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Green Synthesis and Characterization of Biocompatible Silver Nanoparticles using *Stachys lavandulifolia Vahl.* Extract and Their Antimicrobial Performance Study

Abbas Zakeri Bazmandeh[1†], Arash Rezaei[2 †], Hamid Reza Ghaderi Jafarbigloo[3], Amir Mohammad Akbari Javar[4], Ali Hassanzadeh[5], Armin Amirian[6], Mohamad Hadi Niakan[7], Hossein Hosseini Nave[8,9*] and Mohsen Mehrabi[10*]

[1]Department of Medical Nanotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of laboratory Sciences,School of Medicine,Kerman University of Medical Sciences,Kerman,Iran
[3]Department of Basic Science, Payame Noor University, Iran
[4]Department of Mathematics, faculty of Mathematics, Farhangian University, Kerman, Iran
[5] Department of Tissue Engineering and Applied cell Sciences, Tehran University of Medical Sciences, Tehran, Iran
[6]Thoracic and Vascular Surgery Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran
[7]Trauma Research Center, Shahid Rajaee (Emtiaz) Trauma Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
[8]Student Research Committee, Kerman University of Medical Sciences, Kerman, Iran
[9]Department of Microbiology and Virology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[10]Department of Medical Nanotechnology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

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Abstract

Green synthesis of nanoparticles has received extensive attention in the field of nanomedicine as a simple, environment-friendly and cost-effective alternative in comparison with other physical and chemical methods. Silver nanoparticles (AgNPs) are playing an important role in the field of nanomedicine and nanotechnology due to their unique and controllable characteristics. In this research, a biosynthesis approach was used for synthesis of silver nanoparticles (AgNPs). Biosynthesis was carried out by *Stachys lavandulifolia Vahl.* extract as green reducing agent for conversion of Ag^+ to Ag^0. The first visible sign of the synthesis of AgNPs was the change in color of solution from yellowish to reddish brown. The preparation of AgNPs was performed using several techniques such as, UV-vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and dynamic light scattering (DLS). Finally, synthesized AgNPs were evaluated for their antimicrobial activity against four standard bacterial strains using microbroth dilution method. Results of TEM image showed spherical nanoparticles with high crystalline and monodispersity which is very important in medicinal applications. Additionally, DLS analysis demonstrated the ultra-fine size of AgNPs with average size of 164.8 nm. FTIR indicated the role of different functional groups (carboxyl, amine and hydroxyl) in the formation of Silver nanoparticles. A negative zeta potential value of -17.3 mV determined the stability of the silver nanoparticles. AgNPs showed good antimicrobial activity against both gram-positive and gram-negative bacteria, with MICs of 4 to 16 µg mL. In conclusion, the results imply that the synthesized nanoparticles using green nanotechnology could be an ideal strategy to combat multi-drug-resistant pathogenic bacteria.

Keywords: *Stachys lavandulifolia Vahl;* Silver nanoparticle; Green synthesis; Antimicrobial performance

1 Introduction

Resistance to commonly available antimicrobial agents by pathogenic bacteria has been rising at an alarming rate and has become a serious global problem for healthcare (1, 2). Serious infections caused by resistant bacteria such as methicillin-resistant *Staphylococcus aureus* and *Enterobacteriaceae* (3, 4). Nanotechnology has opened a

***Corresponding authors:** (a) Department of Microbiology and Virology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran. E-mail: Hosseini.nave@gmail.com. (b) Mohsen Mehrabi, Department of Medical Nanotechnology, School of Medicine, Shahroud University of Medical Sciences, Shahroud. Iran.
†These authors contributed equally to this article.

