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Stability of Biosynthesised Silver Nanoparticles Using *Achyranthes aspera* Roots and Its Characterization

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A B S T R A C T

The present investigation was aimed to study the biosynthesis, stability and characterization of silver nanoparticles using *Achyranthes aspera* root extract. Synthesis of silver nanoparticles has been done by maintaining different $AgNO_3$ concentrations (0.50, 1.00, 1.50 and 1.84 mM), temperature (25, 45, 75, 105 and 125 ºC) and pH conditions (4, 5, 7, 9 and 10). By analysing the data obtained during stability study, it was found that, combination of $AgNO_3$ of 1.15 mM concentration, temperature at 45 ºC and pH of 9 was the best condition to synthesize the stable Ag NPs for one month. Characterization of synthesized silver nanoparticles was done by zetasizer, UV-Vis spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and atomic force microscopy (AFM). Particle size distribution of zetasizer indicated that the size of the biosynthesized silver nanoparticles was 23.21 nm and UV-Vis spectroscopy showed its absorbance peak at 420 nm, which confirmed the presence of Ag NPs. XRD analysis confirmed that, resultant Ag NPs were face-centered cubic in nature and AFM analysis showed surface area (103.97 μm^2), selected particle height (0.12 µm) and width (1.10 µm). It was concluded that, green synthesis was an eco-friendly and most economical way to produce silver nanoparticles over the chemical and physical methods

Introduction

Nanotechnology is considered as an emerging technology due to the possibility of advanced well-established products and to create new products with totally new characteristics and functions in a wide range of applications. It represents the design, production and application of materials at atomic, molecular and macromolecular scales in order to produce new nano-sized materials (Hahens *et al.*, 2007) and it is mainly concerned with synthesis of nanoparticles of variable size, shape, chemical compositions and controlled dispersity with their potential use for human benefits (Elumalai *et al.*, 2010).

An array of physical, chemical and microbial methods has been used for synthesis of metal nanoparticles of particular shape and size

(Balagurunathan *et al.,* 2011). Many of these methods involve the use of tedious hazardous chemicals or high energy requirements, which are rather difficult and tedious in purification (Ahmed *et al.,* 2014).

Green synthesis provides advancement over chemical and physical methods as it is cost effective, environment friendly, easily scaled-up and further there is no need to use toxic chemicals, high pressure and energy. The biological processes eliminate the elaborate process of maintaining cell cultures and can also be easily scaled-up for large-scale production of nanoparticles (Veeraswamy *et al.,* 2011). During synthesis of nanoparticles, the parameters such as pH, temperature, salt concentration and reducing agent have a significant influence on diameter, size distribution, shape, aggregation, state and stability. Thus, the optical properties of nanoparticles, conductivity and other characteristics may be changed (Kupiec *et al.,* 2011).

Achyranthes aspera is a species of plant in the Amaranthaceae family. It is known as *Uttarani* in kannada language. It is an erect, annual or perennial herb of about 1-2 metre in height and is found as a weed on road sides, field boundaries and waste places throughout India and in South Andaman Islands (Amaladhas *et al.,* 2013). Phytochemical investigations were revealed that, the presence of bioactive compounds like sterols, alkaloids, saponins, sapogenins, cardiac and glycosides in leaves and roots are responsible for the reduction of silver ions to silver nanoparticles (Ag NPs) (Triguna *et al.,* 1992).

It is well known that, silver is an effective antimicrobial agent and possesses a strong antimicrobial activity against bacteria, viruses and fungi. The antimicrobial activity of silver nanoparticles is a result of well-developed surface (Kaviya *et al.,* 2011). Because of their wide spread applications, the scientific community and industry have paid special attention to the synthesis of silver nanoparticles (Tran *et al.,* 2013).

