



Biosynthesis, Characterization, and Antibacterial Activity of Silver Nanoparticles Derived from *Aloe barbadensis* Miller Leaf Extract

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Background: There is a growing commercial demand for nano-formulations due to their prevalence applicability in various areas of bio-nanotechnology. Numerous chemical and physical methods have traditionally been used to synthesize silver nanoparticles, but they are limited due to use of toxic and harmful chemicals, thus drew researchers' attention towards the biosynthesis of the silver nanoparticles by using medicinal plant.

Objective: The present study enlightens the synthesis of silver nanoparticles in an eco-accommodating way by using aqueous Aloe vera leaf extract (AVLE) and evaluate its antimicrobial potential.

Materials and Methods: The synthesis of silver nanoparticles using AVLE was determined by UV–vis spectrum and SEM. The optimization of different reaction conditions was measured, and antibacterial activity was evaluated by the disc diffusion method.

Results: The optimum synthesis of AV-AgNPs showed at a 1mM concentration of silver nitrate, 5:95 ratio of AVLE to silver nitrate solution, pH 8 at ambient temperature for 24 hours. The synthesis was confirmed by UV–Vis spectroscopy maximum absorbance at 400 nm while SEM showed spherical morphology with an average particle size 20-24 nm. The antibacterial activity of AV-AgNPs was measured by disc diffusion method and exhibits significant antibacterial activity against both gram-positive and gram-negative bacteria.

Conclusion: This method appears promising for the biosynthesis of silver nanoparticles by using Aloe vera with potent bactericidal activity, thus suggesting its role in clinical therapeutics and other fields.

Keywords: Aloe vera, Antibacterial, Green synthesis, Plant extract, Silver nanoparticles

1. Background

Microbes designate an important risk to the lives of millions of people around the globe and acting as a mediator for devastating pathogens. Despite the advancement of medical and pharmaceutical sciences, the newer and safer bactericidal agents' preparation is still a major problem in the prevention of spreading various life-threatening infectious diseases (1). Bacteria acquired resistance day by day due to excessive use of antibiotics and these antibiotics developed high toxicity by producing several side effects, thus reducing the quality of life. Whereas, the use of standard microbicidal agents reduced drug bioavailability, low therapeutic index, acquired multiple drug resistance and opposing systemic effects (2). Therefore, it develops the need for new and safer alternatives like metallic nanoparticles. Nanotechnology grows rapidly as an emerging field of

science dealing with nanoparticles (3). Nanoparticles research is inevitable today because of gaining importance in many fields due to its smaller size, various shapes and increased surface area to volume ratio (4). Among all metallic nanoparticles used in various fields, the silver nanoparticles considered as most promising due to having strong bactericidal effects along with antifungal, anti-inflammatory and anti-angiogenesis (5). Silver nanoparticles are immensely used in various consumer products (6).

Nanoparticles synthesized by chemical (7) and physical methods (8) may exert a burden on human and the environment. With the increased presence of nanomaterials in consumer products, large quantities of nanoparticles influenced human health and the environment. To minimize this influence of nanoparticles on human and environment, biological synthesis of

silver nanoparticles is a better alternative and become increasingly popular nowadays (9). Biological synthesis offers an advancement over the other methods as it is cost-effective, nature-friendly, less energy consuming and easy to implement for the wide-range synthesis of nanoparticles (10). Plant extract is extremely attractive as they reduce the need for maintenance of cell culture and downstream processing used in other methods of biological synthesis of nanoparticles (11). The use of plant extracts for the phyto-mediated synthesis of nanoparticles referred to as “green synthesis” and it is believed to play a crucial role in the reduction and stability of nanoparticles (12).

Aloe barbadensis Miller (**Fig. 1**) commonly known as Aloe vera, traditionally uses for a variety of medicinal purposes due to containing hundreds of nutrients and active compounds. Having diverse phytoconstituents Aloe vera reported as an analgesic, anticancer, antidiabetic, antifungal, anti-inflammatory, anti-leishmanial, antimicrobial (13), antimutagenic, antioxidant, antiproliferative, gastric mucosal protective, hepatoprotective, hypolipidemic, immunomodulatory, neuroprotective, radioprotective and wound healer (14).

