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Extracellular Biosynthesis of Silver Nanoparticles Using Aquatic bacterial Isolate and its Antibacterial and Antioxidant Potentials

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ABSTRACT

The use of microorganisms in the synthesis of nanoparticles emerges as an eco-friendly and exciting approach. Silver bionanoparticles (AgNPs) are known to own inhibitory and bactericidal effects. This study focuses on the biosynthesis of silver nanoparticles (AgNPs) using the culture filtrate of ***Enterococcus faecalis S7***, isolated from Al-Bahr El-Pherony, Menoufyia Governorate, Egypt, and evaluation of its antibacterial and antioxidant potency. $AgNO_3$ solution (1 mM) was added to the cell-free culture supernatant, and also the mixture was incubated at 37 °C for 24 h in an orbital shaker (120 rpm). The AgNPs were characterized using UV–visible spectroscopy, X-ray Diffraction (XRD), EDAX, FTIR, and Transmission Electron Microscopy (TEM). Bio-manufactured silver nanoparticles were tested for their antibacterial and antioxidant activity using agar well diffusion and DPPH assays, respectively. The nanoparticles exhibited maximum absorbance at 430 nm in UV–Vis spectroscopy. The XRD spectrum exhibited 2Ɵvalues corresponding to the silver nanocrystals. TEM micrographs revealed the extracellular formation of spherical nanoparticles within the size range of 10–16 nm. The as-formed AgNPs exhibited antibacterial activity against the tested bacterial species *Escherichia coli*, *Salmonella* sp.; *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with a maximum inhibition zone of 30 mm against *S. aureus*. Furthermore, bacterial mediated AgNPs showed antioxidant activity in a dose-dependent manner. The data evaluated by this study provided evidence of AgNPs being a potent antioxidant and antibacterial compounds against both Gram-positive and Gram-negative bacteria. These results suggested that AgNPs can be used as an adjuvant for the treatment of infectious diseases.

INTRODUCTION

Nanotechnology finds its application in various areas of medication, starting from diagnosis, therapeutic drug delivery to the treatment of many diseases. Silver nanoparticles are one of the promising products within the field of nanotechnology because of their wide selection of applications as an antibacterial agent in disinfecting devices, cosmetics, home appliances, and water treatment plants (**Cho *et al.*, 2005**; **Li *et al.*, 2008**; **El-Shanshoury *et al.*, 2011; Abdeen *et al.*, 2014; Kamel *et al.*, 2016; El-Sheekh and El-Kassas, 2016; Masoud *et al.*,**

2018; Ibrahim *et al.*, 2019; El-Shanshoury *et al.*, 2020a,b). Inorganic composites are used as preservatives in various products (**Duran *et al.*, 2007**). Silver nanoparticles are prepared by physical, chemical and biological methods (**Roco, 2005**). Biosynthesis methods, employing microorganisms, have emerged as an easy, clean and viable alternative to chemical and physical methods (**El-Shanshoury *et al.*, 2020a**). An unlimited array of biological resources available in nature, including bacteria, fungi, yeasts, algae, and plants, are used for the synthesis of nanoparticles, which act as reducing and stabilizing agents (**Durán *et al.*, 2011**). These nanoparticles are capped with biomolecules derived from the organism used in the synthesis, which can improve stability and may present biological activity **(Ballotin *et al.*, 2016)**. Prokaryotic bacteria have received the foremost attention in this area (**El-Sheekh *et al.*,2014**). One advantage of using bacteria for synthesis of nanoparticles is simple of handling and their genetic manipulation without much difficulty (**Yusof *et al.*, 2019**).

Silver ions and silver-based compounds are known bactericides and have geared research interests towards nanoparticles as antibacterial agents (**Crabtree *et al.*, 2003**; **Furno *et al.*, 2004; El-Shanshoury *et al.*, 2011; Abdeen *et al.*, 2014; Kamel *et al.*, 2016; Masoud *et al.*, 2018; Ibrahim *et al.*, 2019; Abdallah *et al.*, 2020**). Several studies proposed that silver nanoparticles show efficient antibacterial activity due to the large surface area that comes in contact with the microbial cells and thus, includes a higher percentage of interaction than larger particles of the identical parent material (**Mulvaney *et al.*, 1996**; **Morones *et al.*, 2005**; **Pal *et al.*, 2007**). The mechanisms of action by which Ag-NPs exert their antimicrobial effects are not completely clear, but two main hypotheses have been proposed: (i) a direct interaction of the nanoparticle with the cell membrane, and (ii) the release of ionic silver (**Gugala *et al.*, 2016**). In the first hypothesis, the AgNPs would adhere to the cell membrane via electrostatic attractions between the positive charges of the nanoparticles and the negative charges of the cells as elucidated by **Kim *et al***. (**2007**) or via the interaction of the nanoparticles into the sulfur and phosphorylated proteins present in the cell wall (**Ghosh *et al.*, 2012**), the interaction of the Ag-NPs with the cell membrane leading to its partial disruption. In the second hypothesis, the AgNPs would enter into the cell and lead to the release of silver ions and the subsequent generation of reactive oxygen species (ROS) that would damage the enzymes involved in the cellular oxidation-reduction respiratory chain, and be finally responsible for cell death, also the slow release of silver ions which react with thiol groups of proteins or interfere with DNA replication (**Ivask *et al.*, 2014**; **Jena *et al.*, 2014**). Silver nanoparticles have been reported by several authors as

efficient antioxidants having the ability to scavenging DPPH free radicals (**Mohanta *et al.*,2017**; **Sivasankar *et al.*, 2018; Salari *et al.*, 2019; Abhang, 2020; Rajoka *et al.*, 2020**). Thus, this study aimed to expand the inexperienced synthesis of AgNPs making use of *Enterococcus faecalis* S7 isolated from a water sample of the El-Bahr El-Pherony, Menoufyia Governorate, Egypt and potential affectivity as antibacterial and antioxidant.

MATERIALS AND METHODS

2.1. Bacteria isolation and characterization

The bacteria utilized in this study were isolated from a water sample of the El-Bahr El-Pherony, Menoufyia Governorate, Egypt (N: 30º 22` 27"; E: 30º 59` 47"). Water samples were aseptically collected in sterile brown bottles transported to the laboratory and stored at 4°C until bacteriological analysis completed within 6 h of sampling.