Various instrumental techniques were adopted to characterize the synthesized Ag NPs. The particle size measurement can be obtained by zetasizer, optical properties of the silver nanoparticles can be determined through UV-Visible spectrophotometer, surface morphology by using scanning electron microscope (SEM), crystallinity can be measured by X-ray diffraction (XRD), surface and strength of nanoparticles can be measured by atomic force microscope (AFM) (Joseph *et al.,* 2016).

Materials and Methods

Biosynthesis of silver nanoparticles using *Achyranthes aspera* roots

The biosynthesis of silver nanoparticles using *A. aspera* roots was carried out as described below.

Preparation of *Achyranthes aspera* root extract

A. aspera roots were thoroughly washed using distilled water to remove dirt and soil. Washed roots were cut into small pieces of length 10 mm and dried in a tray dryer (Macro scientific works, Mac 216, Delhi, India) at 50 ± 2 ºC for about 5 days. The dried roots were ground using pulveriser (M/S Sriram Machinery Works, model SRM-108, Tamil Nadu, India) to make them into a fine powder and passed through a 100 mesh sieve (150 µm). Five grams of dried powder was added to 100 ml of distilled water and the mixture was heated at 60 ºC for about 30 min using water bath. Then, it was filtered through filter paper (Whatman No. 1). The filtrate was stored at 4 ºC for further experiments.

Biosynthesis of silver nanoparticles using *Achyranthes aspera* root extract

The root extract of *A. aspera* (10 ml) was diluted with distilled water (90 ml). Further, 1.5 mM $AgNO_3$ solution was prepared and stored in brown bottle. 100 ml of diluted root extract and 100 ml of $AgNO_3$ solution were taken in two separate beakers and heated at 60 °C for 30 min in water bath, cooled and kept for further use.

For synthesis of silver nanoparticles, 85 ml of prepared $AgNO_3$ solution was added to 15 ml of prepared root extract and stirred with glass rod for 10 min. The mixture was heated (45 min) using magnetic stirrer (M/s Tarsons, 6090, Kolkata, India) until colour changed.

Upon heating the chemical reaction took place resulting in colour change in the reactants from pale yellow to dark brown and the mixture was cooled. The appearance of brown colour indicated the formation of silver nanoparticles (Kalidasan and Yogamoorthi, 2014).

Central composite rotatable design (CCRD) and response surface methodology (RSM) can be an effective option for the optimization of variables for the synthesis of silver nanoparticles (Mitra and Meda, 2009). To study the optimum condition for the synthesis of silver nanoparticles, experiment was conducted at different conditions of $AgNO_3$ concentrations (0.50, 1.00, 1.50, 1.83), temperature conditions (25, 45, 75, 105 and 125 °C) and pH (4, 5, 7, 9 and 10).

Centrifugation of biosynthesized Ag NPs was done at 10000 rpm for 30 min using ultra-centrifuge (Beckman Coulter, Optima max-TL, California, USA). The supernatant was collected and stored for further characterization (Kalidasan and Yogamoorthi, 2014).

Characterization of biosynthesized Ag NPs

Particle size analysis

Zetasizer (ZETA Sizer, nano383, Malvern, England) was used to measure average particle size (nm) of Ag NPs. For the particle size analysis, supernatant of centrifuged silver nanoparticles was filled in cuvette up to $3/4^{th}$ of volume and placed in the dynamic light scattering chamber (Das *et al.,* 2014).

Absorbance peak analysis

UV-Visible spectrophotometer refers to absorption spectrophotometer in the ultra-violet and visible spectral region of the electromagnetic spectrum, where molecules undergo electronic transition. Silver nanoparticles were characterized by using UV-Visible spectrophotometer (Schimadzu, UV-1800, Kyoto, Japan). The sample was prepared by diluting 1 ml of Ag NPs into 2 ml distilled water and measured the UV-Visible spectrum of Ag NPs solution (Habibi *et al.,* 2017).

