JJBS

Volume 13, Number 4, December 2020
ISSN 1995-6673
Pages 493 - 498

Jordan Journal of Biological Sciences

# Biogenic Silver Nanoparticle Synthesis, Characterization and its Antibacterial activity against Leather Deteriorates

Savita Kate[1,*], Madhuri Sahasrabudhe [2] and Archana Pethe [3]

[1]Department of Biotechnology, Shivchhatrapati College, [2]Department of Microbiology Maulana Azad College , Aurangabad-431001,
[3]Department of Microbiology, Shivaji College of Arts, Commerce and Science,Akola-444001,M.S., India

Received: October 26, 2019; Revised: December 25, 2019; Accepted: December 31, 2019

**Abstract**

Biogenic Silver Nanoparticles (AgNPs) were synthesized using aqueous plant extract of *Portulaca oleracea* L.(Portulacaceae) at 75°C in 20min. AgNPs were confirmed by UV-visible spectra. Highest absorbance peak was found at 420nm; FTIR showed the peaks at 515.24 $cm^{-1}$ with significant changes upon reduction, biogenic AgNPs. Dmean number was found to be 52.26 nm and zeta potential -29.1mV. AgNPs crystalline particle size was found in range of 28.8 nm to 30.08 nm by XRD. Scanning Electron Microscopy (SEM) analysis confirms AgNPs were spherical in shape. The biogenic synthesized AgNPs showed distinct zone of inhibition against all bacterial leather isolates ranging from 14.66±1 to 20.33±1.52 mm. Minimum inhibitory concentration (MIC) values of the biosynthesized silver nanoparticles against bacterial isolates ranged from 4-20 µg/ml. Experimental outcomes propose that the biogenic silver nanoparticles showed efficient antibacterial activity against leather deteriorates and can be used for preservation of leather.

**Keywords:** leather deteriorates, MIC, SEM, Zeta potential

## 1. Introduction

Nanotechnology is a recent field that is used in research nowadays, creating an impression in all domains of human life. Biogenic synthesis of nanoparticles has proven to be better methods over physical and chemical methods due to slower kinetics and their stabilization. There are multiple opportunities to develop greener processes for the manufacture of nanoparticles. Due to involvement of hazardous chemical, low material conversions, high energy requirement, and difficult, wasteful purification in physical and chemical methods, biosynthesis of silver nanoparticles using plant extract and microorganism has been practiced. The most predominant method for the synthesis of silver nanoparticles is using plant extract owing to easy availability, cost efficiency, eco-friendliness, and non-toxicity (Firdhouse et al., 2012). *Argimone mexicana*, *Tridax procumbens* L., *Jatropa curcas* L., *Calotropis gigantea* L., *Solanum melongena* L., *Datura metel* L., *Carica papaya* L. and *Citrus aurantium* L. leaf extracts were involved in green synthesis of silver nanoparticles, and evaluations of their antimicrobial activities were studied for *Aspergillus flavus*, *Escherichia coli* and *Pseudomonas aeruginosa* and were found to be highly effective ((Khandelwal et al.,2010; Rajasekharreddy et al.,2010). Plants extracts usually include various polyphenols, such as flavonoids, which act as the best reducing agent useful in the synthesis of silver nanoparticles. Luteolin or rosmaric acid reducing substances are found in *Ocimum sanctum*, *Portulaca olerecea*. Reducing agents are most conceivably responsible for the conversion of $Ag^{+}$ to $Ag^{0}$ which occurs during the formation of enol/keto form of those substances. The resulting forms revealed promising antibacterial properties against microorganism such as *Escherichia coli*, *Staphylococcus aureus* and *S. typhi*, *Proteus* spp. (Muthumary et al. 2011).

The leather is a richest source of nutrients for microorganisms. The fibrous proteins present in leather are collagen (98%), elastin (1%) and keratin (1%) (Tissier and Chensais, 2000). Leather making is an important socio-economic activity for several countries throughout the world and used everywhere in daily life. The decomposition of collagen, the major component of leather, has been studied more extensively. Raw hides deteriorated easily and their deterioration depends on a number of factors: time, temperature, moisture content, and the state of the hide. Collagenase activity by *Clostridium* spp., *Bacteriodes* spp., and *Staphylococcus aureus* had earlier been reported (Jaouadi et al. 2013). During the manufacture processes of finished leather from raw hide, the bacterial flora of leather was found to be changed. *Bacillus* spp. was the most prevalent, and was found in nearly all steps of the tanning process. Not surprisingly, *Bacillus* spp., especially *Bacillus cereus* and *Bacillus subtilis* , have been correlated with the majority of cases of deterioration of hides and skins (Baird, 1998). However, not much previous research has been done to inhibit growth of bacterial leather isolates (leather bacterial growth) using pure biogenic silver nanoparticles. Therefore, the present work was undertaken to study biogenic silver nanoparticles synthesis and its antibacterial effect against bacteria isolated indigenously from leather samples.