wide field for the synthesis of new and noble materials for a variety of applications, especially medical and pharmaceutical use the term nanoparticle typically refers to a particle with a size ranging from 1 to 100 nm (5). The antibacterial activity of nanoparticles is due to their high surface area to volume ratio, thereby enabling significant interactions with microbial cell membranes. Silver nanoparticles (AgNPs) has attracted more attention than other metal nanoparticles due to exceptional properties such as surface plasmonic resonance (SPR) and stability, which can be used as catalyst, sensor, anti-angiogenic, anticancer, and especially antibacterial agent (5-8). As mentioned, the exceptional properties of nanoparticles depend on their size and morphology which are determined by the synthesis method. The methods for the synthesis of nanoparticles are mainly grouped into two categories: the physical and chemical approaches. Although these methods are popular, they cause considerable environmental and economic issues. Thus, there is an apparent need for an alternative approach for the synthesis of nanoparticles. The "biological method" was developed as a simple approach as an alternative to physical and chemical methods. Green synthesis method provides several advantages over chemical methods as it is eco-friendly, simple, rapid, safe, clean and cost effective. These features are important in medical applications. A vast number of biological resources including plants, algae, fungi, yeast, and bacteria has been studied so far for the synthesis of nanoparticles (9). The plant-mediated synthesis of AgNPs can be advantageous compared with other biological processes as it is relatively fast, does not require the process of maintaining specific media and aseptic environments (10).

In this work, *Stachys lavandulifolia Vahl.* (Family Lamiaceae) was applied for synthesis of AgNPs as reducer, surfactant and capping agent. This plant was distributed in different regions of Asia and Europe. *Stachys lavandulifolia Vahl.* has been widely used in traditional medicine for treatment of various diseases (11). In this study, green synthesis of AgNPs was done using leaf extracts of *Stachys lavandulifolia Vahl.* The prepared nanoparticles were characterized using several methods such as XRD, UV-vis along with FTIR spectroscopy, TEM and DLS analysis to confirm the formation of nanoparticles. After approval of the structure and purity of AgNPs, antibacterial activity of this nanoparticles was studied on Gram-positive and Gram-negative bacteria.

2 Materials and methods

2.1 Material

In this study silver nitrate ($AgNO_3$) was

2.3 Synthesis of AgNPs with Stachys lavandulifolia Vahl. Extract

For synthesis of AgNPs, 20 mL of different concentrations (5%-20%) of fresh *Stachys lavandulifolia Vahl.* extract was added into 5ml of $AgNO_3$ solution (0.1mM) at room temperature for the reduction of Ag+ ions. The color change of the solution from yellow to reddish brown indicated that the silver nanoparticles were synthesized.

2.4 Characterization

The formation of AgNPs was monitored using UV–vis spectrometer in the range of 250–600 nm. Fourier transform infrared (FTIR) analysis was recorded in the range between 4000 to 400 cm−1 to investigate the functional groups involved in the synthesis of silver nanoparticles. The structural characterization and crystalline nature of AgNPs were analyzed by X-ray diffraction (XRD). The XRD pattern was recorded using a Holland Philips X-ray powder diffractometer using Cu Kα radiation (λ= 0.1542 nm) in the 2θ range of 5°–80°, with operating voltage of 40 kV at a 20 mA current strength. The morphology and average size of AgNPs were analyzed by transmission electron microscopy (TEM). The sample for TEM was prepared by ultrasonic dispersion of the AgNPs in ethanol. Finally, the size distribution of nanoparticles was characterized through dynamic light scattering (DLS).

2.5 Antibacterial performance tests

The minimal inhibitory concentration (MIC) of the AgNPs was determined by micro-broth dilution method using Mueller Hinton broth. Briefly, concentrations with a starting range of 1024 μg/ml to 2 μg/ml of the AgNPs was prepared in the wells of a 96-well plate, and bacterial inoculum with an adjusted bacterial concentration of 1.5×10^6 colony-forming units/ml (CFU/ml) was added to this 2-fold dilution series. The plate was incubated at 37°C for 24 hours. Antimicrobial activity was determined against four reference strains including two Gram-negative bacteria [*Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27852)] and two Gram-positive bacteria [*Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212)]. The ciprofloxacin was used as the positive control for anti-bacterial screening. The lowest concentration of AgNPs that was able to completely inhibit the bacterial growth was considered as the MIC value. The MIC experiments were performed in triplicate against each bacterial strain to confirm the value of MIC for each tested bacterium.