Collected samples were serially diluted using the saline solution (0.85%) and it had been plated on Nutrient agar medium. Then the plates were incubated at 37°C for 24 h. After the incubation period, different morphological bacterial colonies were observed and colonies were randomly selected for the screening of its ability to produce AgNPs. The most potent organism was selected for getting silver nanoparticles (AgNPs). The morphological and physiological characterization of the selected isolate was performed in keeping with methods described in Bergey's manual of determinative bacteriology (**1984).** The biochemical tests employed in the identification and characterization of the isolates include: Gram staining, indole production, methyl red-voges Proskauer, citrate utilization, and urease production tests.

2.2. Molecular identification of the selected bacterial isolate

Molecular identification of the selected strain was carried out by 16S rRNA sequence-based method. Total genomic DNA was isolated from a selected strain for PCR. The quality of the isolated DNA was checked by agarose gel electrophoresis. The genomic DNA was then used as a template for PCR using the primers 16S27F (AGAGTTTGATCCTGGCTCAG) and 16S 1492R (GGTTACCTTGTTACGACTT). The PCR was carried out in a total volume of 50 µL containing 50 ng of genomic DNA, 20 pmol of each primer, 1.25 Units of *Taq* DNA polymerase, 200 µM of each dNTPs and 1× PCR buffer as components. The PCR was performed for 35 cycles in a Mycycler™ (Bio-Rad, USA) with the initial denaturation for 3 min at 94 °C, cyclic denaturation for 30 s at 94 °C, annealing for 30 s at 58 °C and extension for 2 min at 72 °C with a final extension of 7 min at 72 °C. After the PCR, the reaction products were analyzed by agarose gel electrophoresis. The product was then gel purified and was further subjected to sequencing PCR using the Big Dye Terminator Sequence Reaction Ready Mix (Applied Biosystem). After the reaction, the product was purified, precipitated, and was used for

sequence run within the DNA sequencer ABI 310 Genetic Analyzer. The sequence data of 16S rDNA thus obtained was further aligned using BioEdit program. This sequence was then used for BLAST analysis. The 16S rRNA sequence of CS 11 was also used for phylogenetic analysis using the neighbor-joining method in MEGA5.

2.3. Growth of the selected isolate

In the present study, Nutrient broth (NB) media was used for the growth of *Enterococcus faecalis S7*. The flasks containing the bacterial culture were incubated on an orbital shaker at 37°C and agitated at 120 rpm. The growth profile of *Enterococcus faecalis* S7 was studied here within 6 h interval. The culture was then centrifuged at 6000 rpm for 20 minutes to get biomass. The supernatant was collected for further reaction with silver salt ($AgNO_3$) to synthesize nanoparticles.

2.4. Biosynthesis of silver nanoparticles

For nanoparticles synthesis, the obtained supernatant was added separately to the reaction flask containing silver nitrate ($AgNO_3$) at a concentration of 1 mM. The reaction between the supernatant obtained and Ag+ ion was applied at pH7 and 37°C in bright conditions for 24 hours. After 24 hours, the initial yellowish-white color changed to brown color, which showed the synthesis of Ag nanoparticles (**Siddiqi *et al.*,2018**).

2.5. Characterization of silver nanoparticles

The primary confirmation of the silver nanoparticle synthesis was carried out by UV-visible spectroscopy within the range of 200-800 nm. The morphology of the silver nanoparticles was examined by Transmission Electron Microscope (TEM). The chemical composition and crystalline phase of the silver nanoparticles were characterized by Advanced Powder X-Ray diffractometer (D8, Bruker, Germany).

2.5.1. UV-Vis spectrophotometer

The biogeneration of the silver nanoparticles within the reaction mixture was measured by withdrawing 2 ml of the sample at predetermined time intervals and also t he absorbance was measured within the range of 200 to 800 nm at a resolution of 1 nm employing a UV-Vis spectrophotometer (Beckman DU-40) against sterile medium as the blank.

2.5.2. X-Ray Diffraction (XRD) measurement

The biologically synthesized silver nanoparticles were freeze- dried on a lyophilizer and therefore, the powdered sample was used for X-ray diffraction (XRD) analysis. The XRD analysis was performed by X'Pert Pro A Analytical X–ray diffractometer instrument using CuKα radiation (k = 1.54056 Å) within the range of 20-80 at 40 keV.

2.5.3. Transmission Electron Microscopy (TEM) Analysis

Transmission Electron Microscopy (TEM) analysis of synthesized silver nanoparticles was prepared by drop-coating biosynthesized nanoparticles solution on carbon-coated copper TEM grids (400 μm × 40 μm mesh size). Samples were dried and kept under vacuum in desiccators before loading on to a specimen holder. TEM measurements were performed on a Tecnai- 12 (FEI, The Netherlands) electron microscope operated at an accelerating voltage of 120 kV.

2.5.4. Fourier-Transform Infrared (FT-IR) Chemical Analysis

For Fourier-Transform Infra-Red spectroscopy measurements, the biotransformed products present in the extracellular filtrate were freeze-dried and diluted with potassium bromide within the ratio of 1:100. The FT-IR spectrum of samples was recorded on a FT-IR instrument (Digital Excalibur 3000 series, Japan) with diffuse reflectance mode (DRS-800) attachment. All measurements were carried out in the range of 400- 4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.5.5. Energy Dispersive X-Ray Analysis

Energy Dispersive X-Ray Analysis (EDAX) was administrated with the scanning electron microscope Jeol JSM-6360 LA (Armed force central laboratories, Cairo, Egypt) equipped with an EDAX detector operated at an accelerating voltage of 20 keV to perform elemental analysis.

2.6. Determination of antibacterial activity of AgNPs

The AgNPs synthesized from the selected isolate was tested for its antibacterial activity against pathogenic bacteria such as *Escherichia coli*, *Salmonella* sp.; *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by standard well diffusion method in Müller Hinton Agar (MHA) plates. Pure cultures of bacterial pathogens were grown in nutrient broth at 37^{o}C for 18-24 hours. Wells were made on the Müller Hinton Agar plates employing a gel puncture and therefore the plates were inoculated by swabbing the bacterial pathogens to create a confluent lawn of bacterial growth. Then 100 μL of the biosynthesized AgNPs solution was poured on to corresponding well using a micropipette. As a control, 100 μL of 1 mM $AgNO_3$ solution was poured on to the control well. After incubation at 37^{o}C for 24 hours, the diameter of the zone of inhibition in millimeters around each well was measured.