* Corresponding author e-mail: savitaakate@gmail.com.

## 2. Materials and Methods

### *2.1. Isolation and identification of bacteria:*

In the present study deteriorated leather samples were collected from Kedar leather industry, Aurangabad, M.S. in sterilized polythene bags and were brought to the laboratory. Leather deteriorating bacteria were screened by inoculating collected deteriorated leather samples into peptone solution (1% peptone water) incubated at 37°C for 24h and isolation of bacteria was carried out on nutrient agar M002-Hi-Media (Mozotto et al. 2010). To check the ability of different leather isolates to hydrolyze main leather protein collagen, collagen agar plates (1% collagen peptide type I, TC343-Hi-Media and 2% agar Hi-Media) were used and incubated at 37°C for 48h. Bacterial leather isolates showing collagenolytic activity were screened with larger transparent circle around the bacterial colony after incubation by adding a drop of mercuric chloride precipitation reagent (Lili et al. 2010). As per Bergey's manual of systematic bacteriology second edition process, the bacterial leather isolates were identified. The isolates were further subjected for genetic analysis by 16s rDNA sequencing. The sequences obtained were deposited to GenBank for accession number.

### *2.2. Biogenic silver nanoparticles (AgNPs) synthesis*

*Portulaca oleracea* L. aqueous extract (10%) was prepared by boiling fresh washed leaves in sterile distilled water for 10 mins then filtered through whatmann filter paper No.1. To10 ml of silver nitrate solution (1mM )1ml of aqueous extract of *Portulaca oleracea* L. was added then incubated at 75°C for various time intervals 20-35 min. The colour change from yellowish to reddish brown in the reaction mixture was confirmed biosynthesis of nanoparticles (Firdhouse et al., 2013; Mittal, 2012).

### *2.3. Characterization of Silver Nanoparticles*

Characterization of synthesized biogenic silver Nanoparticles was carried out by Ultraviolet-visible spectrophotometry (Elico), Fourier transform infrared spectroscopy **(**FTIR) measurements (Bruker Corporation), Scanning electron microscopy (SEM) , Nanoparticles size analysis, Zeta potential determination (Malvern Zetasizer Nano-ZS) and X-ray diffraction studies (XRD) (D8 advance Bruker axs) (Alzahrani et al., 2015; Khushboo Singh, 2014; Lilyprava Dash, 2013 and Sumitra Chanda, 2013).

### *2.4. Antibacterial activity of synthesized biogenic AgNPs*

#### *2.4.1. Agar well diffusion method*

0.1 ml ($O.D._{600}$ 1.0) of selected bacterial leather isolates was added in different soft agar and mixed well. Seeded soft agar was then plated on basal agar (Muller Hilton agar) plates. After 30 minutes of cooling incubation, inoculated plates were punched using cork borer for obtaining 6mm wells. In one well, 25µl of biogenic silver nanoparticles (0.1g/L) were added. These cultures were also tested against Streptomycin (300mcg), Chloramphenicol (25mcg), Fusidic acid (10mcg), Erythromycin (5mcg), Methicillin (10mcg) and Novobiocin (5mcg) discs (Hi media Pvt. Ltd., India). All inoculated plates were incubated at 37°C for 24h. Diameter of zone of inhibition was measured in mm; the experiments were carried out in triplicates and mean values of zone diameter were determined (Bauer and Kirby, 1966; Awad et al., 2014).

### *2.5. Minimum inhibitory concentration (MIC) of biogenic silver Nanoparticles for bacteria:*

In the present work, minimum inhibitory concentration was determined by standard broth dilution method (Sushmita Deb, 2014 and Zohresh Majidnia, 2012). The MIC was performed in 2ml Muller-Hinton broth(MH) using AgNPs in varying concentrations ranging from 1-10-20µg/ml in test tubes, while one was maintained as blank with AgNPs, not having bacterial suspension (negative control) and second tube having only bacterial suspension, not AgNPs. 0.5 MacFard standard bacterial inoculums were prepared in sterile saline which was equals $10^6$ Cfu/ml. The tubes were then incubated at 37°C for 24 h. After incubation, the MIC was determined by measuring the growth density of test organisms at 600nm on spectrophotometer (Sushmita Deb, 2014; Zohresh Majidnia et al., 2012). MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