3 Result and discussion

In this research, the AgNPs were synthesized by green synthesis method, using *Stachys lavandulifolia Vahl.* extract as both reducer and stabilizer agent. The formation of AgNPs was studied using both visual observation of the color and UV–visible spectral analysis. The $AgNO_3$ solution is colorless (Figure 1a) and there is no absorption peak in the UV-Vis spectrum (Figure 1d). On the other hand, the color of *Stachys lavandulifolia Vahl.* extract was yellow (Figure 1b) and had a weak peak in UV-vis spectrum, that can be attributed to $\pi\rightarrow\pi^*$ transitions of aromatic rings. The color of solution was changed to brown when the $AgNO_3$ was added to yellow extract (Figure 1c). This change in color visually indicates the formation of silver Nanoparticles. Additionally, UV-vis spectroscopy of the colloidal AgNPs in Figure 1d demonstrates a strong peak in 440 nm due to surface plasmon resonance (SPR) of silver nanoparticles formed. Both change of color and SPR band in UV-vis spectra show conversion of Ag^+ in $AgNO_3$ solution to Ag^0 and completion of reduction reaction.

With the objective of finding various functional groups involved in the synthesis of silver nanoparticles FTIR analysis was performed. The FTIR spectrum of biosynthesised AgNPs showed absorption bands at 3432, 2073, 1626, 1636, and 634 cm^{-1} (Figure 2). The absorption band at 3,374 cm^{-1} indicated stretching of the N–H bond of amino groups or presence of attached hydroxyl (–OH) group because of the existence of surface adsorbed alcohols (carbohydrates etc.) and phenols (polyphenols). According to the result of FTIR analysis, C-H stretching of the methyl group can be observed at 2073 cm^{-1} region. The strong peak at 1636 cm^{-1} is related to C=O stretching vibrations of aldehydes, ketones and carboxylic acids (5). Several investigations have reported that functional groups such as alcohol, phenol, and amines act as the capping agent for AgNPs which increases the stability of the nanoparticles (12, 13). Finally, the band at 634 cm^{-1} is related to several aromatic amines. The XRD profile of AgNPs synthesized using the leaf extract of *Stachys lavandulifolia Vahl.* is depicted in Figure. 3. The result demonstrated the face-centered cubic (FCC) for silver crystallites. The diagram of XRD shows four important peaks at 2θ˚= 38.65, 44.60, 64.85 and 77.47 . This result represents silver crystal formation which, respectively, corresponds to 111, 200, 220 and 311. These planes and phase of the Ag nanoparticle are in agreement with the reference file with JCPD= 01-1167. Additionally, there are two unassigned peaks at 2θ˚= 28 and 46 denoted by (•) in Figure. 3 which are thought to be related to crystalline and amorphous organic phases. Hence, the result of XRD pattern approved the successful synthesis of pure silver nanoparticles usage plant extract as the capping agent and surfactant in mild condition. The XRD peaks revealed that AgNPs formed using *Stachys lavandulifolia Vahl.* extract were crystalline and spherical in shape.The average nanosize was calculated by applying Debye–Scherrer formula D = K l/β cosθ which was found to be 36.69 nm (14).