2.7. Estimating Antioxidant Activity

The free radical scavenging activity of silver nanoparticles was measured by the DPPH method (**Oueslati *et al.*, 2020**) against ascorbic acid, a well -known antioxidant. Ascorbic acid served as the positive control and its antioxidant activity was assumed to be 100%. For

that, 1 ml of various concentrations of as-formed AgNPs (25, 50, 75, 100 µl/ml) were mixed with 1 ml of methanolic extract of freshly prepared DPPH. Then the reaction mixture was incubated in dark at room temperature for 30 min and also the absorbance was measured at 517 nm. Percentage inhibition of DPPH scavenging activity was calculated by:

Percentage of inhibition of DPPH Activity = Abs Blank-Abs Sample x 100 Abs Blank Where, Abs Blank = Optical density of Control, Abs Sample = Optical density of sample extract.

RESULTS

3.1. Isolation and identification of the potential isolate

The potential isolate was found to be Gram positive cocci. Based on biochemical characterization and molecular identification, the isolate was identified as *Enterococcus faecalis* S7 (Figure 1; Table 1). The sequence data were subjected to BLAST analysis and also the result showed its maximum identity of 99 % to *Enterococcus faecalis*. *Enterococcus faecalis* S7 was isolated, identified and cultured for the production of biomass. The bacterial isolate of *Enterococcus faecalis* S7 was inoculated in NB media as the production culture and inoculated at 37ºC in an orbital shaker at 121 rpm. The growth profile was then studied at 620 nm with an interval of 6 h, starting from the time of inoculation. The maximum growth kinetics was observed after 24 h of incubation as shown in Figure (2).

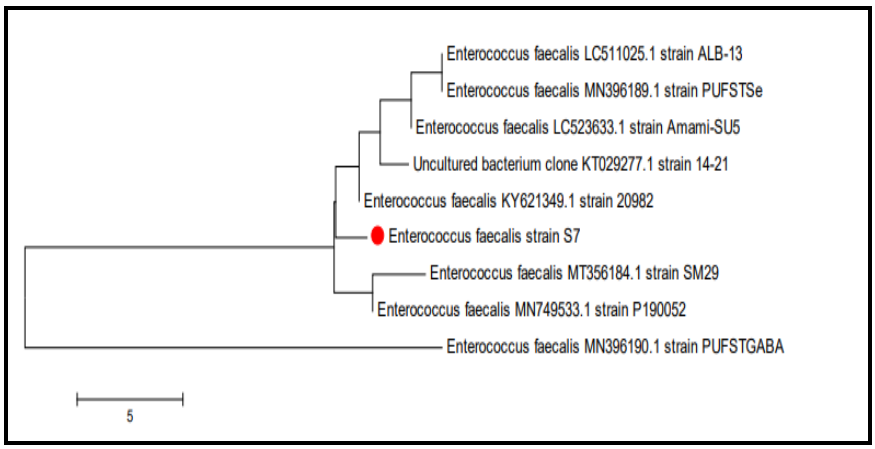

Fig.1. Phylogenetic tree of *Enterococcus faecalis* S7 based on partial sequencing of 16s rRNA

Table 1: Biochemical characteristics of the isolate capable of synthesizing silver nanoparticle

Characteristics of the potential strain	Results
Gram staining	Positive
Morphology	Cocci
Gelatinase	Positive
Voges proskauer	Positive
Indole	Negative
Citrate	Negative
Urease	Negative
Fermentation of	
Glucose	Positive
Mannitol	Positive
Maltose	Positive
Lactose	Positive

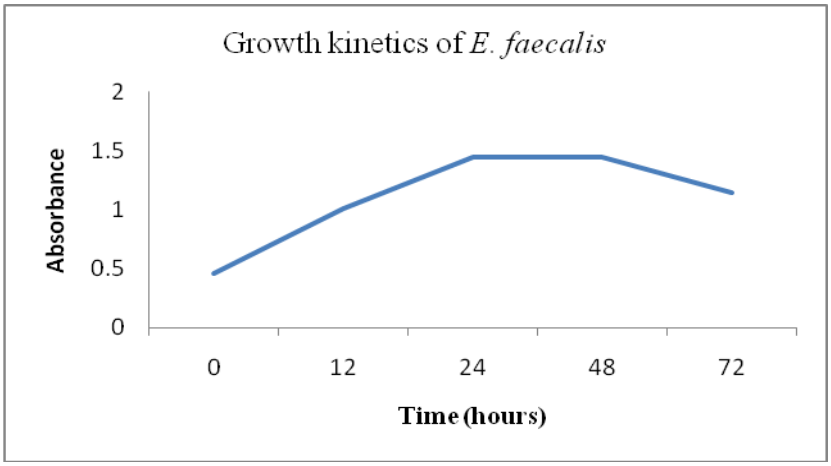

Fig 2. Growth profile of *Enterococcus faecalis* on the NB media

3.2. Characterization of silver nanoparticles usnig UV spectrophotometer

The newly isolated *Enterococcus faecalis* S7 was used for silver nanoparticle synthesis indicated by the color change of reaction mixture from yellow to dark brown (Figure 3 inset). The silver nanoparticles were then characterized by UV–visible spectroscopy. This technique has proved to be very useful for the analysis of metal nanoparticles. A characteristic broad peak of silver nanoparticles was observed within the UV-visible spectra at 420 nm (Figure 3).

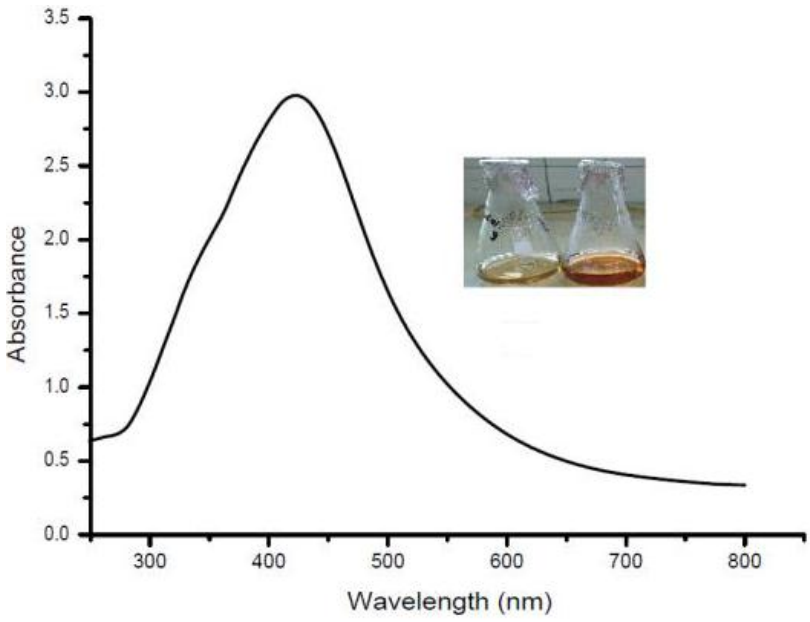

Fig 3. UV-visible spectra of silver nanoparticles synthesized using *Enterococus faecalis* S7 cell free extract