### *2.6. Leather preservation:*

Leather preservation was carried out using biogenic silver nanoparticles as per protocol mentioned by Petica1 (2013) with some modification. Leather samples with the control sample size of 1 × 2 $cm^2$ were sterilized using 70% ethanol for 20 minutes, then leather samples were washed for 30 min with sterile distilled water and were immersed three times in biogenic silver nanoparticles 0.1 g/L at 30°C for 1h, followed by free drying. Treated and untreated samples were exposed to leather isolates then kept in incubator and checked for the efficiency of biogenic AgNps in the form of bacteria growth on samples.

## 3. Result and Discussion

### *3.1. Isolation, screening and identification of leather deteriorates*

From collected fifty deteriorated leather samples, twenty seven bacteria were found to be hydrolyzing keratin, collagen (leather main protein), gelatin and casein at 37°C after 24-48h of incubation. Most proficient five bacterial leather isolates were further identified by morphological, biochemical, 16srDNA sequences(Om gene bio, Pune) submitted to GenBank with accession number: *Arthrobacter creatinolyticus* KJ009396**,** *Bacillus pumilus* KP015747 (H4.9/8), *Bacillus megaterium* KM369985 strain SAK, *Bacillus amyloliquefaciens* KP015745 (strain JS518) and *Bacillus cereus* KP015746 (Lr3/2).*Staphylococcus* sp. and *Brevibacterium* sp. *Pseudomonas* sp. and *Bacillus* sp. from some deteriorated footwear samples reported by Sanchez-Navarro (2013). *Bacillus subtilis, A. awamori, Pseudomonas aeruginosa* were reported from soaking of animal skins (Zambare et al., 2013), Jaouadi et al., (2013) isolated *B. licheniformis* PWD-1, *Brevibacillus brevis.* We isolated *Arthrobacter creatinolyticus* KJ009396from deteriorated leather sample which is the first report.

*3.2. Biogenic Silver nanoparticles (AgNPs) synthesis*

Biogenic AgNPs were synthesized by using *Portulaca oleracea L.* in 20 mins at 75°C at 1:10 ratio of plant extract to 1mM of silver nitrate solution, formation reddish brown colour in reaction mixture after incubation indicates biogenic AgNPs synthesis. Formation of AgNPs at different time intervals may be due to variation in secondary metabolites present in the plant extracts (Firdhouse, 2012). Secondary metabolites play an important role in reduction of silver nitrate to AgNPs and for capping of synthesized AgNPs(P. Lalitha, 2015). Singha (2014) reported AgNPs synthesis at 100°C in 15 mins using *Neptunia oleraceae* plant extract of 1:10 ratio, by using aqueous extract of *Brassica oleracea, capitata*, and *Phaseolus vulgaris* silver nanoparticles were synthesized in dark after 24h (Sushmita Deb,2014). Silver nanoparticles synthesis was reported by Vanaja (2013) from stem extract of *C. aromaticus* in 4 h time of incubation and observed that colour intensity was increased while increasing the time incubation which confirmed increased silver nanoparticles synthesis.