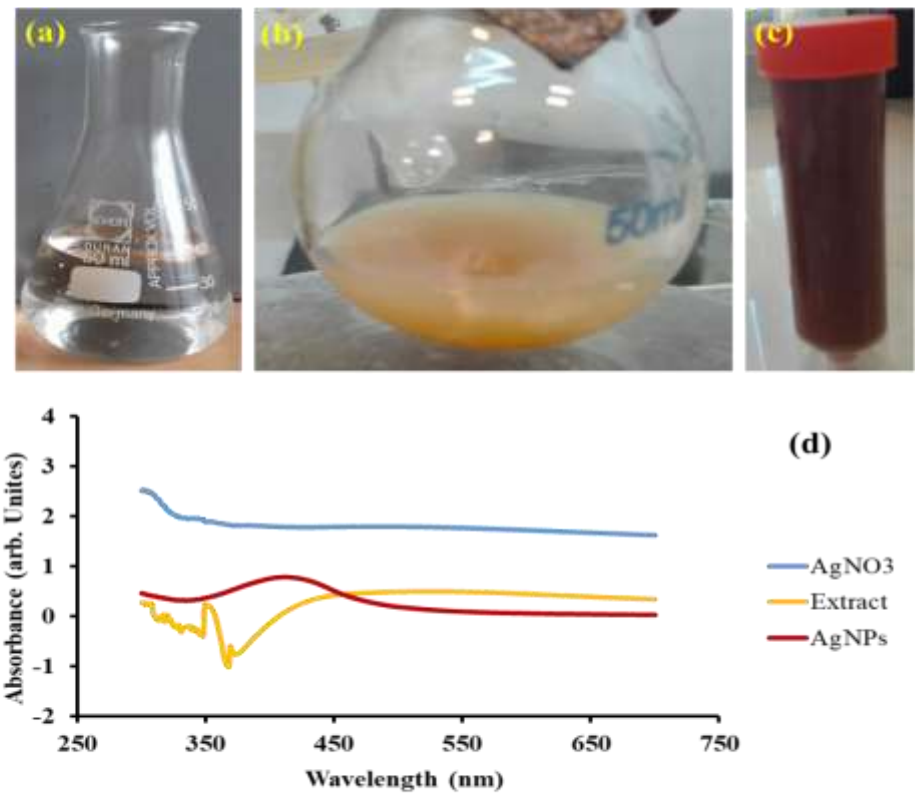

Figure 1: Color of (a) $AgNO_3$ solution; (b) *Stachys lavandulifolia Vahl.* extract; (c) AgNPs suspension and (d) UV-vis spectroscopy of $AgNO_3$, *Stachys lavandulifolia Vahl.* extract, and Ag NPs.