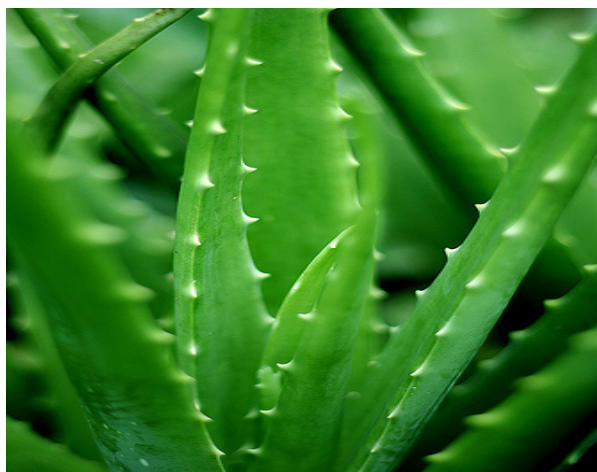


Figure 1. Aloe vera plant

2. Objective

The present study was designed to synthesize silver nanoparticles by using Aloe vera leaf extract and to evaluate its potential anti-bacterial effects. The advantage of using Aloe vera leaf is that it is a well-known, versatile medicinal plant, easily available and well documented potential properties. Moreover, it offers synergistic effects to enhance the antimicrobial properties of the bio-synthesized silver nanoparticles. This study reflects the applications of eco-friendly biogenic silver nanoparticles as a bactericidal agent and represents this method potentially inspiring for large-scale preparation.

3. Materials and Methods

3.1. Materials

The healthy Aloe vera leaf was collected from the University campus, University of Karachi. The clinically isolated microorganisms were procured, from the Department of Microbiology, University of Karachi, Karachi, Pakistan. Silver nitrate (AgNO_3) and other chemicals were used as an analytical grade (Sigma Aldrich).

3.2. Preparation of Plant Extract

The healthy Aloe vera leaf was washed by using distilled water and incised into small pieces. After weighing 10 g leaf, transferred it into a beaker containing 100 ml of distilled water and boiled for 20 min (15). The resulting extract was filtered via Whatman No.1 filter paper and collected, cooled and stored for further use in the refrigerator.

3.3. Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles was conducted by a previously described method with slight modifications (15). 5 mL aqueous Aloe vera leaf extract was mixed with 42.5 mL of 1 mM AgNO_3 solution followed by the addition of 2.5 mL of 0.1% ammonia solution and incubated in dark at room temperature. After 24 hours, the colorless silver nitrate solution changed into faint yellow to reddish brown; indicating the formation of nanoparticles. The obtained Aloe vera mediated silver nanoparticles (AV-AgNPs) were cleansed by repetitive centrifugation at 15,000 rpm for 10 min with distilled water. The obtained AV-AgNPs was collected and re-dispersed in deionized water for the estimation of absorbance, spectrophotometrically.

3.4. Optimization of Different Reaction Parameters

The different reaction parameters were optimized, comprises the concentration of silver nitrate solution, the ratio of Aloe vera leaf extract to silver nitrate solution, pH and reaction time. All parameters were optimized at ambient temperature and absorption spectra were recorded.

3.4.1. Effect of Concentration of Silver Nitrate (AgNO_3)

The synthesis of silver nanoparticles was performed at different concentrations of silver nitrate solution (0.25 mM, 0.5 mM, 0.75 mM, and 1 mM) in a fixed volume of leaf extract (5 mL). The reaction mixture incubated for 24 hours and the absorbance was measured by spectrophotometrically.

3.4.2. Effect of the ratio of Aloe vera Leaf Extract to Silver Nitrate Solution

The synthesis of silver nanoparticles was performed at different ratios of *Aloe vera* leaf extract to silver nitrate solution (1:99, 2:98, 3:97, 4:96, and 5:95) in a constant concentration of silver nitrate solution (1mM). The absorbance was recorded spectrophotometrically, after 24 hours of incubation in the dark at ambient temperature.

3.4.3. Effect of pH

The synthesis of silver nanoparticles was performed, and the pH of the reaction mixture was optimized. The reaction pH was maintained at 2, 5, 7, 8 and 10 pH. The pH was adjusted by using 0.1N HCl or 0.1N NaOH. Then 24 hours of incubation, the absorbance was measured by spectrophotometrically.