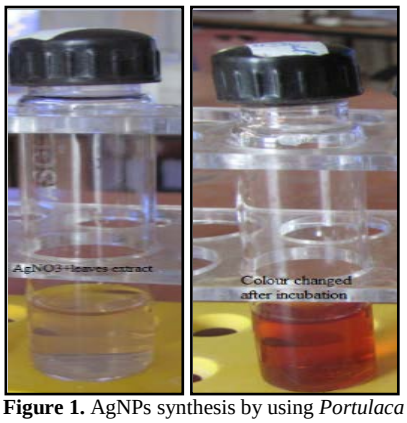


**Figure 1.** AgNPs synthesis by using *Portulaca oleracea L.*

*3.3. Characterization of Silver Nanoparticles*

UV-Vis Spectroscopy

Formation of silver nanoparticles was confirmed by UV-visible spectra, and highest absorbance peak was found at 420 nm (Fig. 2). UV-visible spectra show no evidence of absorption in the range of 400-800 nm for the plant extract. Singha (2014) reported the UV absorption peaks of AgNPs by *N. oleraceae* from 400 to 440 nm clearly indicating the formation of spherical AgNPs by using plants extract. Creation of peak at this range is owing to the phenomenon of surface plasmon resonance, i.e. excitement of surface plasmon present on the outer surface of the metal nanoparticles by applying electromagnetic field; progressively reduced in the peaks specifies no further formation nanoparticles. Higher absorbance spectra is directly proportional to the increasing particle size. Also, it is well recognized that the absorbance of Ag NPs depends mainly upon size and shape. The characteristics of Ag nanoparticles normally appear at a wavelength interval of 400–600nm .UV-Vis spectra of Ag nanoparticles synthesized using the *I. balsamina* aqueous extract evince the blue shift of the absorption band with increasing AgNO3 concentration. This information shows that the Ag nanoparticles have formed in the extract, where the Ag+ has been reduced to Ag0. Proteins and all secondary metabolites of extract play a critical role in both the reducing and capping mechanism for nanoparticle formation (Aritonang F. et at.,2019). The position and shape of the plasmon absorption depends on the particle size and shape (Chanda, 2013).Our biogenic AgNPs showed UV-Vis spectra with highest absorbance peak at 420 nm indicating the formation of spherical AgNPs by using *Portulaca oleracea L.* plants extract.

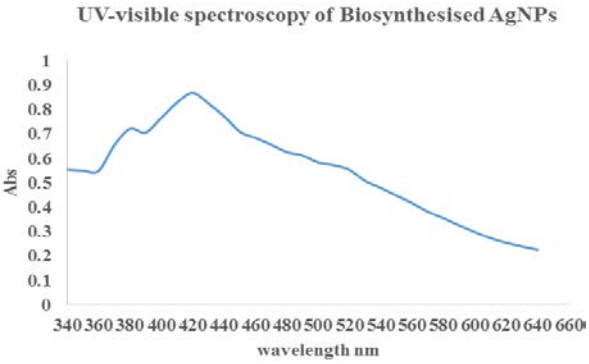


**Figure 2.** UV-visible spectroscopy of biosynthesized silver nanoparticles

*3.4. Fourier transform infrared spectroscopy (FTIR)*

FTIR measurements were carried out to identify the possible biomolecules responsible for reduction, capping and efficient stabilization of silver nano particles and the local molecular environment of the capping agents on the nanoparticles (Chandra, 2013). Fig.3 represents the FT-IR spectra recorded for biosynthesized silver nanoparticle from aqueous extract of *Portulaca oleracea L.*at 75°C conditions to know the functional group responsible for the reduction and stabilization of biogenic silver nanoparticles. The strong peak was observed at 3225.33cm$^{-1}$ may be due to presence of –OH or –NH group. The peak at 1636cm$^{-1}$ and 2114 cm$^{-1}$ revealed the presence of carbonyl and -CN triple bond stretching in proteins respectively. The peaks at 515.24 cm-$^{-1}$ showed significant changes upon reduction. Hence, the presence of these functional groups was responsible for the stabilization of synthesized AgNPs and also acts as reducing and capping agent; the results obtained are in agreement with the result of the Firdhouse et al.(2012). FTIR measurements of biogenic AgNPs by using plant extract in present work as well as the literature cited revealed carboxylic groups and amines from proteins play an important role in stabilization, reduction and capping for AgNPs.

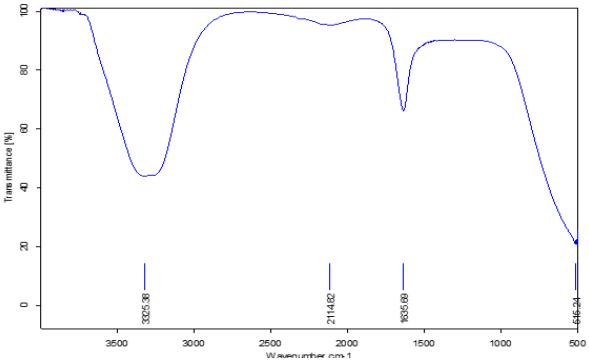


**Figure 3.** FT-IR spectra of biosynthesized silver nanoparticles at 75°C from aqueous extract of *Portulaca oleracea*L.

### 3.5. Particle size Analysis and Zeta potential

Biogenic AgNPs Dmean number 52.26 nm and in master curve distribution Dmean number 134.53 nm (Fig. 4&5) were analyzed at 25°C with an average rate count of 1505 kcps at wavelength 657 nm. Asghari Gholamreza (2014) reported, Statistical distribution of biogenic AgNPs by the *Portulaca oleracea* L. plant part extract form nanoparticles with different sizes; 146 nm, 136 nm and 175 nm for root, leave, and stem extracts, respectively and the fresh leaves aqueous extract of the plant synthesized silver nanoparticles with particle size less than 60 nm (Fridhouse et al., 2012). Shankar et al. (2003) found 50-100 nm size biogenic AgNPs by using *Azadirachta indica.* The present work reported the results obtained are in agreement with the size of the silver nanoparticle cited in literature. Zeta potential of biogenic AgNPs was found to be -29.1mV by Malvern Instruments (Fig. 6) indicates stable AgNPs while Colloidal silver solution (CSS) Zeta potential was -51.46 mV reported by Aurora et al. (2013).