Surface morphology analysis

The morphological features of biosynthesized silver nanoparticles were studied by using scanning electron microscope (SEM) (Carl Zeiss Microscopy, EVO 10, Cambridge, UK). The SEM image of the Ag NPs surface was obtained by scanning it with a high energy beam of electrons in vacuum chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of sample, it produces signals in the form of secondary electrons and back scattered electrons. These signals contain information about sample's surface morphology. Magnification can be adjusted from about 1 to 30,000 times to get clear morphology of silver nanoparticles at the accelerating voltage of 5 to 30 kV with working distance at 10 mm (Haq *et al.,* 2014).

Phase identification analysis

X-ray diffraction (XRD analysis) is a unique method for determination of crystallinity of a compound. Crystalline nature of the silver nanoparticles was measured on X-ray diffraction instrument (M/s Rigaku, Ultima 4, Tokyo, Japan) operated at 30 kV and 100 mA (Plate 6). Spectrum was recorded by CuKα radiation with wavelength of 1.5406 Å in the 2θ range of 20-80°. Silver nanoparticles (~1 g) were uniformly spread on glass sample holder and placed in scanner chamber. The set scan speed and step size of 0.30 º/min and 0.001 s, respectively were fixed. The XRD pattern was recorded for phase identification of silver nanoparticles (Djangang *et al.,* 2015).

Analysis of surface topology

Atomic force microscope (AFM) provides a 3D profile of the surface on a nanoparticle by measuring forces between a sharp probe (< 10 nm) and surface at very short distance (0.20-10 nm probe sample separation). Samples for AFM were prepared by spin-coating the Ag NPs solution into the glass slide. The slide was dried at room temperature and subjected to AFM analysis (Trial SPM, Version 6.4.3, Trieste, Italy) (Hong *et al.,* 2017).

Results and Discussion

Stability of biosynthesised silver nanoparticles using *A. aspera* root extract

During synthesis, addition of root extract of *A. aspera* into the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution from pale yellow to dark brown within reaction duration. This might be due to the reduction of Ag^{+} ions, indicating the formation of Ag NPs.

Biosynthesized silver nanoparticles were checked for their stability by using zetasizer and UV-Visible spectrophotometer for 30 days at an interval of 12 h. Data obtained from the stability study was analysed using central composite rotatable design (CCRD) and as well as Response surface methodology. From the analysed data, it was observed that 1.50 mM $AgNO_3$ concentration, 45 ºC temperature and 9 pH was the best treatment combination (desirability 96.39 %) in terms of stability. During stability study, particle size of the Ag NPs sample prepared with above mentioned best combination was in the range of 19 to 81 nm and absorbance peak was varied from 404 to 434. These results are in good agreement with the results of Vanaja *et al.,* (2013) who reported that, the pH of 8.20 and $AgNO_3$ concentration of 1 mM were favourable in biosynthesis of Ag NPs using *Coleus aromaticus* leaf extract.

Characterization of biosynthesized silver nanoparticles

Particle size analysis

The characterization of biosynthesized silver nanoparticles was done in terms of average particle diameter from the intensity distribution analysis by using zetasizer. The size distribution histogram of zetasizer indicated that, the size of the silver nanoparticles was 23.21 nm (Fig. 1). The variation in particle size was probably due to change in climatic conditions during biosynthesis (Zainala *et al.,* 2013). The size and shape of metal nanoparticles are influenced by a number of factors including pH, precursor concentration, time of incubation and temperature (Umoren *et al.,* 2014).

Kalidasan and Yogamoorthi (2014) reported that, the size of biosynthesized Ag NPs using *A. aspera* root extract was 105 nm. Beg *et al.,* (2016) and Bobbu *et al.,* (2016) reported that, an average particle size of biosynthesized

silver nanoparticles were 19.60 and 25.50 nm using *Pongamea pinnata* seed and *Achyranthes aspera* leaf extract, respectively.