3.3. Characterization of AgNPs by XRD

The XRD pattern of the silver nitrate-treated sample (Figure 4) corresponds to that of silver nanoparticles. The XRD pattern shows four intense peaks in the whole spectrum of 2Θvalues, ranging from 30 to 80. It is important to know the exact nature of the formed silver particles and this can be deduced from the XRD spectrum of the sample. XRD spectra of pure crystalline silver structures and pure silver nitrate have been published by the Joint Committee on Powder Diffraction Standards (file nos. 04-0783 and 84-0713). A comparison of our XRD spectrum with the standard sample confirmed that the silver nanoparticles had been formed in the form of nanocrystals, as was evidenced by the peaks at 2Θ values of 38.25°, 46.37°, 64.60° and 77.62° corresponding to 111, 200, 220 and 311 planes for silver, respectively.

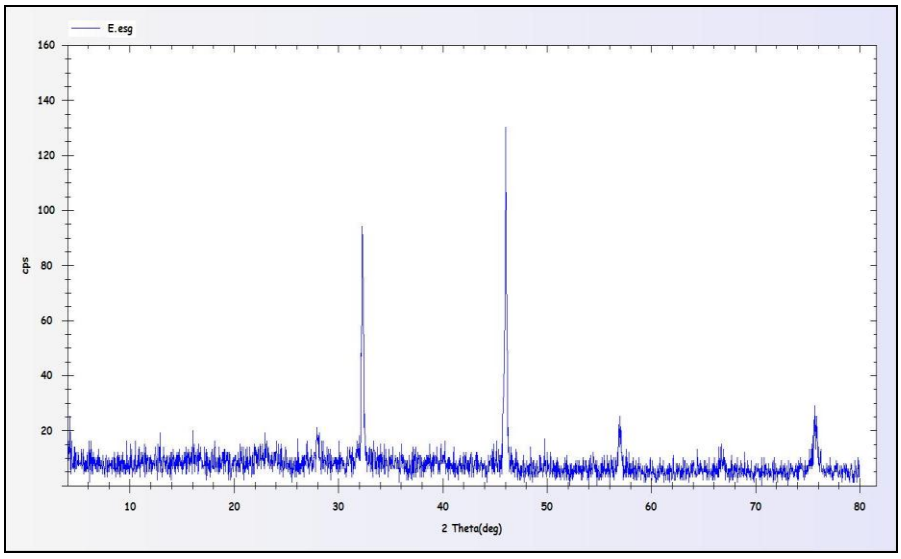

Fig 4. X-ray diffraction pattern of silver nanoparticles.

3.4. FTIR

The reducing/capping proteins accountable for the bioreduction and stabilization of biosynthesized silver nanoparticles were identified using FTIR (Figure 5). The FTIR spectra of silver nanoparticles showed peaks at 3000-3500 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1500-1550 cm^{-1} showed a stretch for C-H bond, peak around 1450-1500 cm^{-1} showed the bond stretch leads for N-H bend primary amines. Thus, the synthesized silver nanoparticles are surrounded by proteins and metabolites with the attachment of the various functional groups.

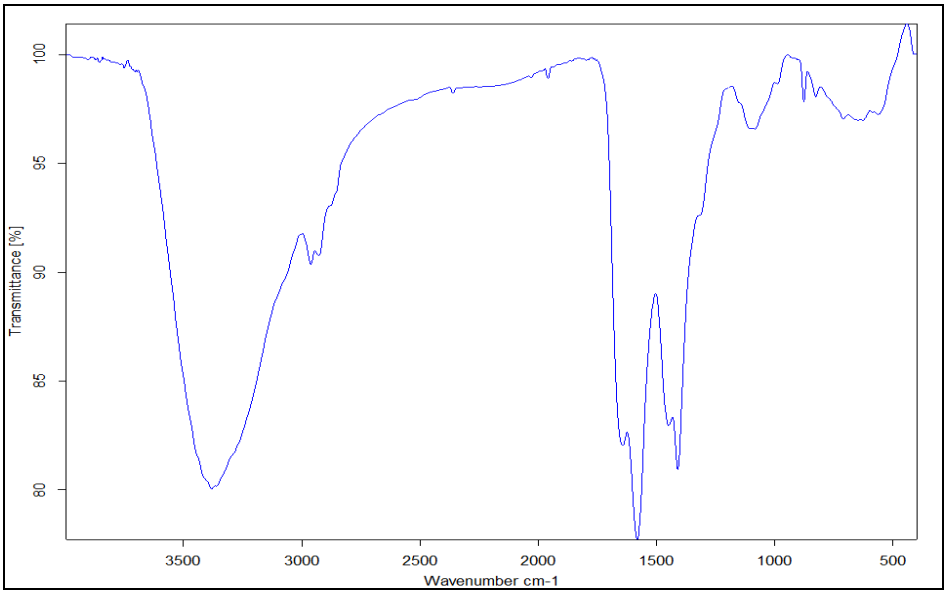

Fig 5. FTIR spectra of silver nanoparticles of silver nanoparticles produced by CFE of *Enterococcus faecalis S7*

3.5. TEM

The shape and size of the silver nanoparticles biosynthesized using cell free extract of *E. faecalis S7* were further analyzed by transmission electron microscopy (TEM), as shown in Figure (6a). TEM micrograph showed a well dispersed AgNPs which is spherical in shape with particle size of 10-16 nm with no agglomeration.

3.6. EDAX

The elemental compositions of the nanoparticles by EDAX (Figure 6b) indicate a sharp peak at 2.9-3.0 keV which shows the presence of silver as base and dominant element. Additional peaks along with those of C and O and N that also appeared throughout the scanning range (0–4 keV) of the spectrum.