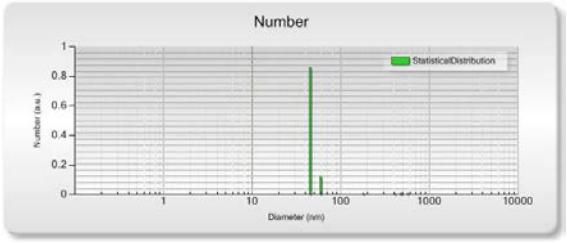


**Figure 4**. Particle size Analysis: Statistical distribution

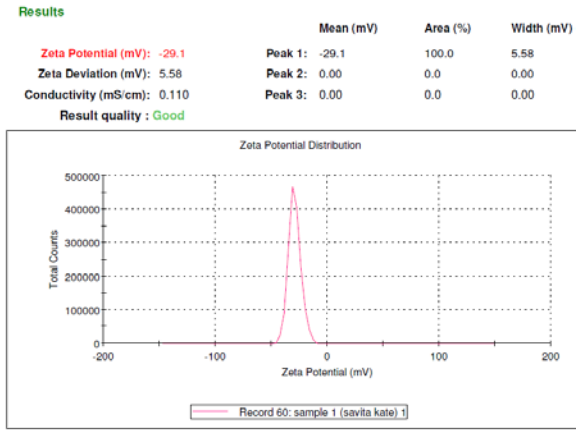


**Figure 5.** Zeta potential analysis of Biogenic AgNPs

### 3.6. X-ray Diffraction Method (XRD)

To calculate the crystalline particle size, crystal structure and formation of silver nano particles was confirmed by using XRD valuable research tool (Bindhu et al., 2013). The XRD pattern silver nanoparticles synthesized from aqueous extract of *Portulaca oleracea L.*(Fig 6) showed the presence of sharp and intense peak at 2θ = 32.20°; according to Scherrer's formula, $D_p=0.94\lambda/\beta_{1/2}.Cos\theta$, an average crystal size of the silver nanoparticles can be estimated from the X- ray wavelength of the Cu Ka radiation (l=1.54A°), the Bragg angle (θ) and the width of the peak at half height (maximum) (β) in radians. The particle size of the synthesized nanoparticles can be calculated using Debye- Scherrer's equation $D_p=0.94\lambda/\beta_{1/2}.Cos\theta$(nm) ,AgNPs size was found in range of 28.8 nm to 30.08 nm which was smaller in size than the reported nanoparticle size32.24 nm (Firdhouse et al.2012).

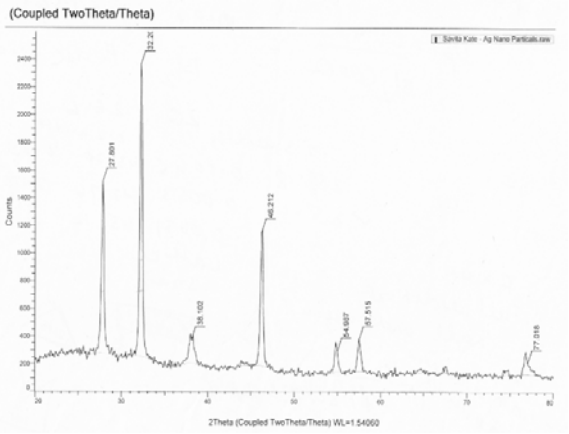


**Figure 6**. X-ray diffraction of Biosynthesized Silver nanoparticles

### 3.7. Scanning Electron Microscop

The SEM analysis gaves a brief idea about the morphology and size of the nano particles. For SEM analysis synthesized silver nanoparticles was coated on a glass substrate. The micrograph showed well separated silver nanoparticles with little agglomeration. The particle size of the synthesized silver nanoparticles varied compared to that of the size obtained from XRD analysis. The analysis confirms the shape AgNPs to be spherical. Similarly, spherical shape silver nanoparticle was reported by using the leaf extract of *Coleus aromaticus* (Vanaja, 2013).