3.4.4. Effect of Time on Reaction Media

The effect of time on reaction media for completion of silver nanoparticles synthesis was studied by monitoring the absorption spectra of incubation time with the interval of 30 minutes, 2 hours, 4 hours, 6 hours and lastly 24 hours.

3.5. Characterization of Silver Nanoparticles

The AV-AgNPs were characterized in a Shimadzu spectrophotometer in the scanning range of 300-700 nm at the interval of 20 nm against double distilled water as a blank. The morphology and particle size of the synthesized silver nanoparticles (AV-AgNPs) were characterized by using scanning electron microscopy (SEM). Briefly, a small amount of the silver nanoparticles synthesized by *Aloe vera* leaf extract (AV-AgNPs) was allowed to dry at room temperature for 24 hours. The sample was plated with gold up to 300°A in auto coater Ion Sputtering Device (JEOL; Japan, Model No. JFC-1500). The gold-coated sample was exposed to scanning electron microscope (JEOL Japan, Model JSM 6380 A), for morphological analysis at an accelerating voltage of 15 kV. Photomicrographs were obtained at magnification ranges from 15000 to 35000.

3.6. Antibacterial Activity

The antibacterial assay for AV-AgNPs was detected by the disc diffusion method (16) by measuring the zone of inhibition against 11 clinical isolates namely, gram-positive bacteria *Bacillus licheniformis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus pyogenes*, and gram-negative bacteria *E. coli* (3 strains), *Klebsiella pneumoniae* (2 strains), *Pseudomonas aeruginosa* and *Salmonella typhi*. The fresh overnight bacterial culture was maintained on Luria Bertani (LB) agar. Freshly prepared

silver nanoparticles (5 μ L) were added to the sterile discs of 5mm diameter and put into the inoculated plates. The AVLE (10%) and AgNO₃ (1 mM) used as a negative control while standard antibiotic gentamicin (20 μ g) as a positive control. The plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured.

4. Results

4.1. Synthesis of Silver Nanoparticles

The color of AV-AgNPs appears to reddish brown within 24 hours of incubation at room temperature (Fig. 2). Similar color changes were found in previous studies (17, 18), hence confirmed the bioreduction of silver nitrate into silver nanoparticles. The corresponding UV-Visible absorption spectrum showed maximum absorption at 400 nm (Fig. 3) and broadening of peak showed the polydispersed nature of particles. These findings agree with other studies (19).

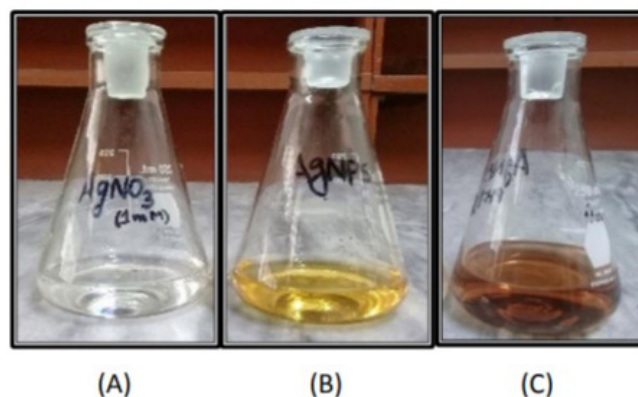


Figure 2. (A) Silver Nitrate Solution; Synthesis of silver nanoparticle after (B) 30 minutes (C) 24hours

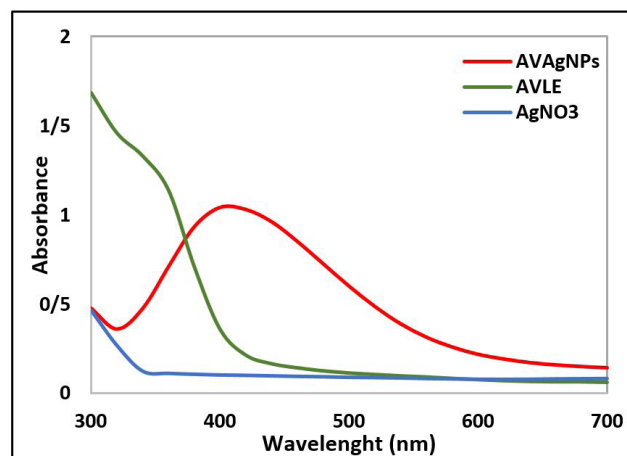


Figure 3. UV-Visible spectrum of AVAgNPs (Aloe vera mediated Silver Nanoparticle), AVLE (Aloe Vera Leaves Extract) and AgNO₃ (Silver Nitrate Solution)

4.2. Optimization of Different Reaction Parameters

Factors affecting the yields of *Aloe vera* mediated silver nanoparticles, including pH, the concentration of silver nitrate, the ratio of *Aloe vera* leaf extract to silver nitrate solution and reaction time were optimized at room temperature (Fig. 4 to 7).

