



Mycological synthesis and characterization of silver nanoparticles by *Aspergillus* species

G. Baskar*, J. Chandhuru, K. Sheraz Fahad, A. S. Praveen, R. Bharathi and S. Fyna

Department of Biotechnology, St. Joseph's College of Engineering, Chennai, India

ABSTRACT

The fungi species have ability to reduce the silver nitrate to silver nanoparticles. Hence the present work was focused on extracellular biosynthesis and characterization of silver nanoparticles synthesized using fungi *Aspergillus niger* and *Aspergillus terreus*. The preliminary detection of silver nanoparticles was carried out by visual observation of colour change from yellow to brown colour. UV absorption spectrum at 420 nm confirms the presence of silver nanoparticles in the filtrate. The functional groups present on the synthesized nanoparticles were found using Fourier Transform Infrared Spectroscopy with the peaks in the range of 560–3690 cm^{-1} . The presence of silver nanoparticles in the sample was confirmed using Energy Dispersive Spectroscopic analysis. Silver nanoparticles synthesized from *A. niger* and *A. terreus* were in the size range of 26.5-100 nm and found to be compactly arranged and spherical in shape.

Keywords: Biosynthesis, Nanoparticles, Fourier Transform InfraRed spectroscopy, Energy Dispersive Spectroscopy, X-ray diffraction.

INTRODUCTION

Nanotechnology is the present hype in our society. Developments in nanotechnology have led to the synthesis of nanoparticles and nanomaterials and use it in various applications. It mainly focuses on developing natural as well as synthetic systems for the production of structures and materials at nano-scale [1]. Compared to bulk materials, nanoparticles have better optical, mechanical, magnetic, electrical, physical and chemical properties. Large surface area to volume ratio is responsible for the enhanced properties of the nanoparticles. Researchers have great interest in studying nanomaterials and nanoparticles as they bridge the gap between bulk materials and atomic or molecular structures. Nanomaterials are useful in developing diagnostic tool, drug delivery system, sunscreens formulation, antimicrobial bandages, disinfectants, nanobiosensors, as catalyst for greater efficiency in current manufacturing process by minimizing the use of toxic materials and an alternative energy production [2,3].

Physical, chemical, mechanical and biological methods are used for synthesis of Nanoparticles and nanomaterials. Some of the physical, mechanical and chemical methods used for synthesizing nanoparticles are co-precipitation [4] sol-gel processing [5], micro-emulsions processing, hydrothermal/solvo-thermal processing [6], microwave processing, sono-chemical processing, and template processing, high temperature solid state reaction, high energy ball milling [7-9], liquid mix process [10], rapid quenching process [11], thermal plasma [12], UV irradiation and lithography. There are many disadvantages in these methods such as being expensive, toxic and involves the use of harmful chemicals. Apart from these disadvantages the produced nanoparticle are less stable and aggregates quickly [13]. This has increased the need to develop high-yield, low cost, nontoxic, and environmentally benign procedures for synthesis of metallic nanoparticles. Thus the use of biological method for synthesizing nanoparticles emerged. Large number of biological resources is available in nature including plants and plant products, algae, fungi, yeast, bacteria, and viruses for synthesis of nanoparticles. Both unicellular and multicellular organisms have been used to produce intracellular or extracellular inorganic nanomaterials [14]. Mostly fungi are chosen instead of bacteria

because of their tolerance and better metal bioaccumulation ability [15]. Other advantages include the ease in the scale up process, economic viability, ability to secrete large amount of enzymes and ease in handling the biomass [16].

Number of researchers has reported on the synthesis of silver nanoparticles from fungi. Biological synthesis of silver nanoparticles from fungus *Trichoderma harzianum* was reported earlier [17]. The use of fungi *Aspergillus fumigatus* for the extracellular biosynthesis of silver nanoparticles have been reported earlier [18, 19]. *Aspergillus terreus* on the other hand has been used for the biomimetic synthesis and characterization of protein capped silver nanoparticles [20, 21]. Thus the fungus was reported as an excellent candidate for the biosynthesis of nanoparticles in a cheap way. Hence the present was focused on the synthesis of silver nanoparticles using culture filtrate of *Aspergillus niger* and *Aspergillus terreus*, characterization of the synthesized nanoparticles.