### 3.8. Antibacterial activity of synthesized biogenic AgNPs

The antibacterial properties of the biogenic synthesized AgNPs were studied, and distinct zone of inhibition was found against all bacterial leather isolates and zone of inhibition ranges from 14.66±1.15 - 20.33±1.52mm in diameter as shown in Table 1; also compared with the diameter of zone of inhibition of known antibiotics such as Streptomycin (300mcg), Chloramphenicol (25mcg), Fusidic acid (10mcg), Erythromycin (5mcg), Methicillin (10mcg) and Novobiocin (5mcg) were used as the positive control. K. Roy et al., (2015) reported antibacterial activity of biogenic silver nanoparticles synthesized by using extract of *Cucumis sativus* and was studied against three bacterial strains *Staphylococcus aureus* (10mm), *Klebsiella pneumoniae* (14mm) and *Escherichia coli* (12mm). They found that antibacterial activity of AgNPs against gram negative was more as compared to gram positive bacteria, and this is due to the release of Ag+ ions from silver nanoparticles. Singh et al. (2015) did the antibacterial assays on *Escherichia coli* (16±0.5mm) and *Pseudomonas aeruginosa* (13 ±0.5mm) using biogenic AgNPs synthesized by green *Phyllanthus niruri* leaves. In the present study, biogenic AgNPs showed highest zone of inhibition against *Bacillus amyloliquefaciens* KP015745 (20.33mm±1.52mm) which was equal to Erythromycin (5mcg), Methicillin (10mcg) antibiotics followed by *Bacillus megaterium* KM369985 strain SAK (17±1.73mm) which was higher than Streptomycin (300mcg), Fusidic acid (10mcg), Erythromycin (5mcg) and *Bacillus pumilus*KP015747 showed complete resistance against Chloramphenicol (25mcg),Erythromycin (5mcg) and Methicillin (10mcg) but biogenic AgNPs showed inhibition (14.66±1.15mm) (Fig.7&8).

**Table 1**.Zone of inhibition shown by different Inhibitors

| Sr. No. | Name of the organism | Zone of inhibition shown by different Inhibitors (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G |
| 01 | *Bacillus megaterium* KM369985 strain SAK | 17±1.73 | 14 | 24 | 23 | 18 | 15 | 21 |
| 02 | *Bacillus cereus* KP015746 | 15±1 | 20 | 25 | 28 | 20 | 25 | 25 |
| 03 | *Bacillus pumilus* KP015747 | 14.66±1.15 | 20 | - | 24 | - | - | 18 |
| 04 | *Bacillus amyloliquefaciens* KP015745 | 20.33±1.52 | 36 | 25 | 27 | 20 | 20 | 26 |
| 05 | *Arthrobacter creatinolyticus* KP015744 | 16.33±1.52 | 24 | 20 | 22 | 21 | 25 | 22 |

Where, A- Biosynthesized silver nanoparticles (20µl), B- Streptomycin (300mcg), C- Chloramphenicol (25mcg),D- Fusidic acid (10mcg), E- Erythromycin (5mcg) F- Methicillin (10mcg) G- Novobiocin (5mcg)

**Figure 7.**Zone of inhibition B. megaterium

**Figure 8.** Zone of inhibition B. pumilus

*3.9. MIC of Silver Nanoparticles for bacteria*

The antibacterial effects of the biogenic silver nanoparticles were measured by determining the minimum concentration needed to inhibit the growth of leather isolates. MIC values of the biosynthesized silver nanoparticles against leather isolates were given in Table2. Leather bacterial isolates in the present study were inhibited at the concentration of 4-20 µg/ml of AgNPs.Chan Yen San et al. (2013) reported the MIC of AgNPs were between 1.6–47, 0.25–7.03, 0.4–30 and 0.4–4.7 µg/mlfor *S. aureus, S. epidermidis, E. coli* and *C. albicans*, respectively. Minimum Inhibitory Concentration (MIC) of AgNPs against *Staphylococcus aureus* was 800µg/ml and for *E.coli* was 300 µg/ml reported by Vivekanandan et al. (2012) in their study, while Ruparelia et al. (2008) found MIC for *E coli* was 40µg/ml, for *S. aureus* 120µg/ml, for *B subtilis* 40µg/ml in his work. Kim et al., (2011) the MIC of AgNPs against *S. aureus* and *E. coli* was 100 µg/ml. In the current work, the biogenic AgNPs was found to be more effective than the literature cited presents.