4.2.1. The Concentration of Silver Nitrate Solution

Results demonstrated that the intensity of absorption spectra of AV-AgNPs increased with increasing the concentration of AgNO_3 (0.25 mM, 0.5 mM, 0.75 mM, and 1 mM) thus, explains the relation of particles synthesis and substrate concentration (20). The optimum concentration of silver nitrate (Fig. 4) found to be 1 mM with the absorbance peak at 400 nm after 24 hours incubation in this study.

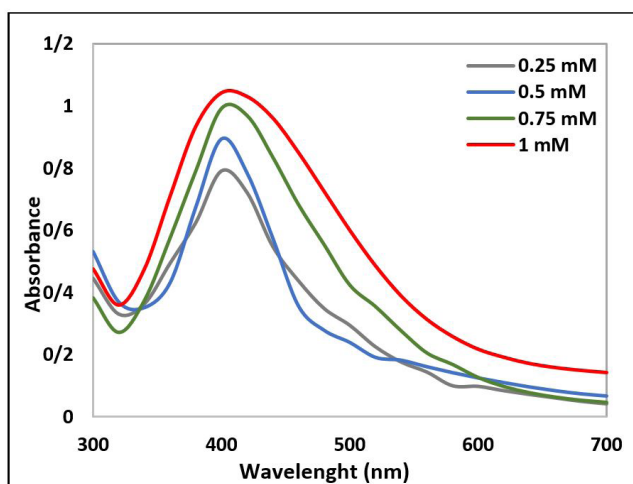


Figure 4. UV-visible spectrum of AgNPs synthesis at a various concentration of silver nitrate solution

4.2.2. Effect of ratio of Aloe Vera Leaf Extract to Silver Nitrate Solution

The reaction mixtures containing the different ratios of *Aloe vera* leaf extract to silver nitrate solution 1:99, 2:98, 3:97, 4:96, and 5:95 in a constant concentration of silver nitrate solution (1 mM) was optimized and result showed that the intensity of absorption spectra of AV-AgNPs increased with increasing the ratio of *Aloe vera* leaf extract to silver nitrate solution. 5:95 ratio gave the maximum formation with the absorbance peak at 400 nm after 24 hours incubation (Fig. 5).

4.2.3. Effect of pH

The solution was adjusted in different pH at a constant concentration of AgNO_3 (1 mM) and *aloe vera* leaf extract (10%). The UV-vis spectra of our study indicated (Fig. 6) that, at acidic and neutral pH (2, 4 and 7 respectively) silver nanoparticles formation was

minimum. While at alkaline pH (8 and 10) formation of silver nanoparticles maximizes and with the increase of pH, the broadening of the peak was observed therefore we select pH 8.0 as optimum pH for this study. The results are in complete correlation with the previously reported studies (21).

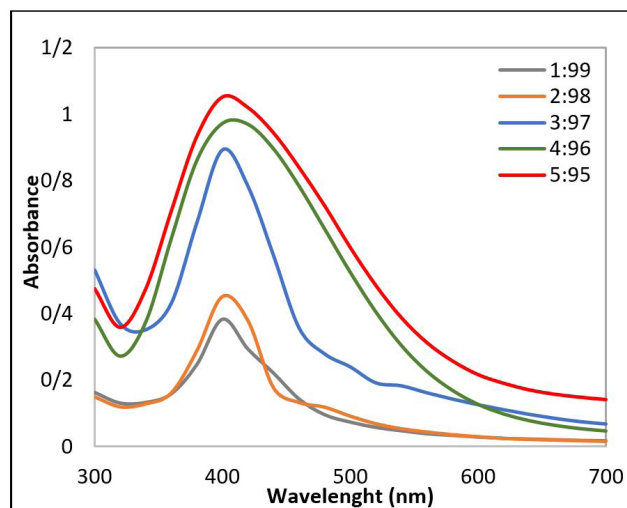


Figure 5. UV-visible spectrum of AgNPs synthesis at a various ratio of aloe vera leaves extract to silver nitrate solution