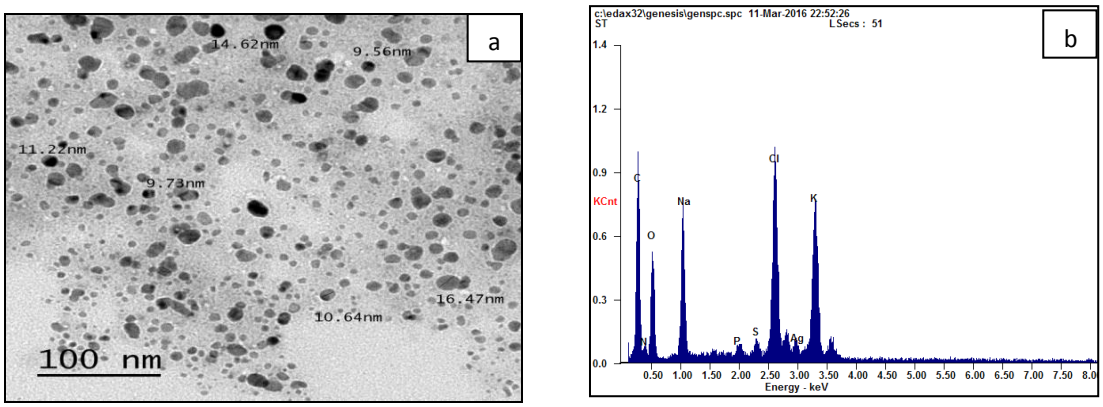

Fig 6. **a)** Microphotograph of the silver nanoparticles synthesized from *Enterococcus faecalis S7* supernatant b) EDAX spectroscope of the synthesized nanoparticles depicts silver as the base element.

3.7. Antibacterial activity of silver nanoparticles

In respect to the antibacterial activity by silver nanoparticles which produced by the tested bacterial isolate, it had wide spectrum activity against Gram-negative and Gram positive bacteria namely; *Vibrio cholera*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli*; showing maximum activity against *S. aureus* with an average diameter of 30 mm (Figure 7).

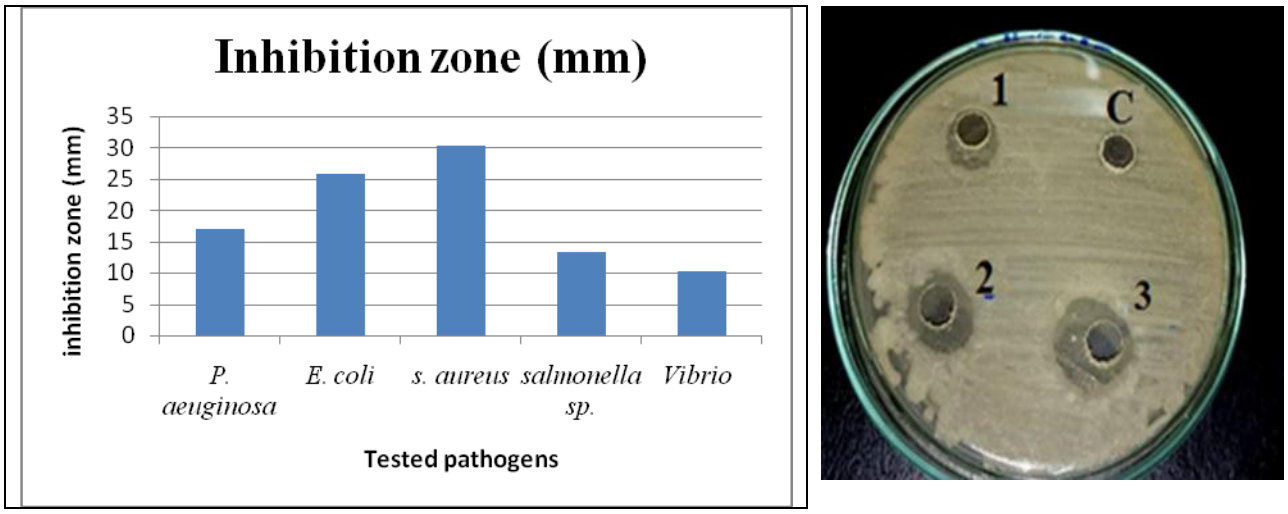

Fig 7. Antibacterial effects of green synthesized AgNPs by cell-free extract against micro-organisms presented as inhibition zones (in mm).

3.8. *In vitro antioxidant activity of silver nanoparticles*

Biosynthesized AgNPs showed promising results as antioxidants using DPPH assay with maximum DPPH inhibition percentage 92.3% at a concentration of 100µl/ml. DPPH activity was increased in a dose-dependent manner, at concentrations 25–75 µl/mL, AgNPs showed a scavenging activity starting from 53% to 72%..

Table 2. DPPH free radical scavenging activity of silver nanoparticles

Concentration (µl/ml)	**Percentage of inhibition (%)**
25	53.3±0.5
50	55.6±0.2
75	72.2±0.7
100	92.3±1.9

DISCUSSION

The antibacterial activity of the AgNPs against wide range of pathogenic bacteria including Gram positive amd Gram negative bacteria such as *Salmonella*, *Pseudomonas*, *Staphylococcus* aureus and *E. coli* is well documented (**Markowska *et al*., 2013; Thuptimdang *et al*., 2015; Singh *et al*., 2018; Kambale *et al*., 2020**). Thus, the AgNPs can be considered as an alternative to be used on surfaces of materials such as dentures, contact lenses, catheters and probes, in order to prevent associated infections (**Roe *et al*., 2008; Qayyum *et al*., 2017**). The applicability of AgNPs could be limited by the high cost

of pure AgNPs suspensions, however biological methods are extensively explored as an environment-friendly and cost effective alternative for the AgNPs production with respect to other convential methods (**Parvataneni, 2019**). Particularly, bacterial assisted biosynthesis has attracted much more attention as a good alternative for metal NPs production, due to the feasibility to use extracellular molecules secreted as intermediaries of metal NPs synthesis (**Mukherjee**, **2008** ;**Siddiqi and Husen, 2016**). Various spectroscopic and microscopic techniques have been used to confirm the synthesis of bacterial derived GNPs including UV-vis spectroscopy, transmission electron microscopy, energy dispersive X-ray analysis, X-ray diffraction, and Fourier-transform-infrared spectra analyses.