**Table 2**. MIC of AgNPs against the selected isolates

| Sr. No. | Name of the isolate | MIC (µg/ml) |
|---|---|---|
| 1 | *Bacillus megaterium* KM369985 strain SAK | 20 |
| 2 | *Bacillus cereus* KP015746 | 5 |
| 3 | *Bacillus pumilus* KP015747 | 5 |
| 4 | *Bacillus amyloliquefaciens* KP015745 | 10 |
| 5 | *Arthrobacter creatinolyticus* KP015744 | 4 |

## 4. Conclusion:

Preservation of leather by application of biogenic AgNPs was studied, and it was found that there was no growth of leather isolates on treated leather samples while untreated samples started to deteriorate by the isolates. Even after six months, there was no deterioration of leather samples, showing the efficiency of biogenic AgNPs in preservation of leather. Hence, biogenic AgNps obtained in the present study can be effectively used for leather preservation.

## Acknowledgements

Authors thank The Principal Dr. P.V. Ashtekar for providing infrastructure facilities to carry out present study.

## Conflict of interest

Authors declare no conflict of interest.

## References

Firdhouse M. and Lalitha P. 2012.Green Synthesis of Silver Nanoparticles Using the Aqueous Extract of *Portulaca oleracea* (L.).*Asian J Pharm Clin Res.*, **6** (1):92-94.

Khandelwal N., Singh A., Jain D., Upadhyay M. K. and Verma H.N.2010. Green synthesis of silver nanoparticles using Argimone mexicana leaf extract and evaluation of their antimicrobial activities. *Digest Journal of Nanomaterials and Bio structures.*,**5** (2): 483 – 489.

Rajasekharreddy P., Rani P.U. and Sreedhar B. 2010.Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach. *J. Nanopart. Res.*, **12**: 1711–1721.

Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K.2011. Biosynthesis of silver nanoparticles using citrus sinensis peel extract and its antibacterial activity. *Spectrochim Acta - Part A Mol Biomol Spectrosc* .,**75**: 594-598.

Tissier C. and Chensais M. 2000. Biocides used as preservatives in the leather industry, Product type 9: fibre, leather, rubber and polymerized materials preservatives. *Emission scenario Documents.*, 1-14.

Nadia Zarai Jaouadi and Hatem Rekik et al. 2013.Biochemical and molecular characterization of a serine keratinase from *Brevibacillus brevis US575* with promising keratin biodegradation and Hide dehairing activities. *PLOSOne*., **8** (10):1-17.

Baird D. H. 1998.The Microbial Decomposition of Chromium. Master of Science in Microbiology, University of Canterbury.

Lili Liu, Meihu Ma, Zhaoxia Cai Xieli Yang and Wentao Wang. 2010. Purification and Properties of a Collagenolytic Protease Produced by *Bacillus cereus MBL13* Strain. *Food Technol. Biotechnology*., **48** (2): 151–160.

Lalitha P. and Firdhouse M. Jannathul.2015. Biocidal potential of biosynthesized silver nanoparticles against fungal threats. *J Nanostructure Chem.*,**5**: 25–33.

Mittal Subhangi.2012. Biosynthesis of Silver Nanoparticles (AgNPs) using waste fruit Peel as an antimicrobial drug agent. Department of Botany, Faculty science, Dayalbagh Educational Institute Dayalbagh Agra-28110.

Alzahrani Eman.2015. Eco-Friendly Production of Silver Nanoparticles from Peel of Tangerine for Degradation of Dye. *World Journal of Nano Science and Engineering.*, **5**:10-16.

Singh K, Panghal M, Kadyan S, Chaudhary U and Yadav P.2014.Antibacterial Activity of Synthesized Silver Nanoparticles from Tinospora cordifolia against Multi Drug Resistant Strains of Pseudomonas aeruginosa Isolated from Burn Patients. *J Nanomed Nanotechnol.*, **5**(2): 1-6.

Dash L.2013.Biological Synthesis and Characterization of Silver Nanoparticles using Bacillus thuringiensis. Department of Life Science National Institute of Technology Rourkela-769008, Orissa, India.

Aritonang H., Koleangan H. and Wuntu A.2019. Synthesis of Silver Nanoparticles Using Aqueous Extract of Medicinal Plants' (*Impatiens balsamina* and *Lantana camara*) Fresh Leaves and Analysis of Antimicrobial Activity. *International Journal of Microbiology* Volume 2019, Article ID 8642303, 8 pages https://doi.org/10.1155/2019/8642303.

Chanda S.2013.Silver nanoparticles (medicinal plants mediated): A new generation of antimicrobials to combat microbial pathogens- a review. *FORMATEX*., 1314-1323.