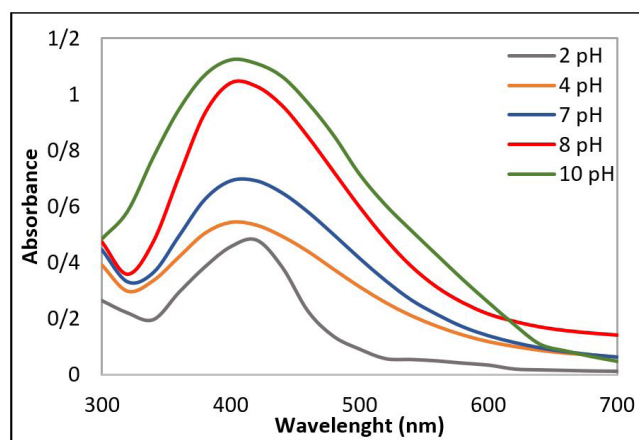


Figure 6. UV-visible spectrum of AgNPs synthesis at different pH

4.2.4. Effect of Reaction Time

Results displayed that with the passage of time, absorbance of reaction media was increases (Fig.7) and demonstrating the progress in the formation of AgNPs. Change in color was observed by subsequently adding the silver nitrate solution to the *Aloe vera* leaf extract. After 30 min, the color of the solution becomes faint yellow and turning reddish brown till 24 hours. After 24 hours the color nearly constant, indicating that no silver salt was left for further reaction. Previous findings also stated that with the passage of time the synthesis of silver nanoparticles increased without changing in

peak wavelength (22). These results are in complete association with other studies (20, 23).

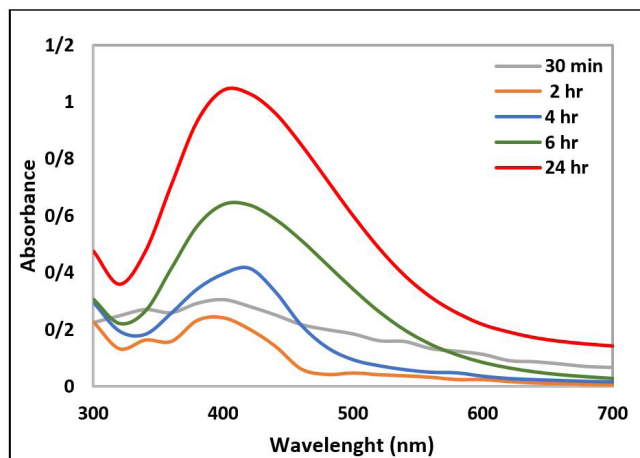


Figure 7. UV-visible spectrum of AgNPs synthesis at different reaction time

4.3. Characterization of Biosynthesized Silver Nanoparticles

The characterization of AV-AgNPs by SEM provides morphology and size of the silver nanoparticles. SEM result revealed that the AV-AgNPs was spherical in shape, and the average size of the AV-AgNPs was ranged between 20-24 nm (Fig. 8).

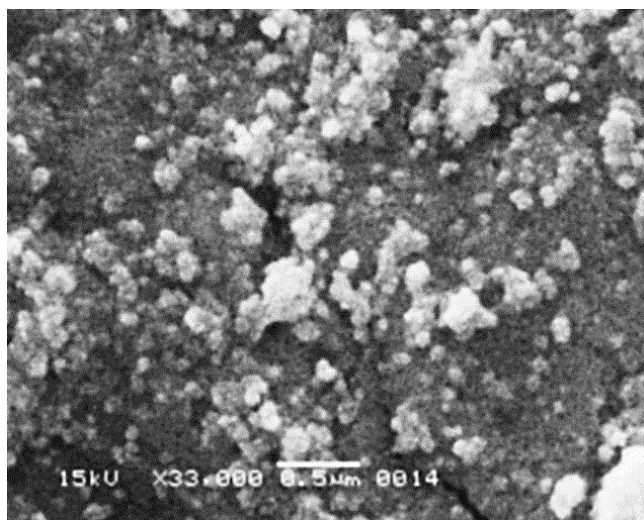


Figure 8. SEM image of AV-AgNPs

4.4. Antibacterial Activity

The AV-AgNPs displayed the highest zone of inhibition against *E. coli* (strain 3) and *Klebsiella pneumoniae* (strain 1) than the standard antibiotic, Gentamicin. *Bacillus licheniformis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *E. coli* (Strain 1 and 2), *Klebsiella pneumoniae* (Strain 2), *Pseudomonas*