Absorbance analysis

The UV-Visible absorption spectra of biosynthesized silver nanoparticles exhibited characteristic surface plasmon resonance (SPR) band centered at wavelength of 420.80 nm and absorbance of 1.17 (Fig. 2). This observed intense band was attributed due to the excitation of free electrons in the nanoparticles which indicated the presence of silver nanoparticles.

Similar results were reported by Hafez *et al.*, (2017), Halawani (2017) and Sivakumari *et al.*, (2018) reported SPR band for biosynthesized silver nanoparticles using *Morus nigra* leaf extract (425 nm), *Zizyphus spinachristi* L. leaf extract (414 nm) and *Achyranthes Aspera* (450 nm).

Surface morphology analysis

The clear magnified (8.07 KX) SEM image at the accelerating voltage of 10.00 kV with working distance of 9.50 mm, showed that, uniformly distributed silver nanoparticles were spherical in shape (Fig. 3).

Fig.1 Particle size analysis of biosynthesized Ag NPs using zetasizer

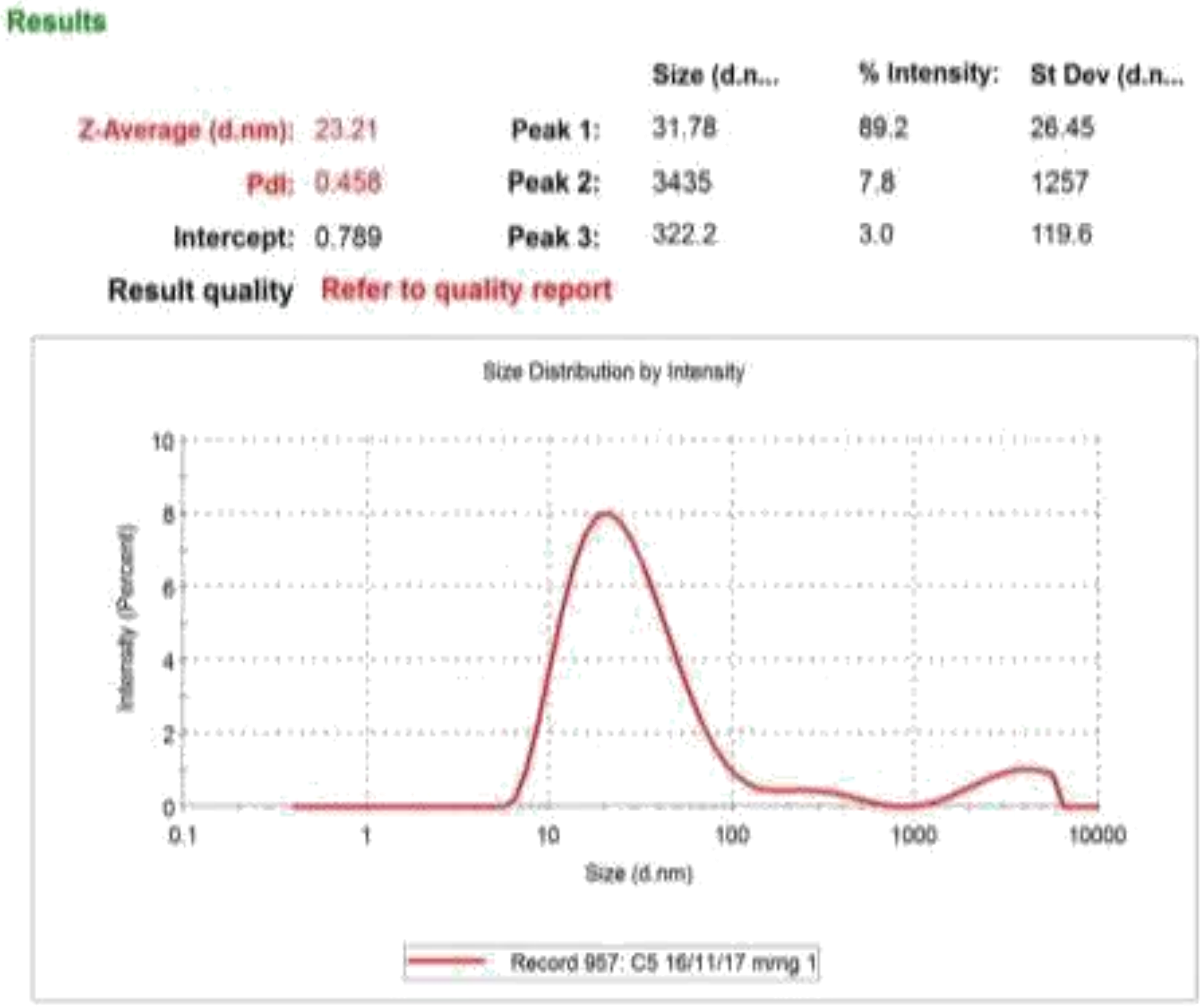


Fig.2 Absorbance analysis of biosynthesized Ag NPs using UV-Visible spectrophotometer

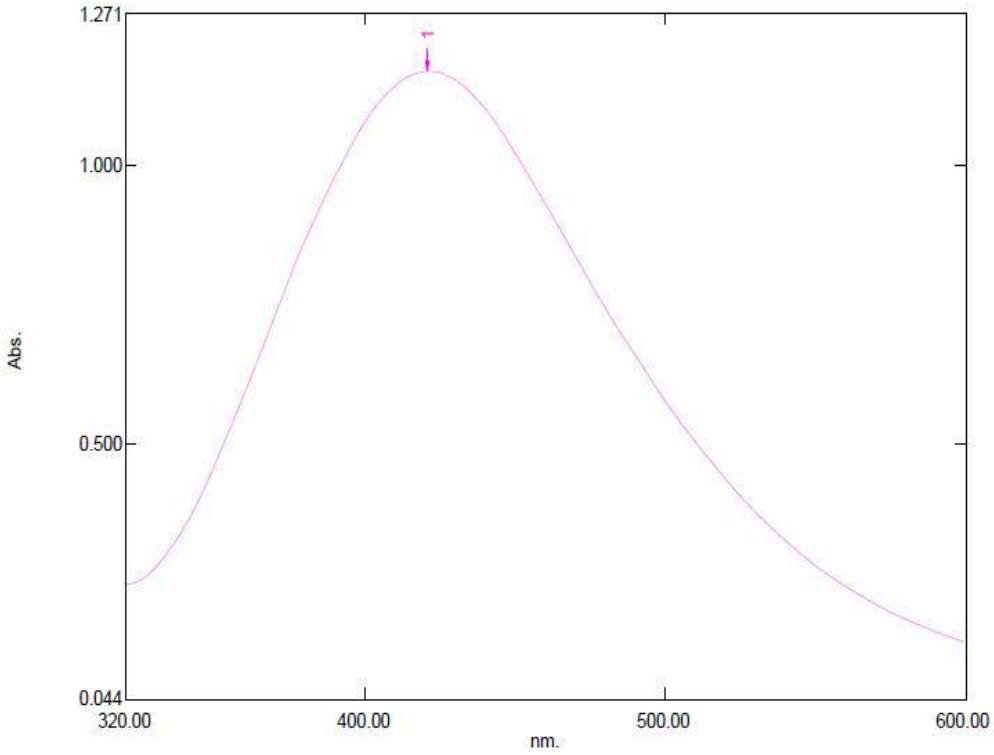


Fig.3 Morphology of biosynthesized Ag NPs analysed using scanning electron microscopy (SEM)