In this study, a simple method for AgNPs biosynthesis was carried out using a freshwater bacterial isolate *Enterococcus faecalis* S7. AgNPs were characterised by identification of their surface plasmon resonance (SPR) using UV-Vis spectroscopy. SPR peaks typically occur between 390 and 530 nm and can be correlated with the size of the AgNPs. This shows that biosynthesis of silver nanoparticles using culture supernatant of *E. faecalis* is analogous to *Enterobacteria* and *K. pneumonia* (**Shahverdi *et al.*, 2007**; **Mokhtari *et al.*, 2009**) lactic acid bacteria LCM5 (**Matei *et al.*, 2020**). Microbial culture extracts contain compounds that are not only capable of reducing the Ag^+ ions but also act as natural capping agents as proved by the FTIR spectrospopic analysis. These biological extracts contain a mixture of biomolecules like enzymes, proteins, amino acids, carbohydrates and vitamins that can be responsible for the reduction and capping of the Ag^+ ions (**Deepak *et al.*, 2011**). The FTIR analysis confirmed the presence of carbonyl groups from the amino acid residues and proteins having the stronger capability to bind the metal designating the proteins which can probably reduce the metal to form metal nanoparticles, i.e. capping of silver nanoparticles which suggests that biological molecules have the capability to perform dual functions of formation and stabilization of silver nanoparticles. Among the enzymes, Nitrate reductase is among the widely accepted enzymes responsible for the synthesis of the nanoparticles. Nitrate reductase is an enzyme that is responsible for the conversion of nitrate to nitrite and through this process of catalysis, an electron shuttles to the incoming silver ions (**Kalimuthu *et al.*, 2008**). The size and morphology of the AgNPs were investigated through TEM imaging. The elemental compositions of the nanoparticles was analyezed by EDAX which indicated a sharp peak at 2.9-3.0 keV . Additional peaks along with those of C and O and N that also appeared throughout the scann ing range (0–4 keV) of the spectrum suggesting that the biological origin molecule i.e.; enzymes or proteins were attached with the biosynthesized Ag-NPs, further the presence of protein or enzyme like molecule.

Silver nanoparticles showed a potent antibacterial activity against wide range of bacteria including *Vibrio cholera*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli*. This study gave similar results with many authors, **Abdeen *et***

al. (**2014**), **Kamel *et al***. (**2016**), **Masoud *et al***. (**2016**) and **Siddique *et al***. (**2020**) who reported that metal nanoparticles are termed as a new generation of antimicrobials (**Rai *et al.,* 2009**). They proved that AgNPs inhibited the growth of Gram positive and negative bacteria and possess effective antimicrobial activity against multi drug resistant strains of *Klebsiella pneumoniae*, *E. coli*, *Bacillus cereus*, *S. aureus*, *Salmonella*, *Pseudomonas*, *Moraxella*, *Acientobacter*, *Enterococcus*, and *St. pneumonia.*

There are different theories proposed to judge the mechanism of action of AgNPs; (1) silver ions may interact with the thiol groups of some of the major enzymes and inactivate them, (2) silver ions exhibit an oligo dynamic effect by denaturing the cellular proteins, inhibition of DNA replication, and alteration plasma membrane permeability (**Feng *et al.,* 2000**), (3) other theories showed that there have been some structural changes within the cell membrane due to silver ions, which were liable for the bactericidal activity of the AgNPs. The major mechanism through which silver nanoparticles manifested antibacterial efficacy is by anchoring to and penetrating the bacterial semi-permeable membrane, and possibly cause further damage by interacting with sulphur and phosphorus containing compounds, including DNA (Singh *et al.*,2009). High surface area to volume ratio causes high bactericidal activity of AgNPs compared with bulk silver metal (**Cho *et al.,* 2005**; **Panyala *et al.,* 2008**).

Furthermore, AgNPs showed a strong antioxidant activity in a dose dependant manner. The observed free-radical-scavenging potential of AgNPs might be attributed to two different mechanisms for the deactivation of free radicals: hydrogen atom transfer (HAT) and electron transfer (ET). These reactions can occur simultaneously. The dominant mechanism is determined by the structure and properties of the antioxidant compound. Hence, the presence of those secondary metabolites and Ag^{+} ions leads to the antioxidant activities through HAT and single-electron-transfer mechanisms simultaneously. The above-mentioned results are indicative of substantial antioxidant potential of AgNPs. Significant free radical scavenging activity using cell free extracts has been reported before (**Vorobyouva *et al*., 2020**).

DPPH is widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, the synthesized silver nanoparticles showed potential free radical scavenging activity. The utilization of DPPH provides a simple and rapid way to evaluate antioxidant activity. Results of DPPH reduction is shown in Table 3. The synthesized silver nanoparticles showed a good capacity of scavenging the DPPH free radical. The antioxidant activities of the individual compounds, present in the extract may depend upon structural features, may be the number of phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, free carboxylic groups, and other structural features

CONCLUSION

The current study revealed that silver nanoparticles can be bio-fabricated employing a very simple, inexpensive; eco-friendly method using *E. faecalis* S7 cell-free extract. The TEM analysis showed that the sizes of the synthesized AgNPs ranged from 10 to 16 nm. This technique revealed that the cell free extracts can be used as an efficient stabilizing reducing and capping agent for the synthesis of AgNPs, thereby providing stability to the silver nanoparticles. The biosynthesized AgNPs were crystalline in nature as evident from the XRD spectral analysis. AgNPs exhibited high antioxidant and antimicrobial potential, in a dose-dependent manner. Furthermore, the results of this study suggested that AgNPs may be proved to be an efficient component in various biomedical applications.

REFERENCES

Abdallah, M.; Benoliel, C.; Jama, C.; Drider, D.; Dhulster, P. and Chihib, N.E. (2014). Thermodynamic prediction of growth temperature dependence in the adhesion of *Pseudomonas aeruginosa* and *Staphylococcus aureus* to stainless steel and polycarbonate. J. Food Prot., 77(7): 1116–1126.

Abdeen, S.; Geo, S.; Sukanya, S. and Praseetha P.K. (2014). Biosynthesis of silver nanoparticles from *Actinomycetes* for therapeutic applications. Int. J. Nano. Dimens., 5: 155-162.

Abhang, P. (2020). Antimicrobial and Antioxidant Properties of Silver Nano-particles Synthesized from *Escherichia Coli*. Sci. Technol. Develop., pp.55-61.

Ballotin, D.; Fulaz, S.; Souza, M.L.; Corio, P.; Rodrigues, A.G. and Souza, A.O. (2016). Elucidating protein involvement in the stabilization of the biogenic silver nanoparticles. Nanoscale Res. Lett., 11: p. 313.