aeruginosa and *Salmonella typhi* were found moderately susceptible to AV-AgNPs with a relatively smaller zone of inhibition when compared to gentamicin (Fig.9, Table 1). The AV-AgNPs displayed the inhibition zone against *Klebsiella pneumoniae* (strain 1) and *Streptococcus faecalis* even when these microbes showed resistance against gentamicin. Furthermore, AV-AgNPs showed more bactericidal activity compared with the $AgNO_3$ solution while aqueous aloe vera leaf extract (10%) did not show any inhibition towards any of the tested organisms.

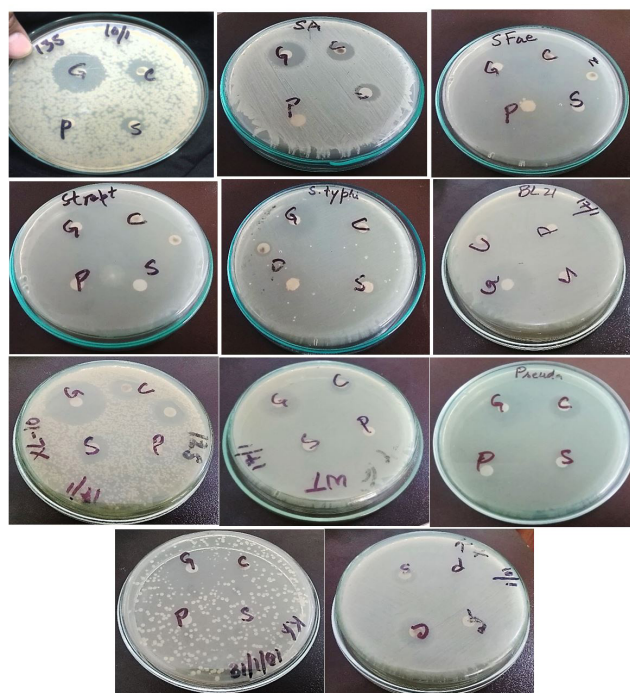


Figure 9. Antibacterial Activity of AV-AgNPs

5. Discussion

Biological synthesis of nanoparticles via plant extract is presently being practiced due to evasion of holding up the microbial culture and being monotonous when contrasted with microbes (24). Many nanoparticles like gold, silver, copper, palladium, zinc and lots of others are synthesized by using plant extracts (25). The main mechanism in the synthesis of nanoparticles via a plant involves the reduction of silver ions into particles due to the presence of phytoconstituents in the extract (26). The *Aloe vera* leaf extract was successfully used as a reducing and stabilizing agent for the synthesis of aloe vera mediated silver nanoparticles (AV-AgNPs). The synthesis was confirmed by the color change, that was owed to the excitation of surface plasmon resonance of the formed silver nanoparticles (27). The factors that affect the synthesis of silver nanoparticles were included pH, the concentration

Table 1. Zone of Inhibition (mm diameter)

| S. No. | Bacterial strain | Zone of Inhibition (mm diameter) | | | |
|---------------|---|----------------------------------|--------------------------|------------------------|--------------------------|
| | | AVLE 10% | AgNO ₃ 1mM | AV-AgNPs 70 µg/disc | Gentamicin 20 µg/disc |
| Gram-positive | | | | | |
| 1. | <i>Bacillus licheniformis</i> | NA | 9 | 14 | 24 |
| 2. | <i>Staphylococcus aureus</i> | NA | 11 | 14 | 18 |
| 3. | <i>Streptococcus faecalis</i> | NA | NA | 09 | NA |
| 4. | <i>Streptococcus pyogenes</i> | NA | NA | 09 | 15 |
| Gram-negative | | | | | |
| 5. | <i>Salmonella typhi</i> | NA | 8 | 06 | 15 |
| 6. | <i>Escherichia coli</i> BL-21 (strain 1) | NA | 6 | 10 | 22 |
| 7. | <i>Escherichia coli</i> XL-10 Gold (strain 2) | NA | 10 | 19 | 30 |
| 8. | <i>Escherichia coli</i> WT (strain 3) | NA | 8 | 20 | 18 |
| 9. | <i>Pseudomonas aeruginosa</i> | NA | 9 | 15 | 17 |
| 10. | <i>Klebseilla pneumoniae</i> (strain 1) | NA | 15 | 20 | 18 |
| 11. | <i>Klebseilla pneumoniae</i> (strain 2) | NA | 12 | 15 | NA |