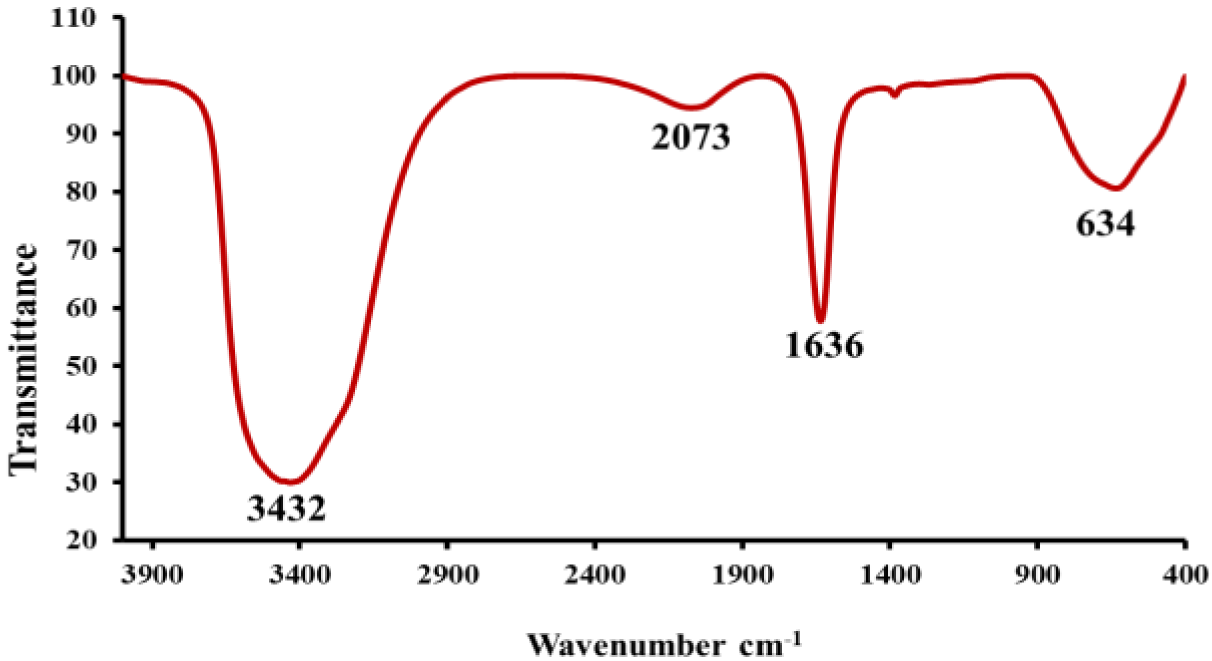

Figure 2: FTIR spectroscopy of green silver nanoparticles

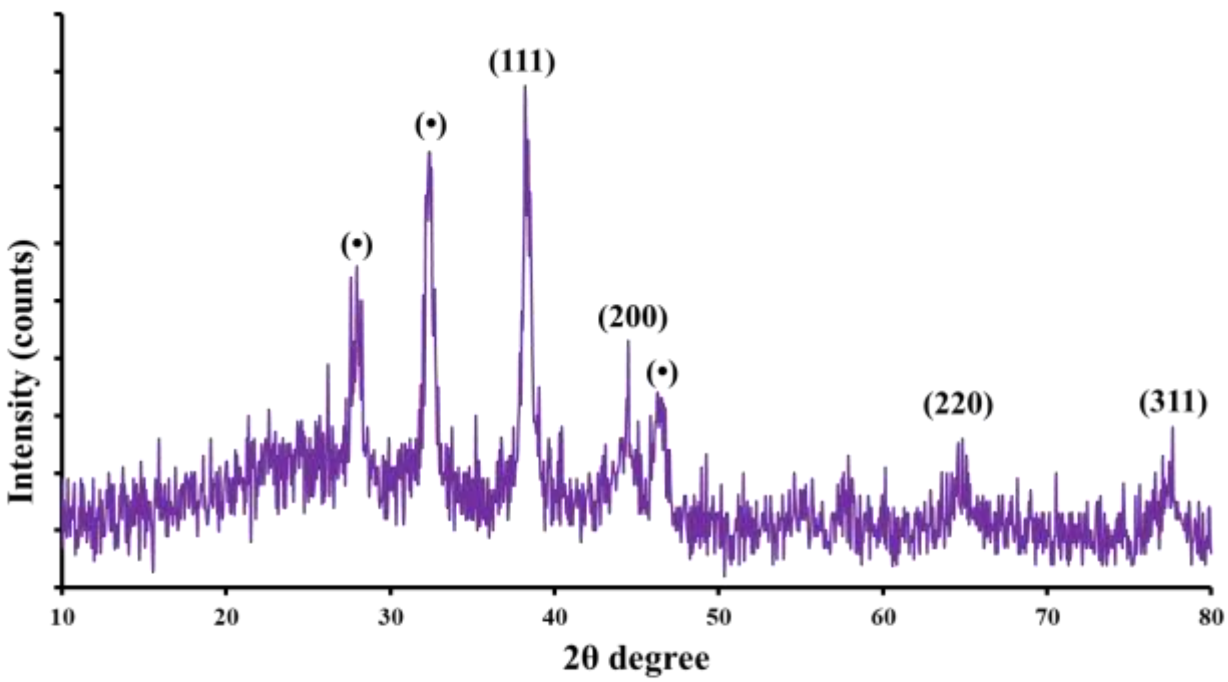

Figure 3: XRD pattern of biosynthesized AgNPs using *Stachys lavandulifolia Vahl.* extract