Cho, M.; Chung, H.; Choi, W. and Yoon, J. (2005). Different inactivation behaviors of MS-2 phage and *Escherichia coli* in TiO_2 photocatalytic disinfection. Appl. Environ. Microbiol., 71: 270-275.

Crabtree, J.H.; Burchette, R.J.; Siddigi, R.A.; Huen, I.T.; Hadnott, L.L. and Fishman, A. (2003).The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. Perit Dial Int., 23:368–374.

Deepak, V.; Kalishwaralal, K.; Pandian, S.R.K. and Gurunathan, S. (2011). An insight into the bacterial biogenesis of silver nanoparticles, industrial production and scale-up. In Metal nanoparticles in microbiology (pp. 17-35). Springer, Berlin, Heidelberg.

Durán, N.; Marcarto, P.D.; De Souza, G.I.H.; Alves, O.L. and Esposito, E. (2007). Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J. Biomed. Nanotechnol., 3: 203–208.

Durán, N.; Marcato, P. D.; Durán, M.; Yadav, A.; Gade, A. and Rai, M. (2011). Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi and plants. Appl. Microbiol. Biotechnol., 90: 1609–1624.

El-Shanshoury, A.; Darwesh, O.; Sabae, S.; Awadallah, O. and Hassan, S. (2020a). Bio-manufacturing of selenium nanoparticles by *Bacillus subtilis* isolated from Qarun Lake and evaluation their activity for water remediation. Res. Appl.Chem., 10 (4): 5834-5842.

El-Shanshoury, A.R.; ElSilk S.E. and Ebeid M.E. (2011). Extracellular biosynthesis of silver nanoparticles using *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Streptococcus thermophilus* ESh1 and their antimicrobial activities. ISRN Nanotechnol., 2011.

El-Shanshoury, A.R.; El-Zeiny E. Ebeid, E.E.; Elsilk, S.E.; Mohamed, S.F. and Ebeid, M.E. (2020b). Biogenic synthesis of gold nanoparticles by bacteria and utilization of the chemical fabricated for diagnostic performance of viral Hepatitis C Virus-NS4. Lett. Appl. NanoBioSci., 9 (3): 1395-1408.

El-Sheekh, M.; Daboor, S.; Swelim MA and Nohamed, S. (2014). Production and characterization of antimicrobial active substance from *Spirulina platensis*. Iran. J. Microbiol., 6 (2): 112-119.

El-Sheekh, M.M. and El-Kassas, H. (2016). Algal production of nano-silver and gold: Their antimicrobial and cytotoxic activities: A review. J. Genet. Eng. Biotechnol., 14 (2): 299-310

Feng, Q.L.; Wu, J.; Chen, G.Q.; Cui, F.Z.; Kim, T.N. and Kim, J.O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res., 52: 662-668.

Furno, F.; Morley, K.S.; Wong, B.; Sharp, B.L.; Arnold, P.L.; Howdle, S.M.; Bayston, R.; Brown, P.D.; Winship, P.D. and Reid, H.J. (2004). Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. J. Antimicrob. Chemother., 54:1019–1024.

Ghosh, S.; Patil, S.; Ahire, M.; Kitture, R.; Kale, S.; Pardesi, K.; Cameotra, S.S.; Bellare, J.; Dhavale, D.D. and Jabgunde, A. (2012). Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. Int. J. Nanomed., 7: 483–496.

Gugala, N.; Lemire, J.; Chatfield-Reed, K.; Yan, Y.; Chua, G. and Turner, R.J. (2016). Using a chemical genetic screen to enhance our understanding of the antibacterial properties of silver. Genes., 9: p. 344.

Ibrahim, E.; Fouad, H.; Zhang, M.; Zhang, Y.; Qiu, W.; Yan, C.; Li, B.; Mo, J. and Chen, J. (2019). Biosynthesis of silver nanoparticles using endophytic bacteria and their role in inhibition of rice pathogenic bacteria and plant growth promotion. RSC Advances., 9(50): 29293-9.

Ivask, A.; Kurvet, I.; Kasemets, K.; Blinova, I. and Aruoja, V. (2014). Size-dependent toxicity of silver nanoparticles to bacteria, yeast, algae, crustaceans and mammalian cells *in vitro*. PLoS ONE., 9(7): e102108

Jena, J.; Pradhan , N.; Nayak, R.R.; Dash, B.P.; Sukla, L.B.; Panda, P.K. and Mishra, B.K. (2014). Microalga *Scenedesmus* sp. a potential low-cost green machine for silver nanoparticle synthesis. J. Microbiol. Biotechnol., 24: 522–533.

Kambale, E.K..; Nkanga, C.I.; Mutonkole, B.P.I.; Bapolisi, A.M.; Tassa, D.O.; Liesse, J.M.I.; Krause, R.W. and Memvanga, P.B. (2020). Green synthesis of antimicrobial silver nanoparticles using aqueous leaf extracts from three Congolese plant species (*Brillantaisia patula, Crossopteryx febrifuga* and *Senna siamea*). *Heliyon*, *6*(8): p.e04493.

Kamel, Z.; Mahmoud M. and Namoury, N. (2016). Biosynthesis, characterization, and antimicrobial activity of silver nanoparticles from actinomycetes. Res. J. Pharm. Biol. Chem. Sci.,7: 119-127.

Kim, J.S.; Kuk, E.; Yu, K.; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.Y.; Kim, Y.K.; Lee, Y.S.; Jeoong, D.H. and Cho, M.H. (2007). Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol. Med., 3: 95–101.

Krieg, N.K. (Ed) (1984). Bergey's Manual of Systematic Bacteriology. Baltimore, Hang Kong, London and Sydney. 1: 172 – 173 and 414.

Li, Q.; Mahendra, S.; Lyon, D.; Brunet, L.; Liga, M.; Li, D. and Alvarez, P. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. Water Res., 42: 4591-4602.

Markowska, K.; Grudniak, A.M. and Wolska, K.I. (2013). Silver nanoparticles as an alternative strategy against bacterial biofilms. Acta Biochim Pol. , 60(4):523–30.

Masoud, E.M.; El-Bellihi, A.A.; Bayoumy, W.A. and Mohamed, E.A. (2018). Polymer composite containing nano magnesium oxide filler and lithiumtriflate salt: an efficient polymer electrolyte for lithium ion batteries application. J. Mol. Liq., 260*:*237-244.

