29. Delahaye C: Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis*. Journal of Medicinal and Biological sciences 2009; 3: 1-7.
30. Esmaeillou M: Vancomycin capped silver nano particles as an antibacterial agent against multi drug resistant bacteria. Advanced Pharmaceutical Bulletin 2017; 7(3): 479-83.
31. Thilagam M, Tamilselvi A, Chandra Sejkeran B and Rose C: Phytosynthesis of silver nanoparticles using medicinal and dye yielding plant of *Bixa orellana* L. leaf extract. J of Pharmaceutical & Scientific Innovation 2013; 2(4): 9-13.
32. Eugino M, Muller N, Frases S, Almeida-Paes R, Mauricio LMTR, Lemgruber L, Farina M, de-Souza W and Anna CS: Test-derived biosynthesis of silver/silver chloride nanoparticles and their anti-proliferative activity against bacteria. RSC Adv 2016; 6: 9893-04.
33. Rajput S, Werezuk R, Lange RM and McDermott MT: Fungal isolate optimized for biogenesis of silver nanoparticles with enhanced colloidal stability. Langmuir 2016; 32: 8688-97.
34. Zhang JZ and Nogues C: Plasmonic optical properties and applications of metal nanostructures. Plasmonics 2008; 3: 127-50.
35. Nakamoto K: Infrared and Raman spectra of inorganic and coordination compounds, part a and part B, John Wiley & Sons, Inc. USA, Edition 6th, Vol. set 2, 2009.
36. Prabhu S and Poulose EK: Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, & toxicity effects. Int Nano Lett 2012; 2: 32.
37. Hong X, Wen J, Xiong X and Hu Y: Shape effect on the antibacterial activity of silver nanoparticles synthesized *via* a microwave-assisted method. Environ Sci Pollut Res. 2016; 23: 4489-97.
38. Agnihotri S, Mukherji S and Mukherji S: Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy. RSC Adv 2014; 4: 3974-83.
39. Saravanan M, Amelash T, Negash L, Gebreyesus A, Selvaraj A, Rayar V and Deekonda K: Extracellular biosynthesis and biomedical application of silver nanoparticles synthesized from Baker's yeast. Int J Res Pharm Biomed Sci 2013; 4: 82228.
40. Bonnigala B, Aswani Kumar YVV, Vinay Viswanath K, Joy Richardson P, Mangamuri UK and Poda S: Anticancer activity of plant mediated silver nanoparticles on selected cancer cell lines. J Chem Pharma Res 2016; 8: 276-81.

How to cite this article:
Kothari R and Nagraj: Synergistic effect of bio-conjugate silver nanoparticles with antibiotic: antibacterial agent against multi-drug resistance bacteria. Int J Life Sci & Rev 2019; 5(8): 117-24. doi: 10.13040/IJPSR.0975-8232.IJLSR.5(8).117-24.