Bauer A.W, Kirby W.M., Sherris J.C. and Turck M.1966.Antibiotic susceptibilitytesting by a standardized single disk method. *Am J Clin Pathol.*, **45:**493-496.

Awad A., Hendi A., Khalid M., Ortashi O., Dalia F., Elradi A., Lamia E., Eisa., Shorog-lahieb A., Al-Otiby, Merghani M. and Awad A. G. 2014.Silver nanoparticles biogenic synthesized using an orange peel extract and their use as an anti-bacterial agent.*International Journal of Physical Sciences*.,**9**(3):34-40.

Deb S.2014. Synthesis and Characterization of Silver Nanoparticles Using Brassica oleracea capitata (Cabbage) and Phaseolus vulgaris (French Beans): A Study on their Antimicrobial Activity and Dye Degrading Ability. *International Journal of Chem Tech Research.*, **6**(7):3909-3917.

Idris A., Majidnia Z. and Valipour P. 2013. Antibacterial Improvement of Leather by Surface Modification using Corona Discharge and Silver Nanoparticles Application. *Journal of Science and Technology*. 1-15.

Petica A., Gaidau Ma C. J., Simion D. and Niculescu M. 2013. Antimicrobial Electrochemically Obtained Nanosilver Solutions for Leather and Furskin Treatment. *REV. CHIM. (Bucharest)*., **64**(11): 1329-1334.

Sanchez-Navarro M. M., Pérez-Liminana M. A., Cuesta-Garrote N., Maestre-Lopez M. I., Bertazzo M., Martinez-Sanchez M. A., Orgiles-Barcelo C. and Aran-Ais F.2013. Latest Developments in Antimicrobial Functional Materials for Footwear. *FORMATEX*. 102-113.

Zambare V.P., Nilegaonkar S.S. and Kanekar P.P.2013. Protease Production and Enzymatic Soaking of Salt-Preserved Buffalo Hides for Leather Processing.*IIOAB Letters*., **3**: 2161-3702.

Singha Sumita., Neog Kundal., Kalita Partha Pratim., Talukdar Nayan and Sarma Manash Pratim.2014.Biological synthesis of silver nanoparticles by *Neptunia oleraceae. International Journal of Basic and Applied Biology.*,**2**(2):55 – 59.

Vanaja M., Rajeshkumar S., Paulkumar K., Gnanajobitha G., Malarkodi C. and Annadurai G.2013. Phytosynthesis and characterization of silver nanoparticles using stem extract of *Coleus aromaticus.International Journal of Materials and Biomaterials Applications.*,**3**(1): 1-4.

Asghari Gholamreza., Jaleh Varshosaz and Nafiseh Shahbazi.2013. Synthesis of silver nanoparticle using *Portulaca oleracea L.*L. extracts .*Nanomedicine Journal.*, **1**(2):94-99.

Shankar S. S., Ahmad A., Sastry M. 2003. Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles. *Biotechnology Progress.*, **19** (6): 1627–1631.

Bindhu M. R. and Umadevi M.2013.Synthesis of monodispersed silver nanoparticles using Hibiscus cannabinus leaf extract and its antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.*, **101:184**-190.

Roy K., Sarkar C.K. and Ghosh C.K.2015.Single-Step Novel Biosynthesis of Silver Nanoparticles Using *Cucumis Sativus* Fruit Extract and Study of its photcatalytic and antibacterial Activity. *Digest Journal of Nanomaterials and Biostructures.*,**10**(1):107 – 115.

Singh A. and Dhaliwal H.2015.Green Synthesis of Silver Nanoparticles Using Phyllanthus Niruri Leaf Extract and Evaluation of Their Antimicrobial Activities. *European Journal of Environmental Ecology.*, **2**(1):9-13.

San Chan Yen and Don Mashitah Mat.2013.Biosynthesis of Silver Nanoparticles from *Schizophyllum Commune* and in-vitroAntibacterial and Antifungal Activity Studies.*Journal of Physical Science.*, **24**(2):83-96.

Vivekanandan K.Ea., Rajb K. Gokul., Kumaresana S. and M. Pandib.2012. Biosynthesis of silver nanoparticle activity against bacterial strain, cephalexin antibiotic synergistic activity. *INT J CURR SCI.*, **4**:1-7.

Kim, Soo-Hwan., Hyeong-Seon Lee., Deok-Seon Ryu., Soo-Jae Choi and Dong-Seok Lee.2011. Antibacterial Activity of Silver-nanoparticles Against *Staphylococcus aureus* and *Escherichia coli. Korean J. Microbiol. Biotechnol.*,**39**(1):77–85.