The morphology and size of green synthesized AgNPs were studied by TEM. The size and shape of the synthesized silver nanoparticles significantly affect their antimicrobial activity. TEM images of the synthesised AgNPs are shown in Figure 4. TEM images confirm the formation of spherical Ag nanoparticles (Figure 4a). According to Figure 4b, spherical nanoparticles have high monodispersity and crystallinity, without any aggregation. Based on the theory for crystal nucleation and growth, various types of organic compounds have basically a role in formation of crystalline nanoparticle as the capping agent. The polyphenol compounds can control the formation and growth of the primary core of AgNps in overall reaction which was clearly illustrated in scheme 1. Besides, the particle size of nanoparticles was estimated about 150 nm, and it was fine and suitable for medical applications.

Size distribution and average size of nanoparticles was measured using histogram of DLS analysis in Figure 5a. The measured average size is 164.8 nm which shows the monodispersity of particles in green strategy for synthesis of nanomaterials. Also, this size distribution is in agreement with the SPR peak in UV-vis spectroscopy. This definite size is matched with TEM image and confirms antimicrobial activity of AgNPs.

Moreover, zeta potential was performed to study the dispersion surface charge of AgNPs, which was covered with bio-molecular of the plant extract and that caused the nanoparticles stable in colloidal solution. According to the result in Figure 5b zeta potential diagram shows a negative charge with a value of -17.3 mV and this value leads to stability of nanoparticle and prevents the aggregation and agglomeration of silver nanoparticles and is also in agreement and comparable to the former studies (15, 16).

In order to evaluate the antibacterial activity of the silver nanoparticles synthesized using *Stachys lavandulifolia Vahl.* extract, we employed microbroth dilution method. For the bacterial strains *E. faecalis* 29212, *S. aureus* 25923, *P. aeruginosa* 27853 and *E. coli* 25922, the MIC values of silver nanoparticles were 8, 8, 4 and 4 μg mL, respectively. The antibacterial activity of AgNPs has been well documented in previous studies (14). However, the comparison of the results of antimicrobial studies is difficult as there is no standard method for the determination of antibacterial activity of AgNPs and different methods have been used by the researchers. In this study, AgNPs exhibited excellent antibacterial activity against both gram-positive and gram-negative bacteria, but the gram-negative bacteria were more sensitive to the nanoparticles than the gram-positive bacteria. These results are consistent with those of other studies, which also showed that Gram-positive strains exhibited greater resistance against nanoparticles in comparison with Gram-negative ones. This may be due to the difference in their cell wall structure. The Gram-positive bacterial cell wall consists of a thick peptidoglycan layer, while Gram-negative bacteria have thin peptidoglycan layer which allows easier penetration of AgNPs into the cell wall of Gram-negative bacteria (14, 17).

As the results showed, the synthesized AgNPs indicated a strong inhibition effect on several types of gram-positive and gram-negative bacteria with penetration in the bacteria cell due to the small size and high ratio of the surface per volume which leads to wide application of silver nanoparticles in the production of various antibacterial products.