NA: No Antibacterial activity

of silver nitrate, the ratio of *Aloe vera* leaf extract to silver nitrate solution and reaction time at ambient temperature. pH considered an important parameter for the synthesis of the silver nanoparticles by influencing the electrical charges present in the extract. It also acts as capping and stabilizing agents. pH can change the size and shape of silver nanoparticles (28). The alkaline pH favors the maximum synthesis of silver nanoparticles likely associated to be activation of biomolecules or more functional groups were present in plant extract under the alkaline conditions (29).

The association of silver nanoparticles synthesis was found with different concentration of AgNO₃ and ratio of *Aloe vera* leaf extract to silver nitrate solution (30). The substrate concentration (AgNO₃) also directs the morphology of silver nanoparticles (31). 5:95 was considered as the optimum ratio for maximum particle synthesis may be due to the quantity of reducing and capping agents present in extract being enough for silver nitrate reduction (32). Similar outcomes were found by Verma *et al.*, (23) when they used an aqueous extract of *Solanum nigrum* leaf extract. The concentration of biomolecules reduces and cap the silver nanoparticles and protecting them from accumulation, resulting in symmetrical nanoparticles formation (21). The optimum reaction time was found to be 24 hours for this study. The reaction time depends to utilizes the all silver ions present in the reaction mixture will be converted into nanoparticles (33).

Scanning electron microscopy is well known to investigate the morphology and size of nanoparticles. In the present study, the AV-AgNPs was spherical in shape, and the average size was ranged between 20-24 nm (Figure 8). Pareetha *et al* suggested that the morphology of silver nanoparticles persuaded by a hydrogen bond and electrostatic interactions found in plant extract (34).

The aloe vera mediated silver nanoparticles (AV-AgNPs) showed efficient anti-bacterial activity against clinically isolated Gram-positive and Gram-negative bacteria (Table, 1) hence has a great potential in biomedical applications (35, 36). Various investigations establish a mechanism of action of AgNPs against pathogenic microbes including the capability of silver nanoparticles, release silver ions and having the specific surface to volume ratio increases their probability of interaction with microorganisms consequently endorsing the dissolution of silver ions thereby improving its biocidal effectiveness (37). On the other hand, Slavin *et al* (38) reported the oxidation of silver nanoparticles by the oxygen and reduce the Ag into Ag⁺ ions and generating reactive oxygen species (ROS) thus disrupted the membrane integrity. Other mechanism relating AgNPs and cytoplasmic organelles interactions by thiol groups (-SH) of surface proteins and enzymes causing the deterioration of cellular membrane and mitochondrial ATP synthesis. This leads to ROS generation, provoke oxidative stress and irreversible damage to DNA (39).

6. Conclusion

In our study, we concluded that the AgNPs were successfully synthesized from the *Aloe vera* leaf extract with a characteristic peak at 400 nm, average diameters ranging from 20-24 nm with spherical morphology. These AV-AgNPs exerts significant antimicrobial activity against clinically isolated both Gram-positive and Gram-negative bacterial strains suggests that the synthesized AV-AgNPs were an excellent bactericidal agent. This green engineered technique using extracts gives new ways to the advancement of AgNPs with controlled size and shape.

Abbreviations

AVLE: Aloe vera leaf extract

AV-AgNPs: Aloe vera mediated silver nanoparticles

UV-vis spectrum: Ultraviolet-visible spectrum

λ_{\max} : Maximum wavelength at which maximum absorbance demonstrated

SEM: Scanning electron microscopy

Acknowledgments

We are thankful to Centralized Science Laboratory (CSL), University of Karachi for assistance in Scanning electron microscopy (SEM).

Conflicts of Interest

There is no conflict of interest to declare.

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