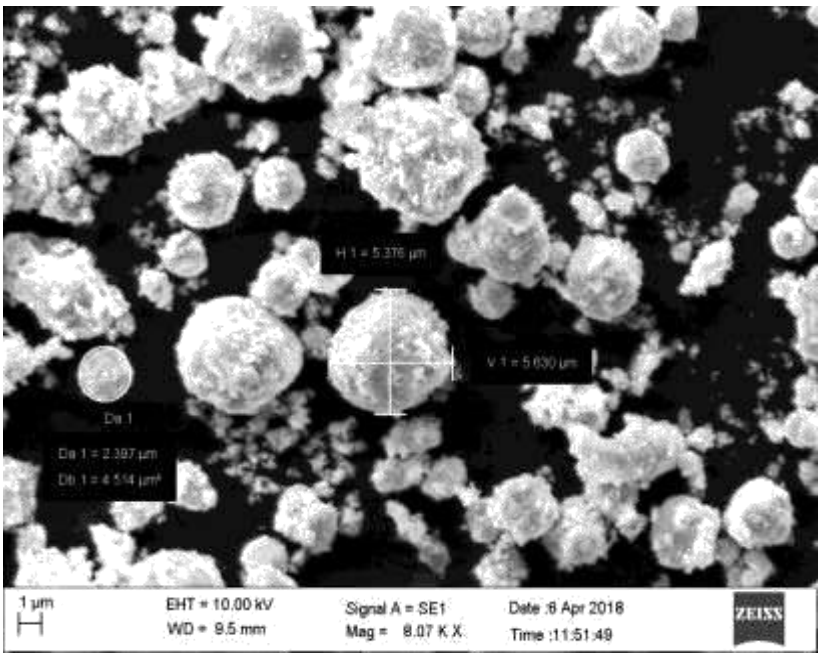


Fig.4 XRD pattern of biosynthesized Ag NPs using *Achyranthes aspera* root extract

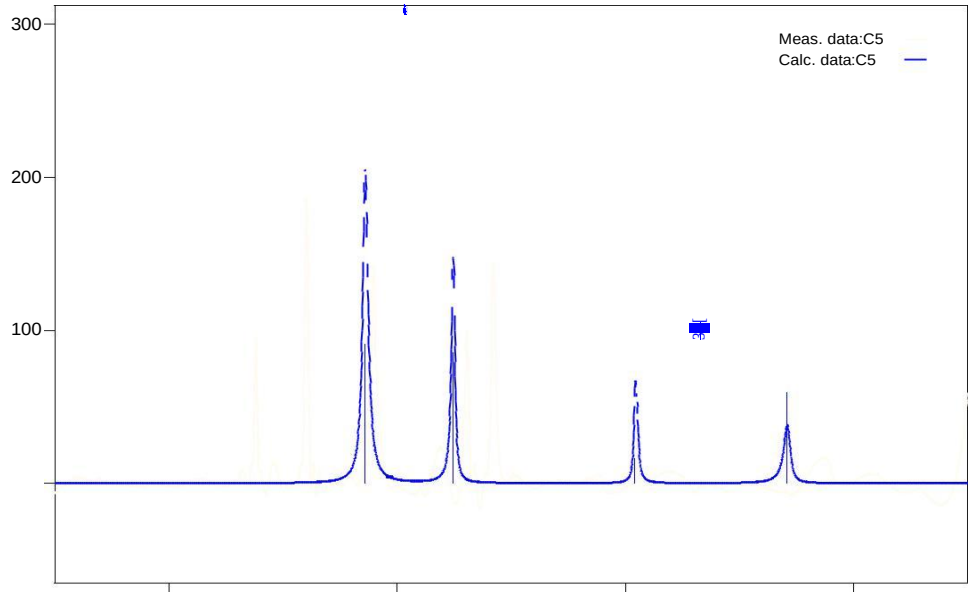
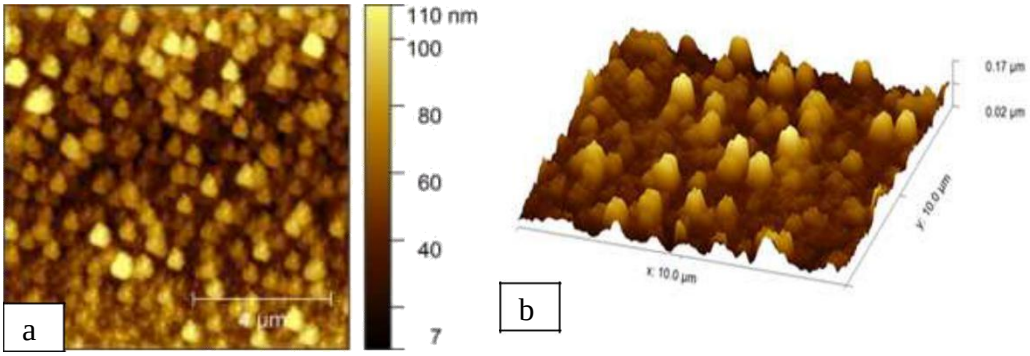