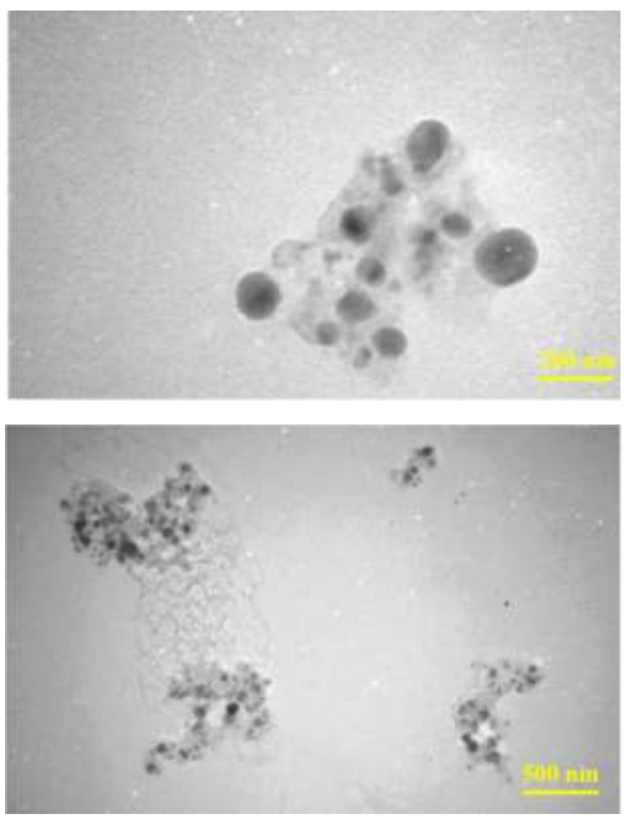

Figure 4: TEM image of synthesized AgNPs a) high-magnification; and b) low magnification

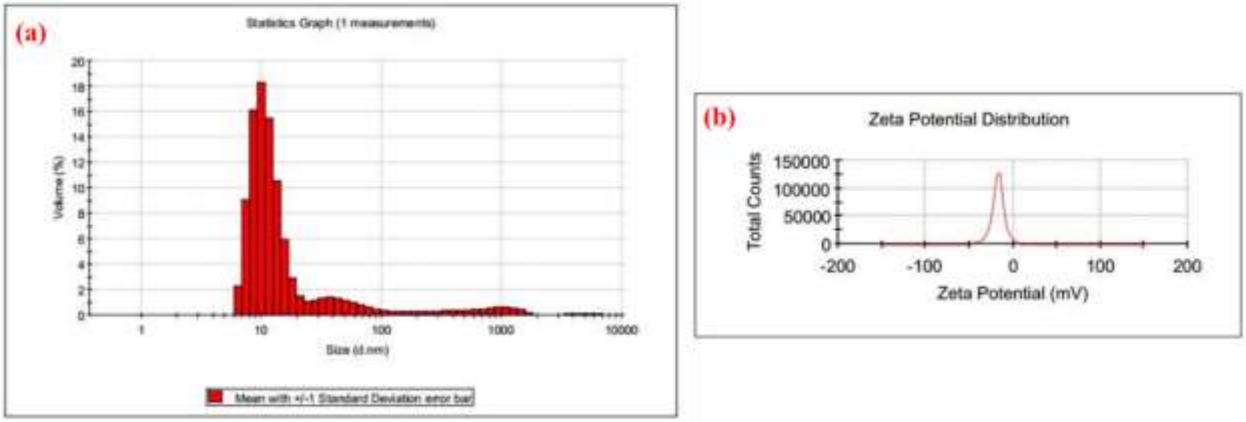

Figure 5: (a) DLS and (b) zeta potential of synthesized AgNPs using *Stachys lavandulifolia Vahl.* extract

4 Conclusion

Silver nanoparticles were successfully synthesized using green approach by *Stachys lavandulifolia Vahl.* extract as non-toxic and green reducing agent. After this, nanoparticles were characterized using various methods, for example FTIR, XRD, Uv-vis spectroscopy, TEM and DLS; this analysis approved the successful synthesis of nanoparticles with high purity. Therefore, XRD pattern demonstrated the crystalline cubic phase and also spherical shape and morphology was observed in TEM image with the estimated size about 32 nm in diameter. Moreover, DLS showed a very fine size of AgNPs 164.8 nm in diameter which is in agreement with SPR properties of AgNPs in Uv-vis spectroscopy. All analyses together showed the fundamental role of the plant extract as the capping agent and surfactant to control the size of AgNPs and also as the stabilizer of Ag^0 in colloidal solution. The biosynthesized nanoparticles also exhibited a high antibacterial activity against the tested strains. Therefore, this method is very safe, simple, eco-friendly and cost-effect for synthesis of biocompatible AgNPs in a large scale for medical application.

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