Matei, A.; Matei, S.; Matei, G.; Cogălniceanu, G. and Cornea, C. (2020). Biosynthesis of silver nanoparticles mediated by culture filtrate of lactic acid bacteria, characterization and antifungal activity. EuroBiotech. J., 4(2): 97-103.

Mohanta, Y.K.; Panda, S.K.; Jayabalan, R.; Sharma, N.; Bastia, A.K. and Mohanta, T.K. (2017). Antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles synthesized by leaf extract of *Erythrina suberosa* (Roxb.). Front. Mol. Biosci., 4: 14.

Mokhtari, N.; Daneshpajouh, S.; Seyedbagheri, S.; Atashdehghan, R.; Abdi, K.; Sarkar, S.; Minaian, S. Shahverdi, H.R and Shahverdi A.R. (2009). Biological synthesis of very small silver NPs by culture supernatant of *Klebsiella pneumonia*: The effects of visible-light irradiation and the liquid mixing process. Mater. Res. Bull., 44(6): 1415–1421

Morones, J.R.; Elechiguerra, J.L.; Camacho, A.; Holt, K.; Kouri, J.B.; Tamorez, J.T. and Yacaman, M.J (2005). The bactericidal effect of silver nanoparticles. Nanotechnol., 16:2346–2353.

Mukherjee, P.; Roy, M.; Mandal, B.P.; Dey, G.K.; Mukherjee, P.K.; Ghatak, J.; Tyagi, A.K. and Kale, S.P. (2008). Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. Nanotechnol., 19(7): p. 075103.

Mulvaney, P. (1996). Surface plasmon spectroscopy of nanosized metal particles. Langmuir., 12:788–800.

Oueslati, M.H.; Tahar, L.B. and Harrath, A.H. (2020). Catalytic, antioxidant and anticancer activities of gold nanoparticles synthesized by kaempferol glucoside from *Lotus leguminosae*. Arab. J. Chem., 13(1): 3112-3122.

Pal, S.; Tak, Y.K. and Song, J.M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram negative bacterium *Escherichia coli*. Appl. Environ. Microbiol., 73:1712–1720.

Panyala, N.R.; Peña-Méndez, E.M. and Havel, J. (2009). Gold and nano-gold in medicine: overview, toxicology and perspectives. J. Appl. Biomed., 7(2):75-91.

Parvataneni, R. (2019). Biogenic synthesis and characterization of silver nanoparticles using aqueous leaf extract of *Scoparia dulcis* L. and assessment of their antimicrobial property. Drug Chem Toxicol., 1–15.

Qayyum, S.; Oves , M. and Khan, A.U. (2017). Obliteration of bacterial growth and biofilm through ROS generation by facilely synthesized green silver nanoparticles. PloS one., 12(8):e0181363.

Rai, M.; Yadav, A. and Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv., 27: 76–83.

Rajoka, M.S.R.; Mehwish, H.M.; Zhang, H.; Ashraf, M.; Fang, H.; Zeng, X.; Wu, Y.; Khurshid, M.; Zhao, L. and He, Z. (2020). Antibacterial and antioxidant activity of exopolysaccharide mediated silver nanoparticle synthesized by *Lactobacillus brevis* isolated from Chinese koumiss. Coll. Surf. B Biointerface.*;* 186: p.110734.

Roco, J. (2005). Trends in nanotechnology patents. Nanopart. Res., 7: 707–712.

Roe, D.; Karandikar, B.; Bonn-Savage, N, .;Gibbins, B. and Roullet JB. (2008). Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. J Antimicrob Chemother., 61(4):869–76.

Salari, S.; Bahabadi, S.E.; Samzadeh-Kermani, A. and Yosefzaei, F. (2019). *In-vitro* Evaluation of Antioxidant and Antibacterial Potential of Green Synthesized Silver Nanoparticles Using *Prosopis farcta* Fruit Extract. Iran. J. Pharm. Res., 18(1):430.

Shahverdi, A. R.; Minaeian, S.; Shahverdi, H. R.; Jamalifar, H. and Nohi, A.A. (2007). Rapid synthesis of silver nanoparticles using culture supernatants of *Enterobacteria*: a novel biological approach. Process Biochem., 42(5): 919–923.

Siddiqi, K.S. and Husen, A.(2016). Fabrication of metal nanoparticles from fungi and metal salts: scope and application. Nanoscale Res Lett., 11(1): p. 98.

Siddiqi, K.S.; Husen, A. and Rao, R.A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. J. Nanobiotechnol., 16(1):1-28.

Siddique, M.H.; Aslam, B.; Imran, M.; Ashraf, A.; Nadeem, H.; Hayat, S. and Qureshi, U. (2020). Effect of Silver Nanoparticles on Biofilm Formation and EPS Production of Multidrug-Resistant *Klebsiella pneumoniae*. BioMed. Res. Int., 2020:1-9.

Singh, P.; Pandit, S.; Beshay, M.;Mokkapati, V.R.S.S.; Garnaes, J.; Olsson, M.E.; Sultan, A.; Mackevica, A.; Mateiu, R.V.; Lütken, H. and Daugaard, A.E. (2018**).** Anti-biofilm effects of gold and silver nanoparticles synthesized by the *Rhodiola rosea* rhizome extracts. Artif Cells Nanomed Biotechnol., 46(sup3):S886–99.

Singh, R. and Lillard Jr, J.W. (2009). Nanoparticle-based targeted drug delivery. Exp. Mol. Pathol., 86:215–223.

Sivasankar, P.; Seedevi, P.; Poongodi, S.; Sivakumar, M.; Murugan, T.; Sivakumar, L.; Sivakumar, K. and Balasubramanian, T. (2018). Characterization, antimicrobial and antioxidant property of exopolysaccharide mediated silver nanoparticles synthesized by *Streptomyces violaceus* MM72. Carbohydr. Polym., 181:752-759.

Thuptimdang, P.; Limpiyakorn, T.; McEvoy, J.; Pru¨ß, BM. and Khan, E. (2015). Effect of silver nanoparticles on *Pseudomonasputida* biofilms at different stages of maturity. J. Hazard. Mater., 290:127–33.

Vorobyova, V.; Vasyliev, G. and Skiba, M. (2020). Eco-friendly "green" synthesis of silver nanoparticles with the black currant pomace extract and its antibacterial, electrochemical, and antioxidant activity. Appl. Nanosci.; pp.1-12

Yusof, H.M.; Mohamad, R. and Zaidan, U.H. (2019). Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. J. Animal Sci. Biotechnol., 10 (1): 57pp.