Fig.5 a) 2D and b) 3D images of standard Ag NPs using AFM



Some of the larger particles might be present because of aggregation due to the presence of cell components on the surface of nanoparticles and acted as capping agent (Vanaja *et al.*, 2013). The present results are in good agreement with the findings of Kalidasan and Yogamoorthi (2014) who reported that, the biosynthesized Ag NPs were in spherical shape. Sivakumari *et al.*, (2018), Allafchian *et al.*, (2016) and Premasudha *et al.*, (2015) for biosynthesized Ag NPs (spherical shape) using *A. aspera*, *Phlomis* leaf extract and *Eclipta alba* leaf extract as reducing agent, respectively.

Phase identification analysis

XRD pattern showed four distinct diffraction peaks at 37.18º, 44.90º, 60.86º and 74.16º that were corresponding to (111) (200) (220) and (311) reflections planes of biosynthesized silver nanoparticles, respectively. The highest peak was observed at 37.18º (111) reflection (Fig. 4). The XRD study confirmed that, the resultant nanoparticles were face centred cubic in nature and intensity of the peaks reflected high degree of crystallinity of silver nanoparticles. The peaks observed during XRD analysis were due to the presence of

organic compounds in the extract and intensity of the peaks denoted the degree of crystallinity of the particles (Halawani, 2017). The unassigned peaks could be due to the crystallization of bio-organic phase on the surface of the nanoparticles (Ahmad and Sharma, 2012). Similar findings were also reported by Halawani (2017) who reported that, the silver nanoparticles biosynthesized using *Zizyphus spinachristi L.* aqueous leaf extract were face centred cubic in nature.

Surface topology analysis

Surface topology of biosynthesized silver nanoparticles was studied by atomic force microscope (AFM). AFM micrographs with a scanning area of 10 × 10 µm of silver nanoparticles in 2D and 3D images of the biosynthesized Ag NPs samples showed spherical particles with different sizes (Fig. 5). Height and width of the arbitrarily selected biosynthesized Ag NPs was 0.11 and 1.10 µm, respectively. Other parameters such as roughness average of about 56.16 nm and root mean square roughness of about 66.85 nm were recorded for biosynthesized Ag NPs. Some nanoparticles were agglomerated in the sample which might be due to the deposition of the silver nanoparticles on the surface tending to form cluster together during AFM analysis. Also, the shape of the tip of AFM might cause misleading cross sectional views of the sample (Alahmad, 2013). Similar results were observed by Yadav *et al.*, (2015) who reported that, the AFM analysis for biosynthesized Ag NPs using bacteria *Pseudomonas sp.* Hong *et al.*, (2017) showed the AFM micrographs for silver thin films. The biosynthesis of silver nanoparticles using *Achyranthes aspera* root extract is an environmental friendly, simple and economically efficient route for synthesis of nanoparticles which could be an alternative to chemical and physical methods. The stable Ag NPs were found at optimum conditions of $AgNO_3$ of 1.50 mM, temperature at 45 ºC and pH of 9 for a period of 1 month.

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