EXPERIMENTAL SECTION

2.1. Fungi used and growth conditions

The fungal cultures *A. niger*, *A. fumigatus*, *A. terreus* and *A. aculeatus* were obtained from Institute of Microbial Technology, Microbial Type Culture Collection and Gene Bank, Chandigarh, India. All the fungal cultures were subcultured by growing on Czapek agar slants for 96 h at 32°C and refrigerated at 4°C.

2.2. Materials used

Mycological peptone, dextrose and agar were purchased from Himedia, Mumbai, India.

2.3. Mycological synthesis of silver nanoparticles using *A. niger* and *A. terreus*

The fungi *A. niger* and *A. terreus* was inoculated into two different 500 ml Erlenmeyer flasks containing 200 ml Czapek-Dox liquid medium containing (g/100ml of distilled): glucose (3 gm), sodium nitrate (2 gm), potassium chloride (0.052 gm), di-potassium hydrogen sulphate (0.15 gm), zinc sulphate (0.001 gm), copper sulphate (0.001 gm), ferrous sulphate (0.001 gm) and magnesium sulphate (0.052 gm) at pH 6.2. The fungal cultures were grown aerobically by agitating in an orbital shaker at 160 rpm at 32°C for 4 days. After the incubation time the fungal cultures were filtered under vacuum through Whatman#2 filter paper. 1 mM AgNO₃ salt was added to the filtrate obtained from both the cultures and kept in a shaking incubator at 150 rpm at 32°C for 2 days. Deposition of brown coloured precipitate and presence of brown coloured suspended particles in both the flask confirmed the occurrence of transformation process. The suspended particles and precipitate in both the flasks were separated by centrifugation at 10,000 rpm for 10 min and lyophilized.

2.4. Characterization of synthesized silver nanoparticles

Various properties of the synthesized silver oxide nanoparticles were investigated. Optical properties of the nanoparticles were analyzed by using UV-Visible spectroscopy. UV-Visible spectrum was recorded on SYSTRONICS Double Beam UV-Visible spectrophotometer 2201. The spectrum values were obtained between the wavelength range 200 to 900 nm. The characteristic functional groups present on the molecules of synthesized nanoparticles were analyzed using Fourier Transform-Infra Red (FT-IR) spectroscopy. FT-IR spectroscopy was measured on BRUKER α -T FT-IR Spectrometer. The samples were mixed with KBr (binding agent) and were made into discs at high pressure using hydraulic press. These discs were scanned in the range of 500 to 4000 cm⁻¹ to obtain FT-IR spectra. Structural characterization was analyzed in order to obtain information about particle size, crystal structure and surface morphology using X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). Purity of the nanoparticles is studied using Energy dispersive X-ray analysis (EDX). XRD patterns were recorded on a XPERT-PRO diffractometer. This diffractometer uses Cu-K as an anode, acts as a X-Ray source (wavelength = 1.54060 Å), operating with Cu- tube radiation at 40 Kv and 30 mA. The scan step for 2 θ was 0.0170° with a scan step time of 38.1 sec. Size of the silver nanoparticles were examined under QUANTA 200 SEM, magnification range 35 to 30,000.

RESULTS AND DISCUSSION

The silver nanoparticles produced using *A. niger* and *A. terreus* culture filtrate was characterized by UV-Visible spectrophotometer, FT-IR, XRD, SEM and EDX and the results are discussed.

3.1. Visual observation of synthesized silver nanoparticles

The colour of the culture filtrate was changed from colourless liquid solution to brownish solution after 48 hr of incubation with silver nitrate. This colour change is due to the excitation of surface plasmon resonance (SPM), indicates the reduction of silver nitrate ions by the proteins present in fungal culture filtrate which resulted in the formation of silver nanoparticle.

3.2. UV spectrum analysis of synthesized silver nanoparticles

UV spectrum analysis was done to investigate surface plasmon resonance. The presence of silver nanoparticles in culture filtrates was confirmed by an absorption peak obtained at 410 nm for the sample obtained from *A. niger* and absorption peak at 420 nm for the sample obtained from *A. terreus* as shown in Figure 1 (a) and (b).

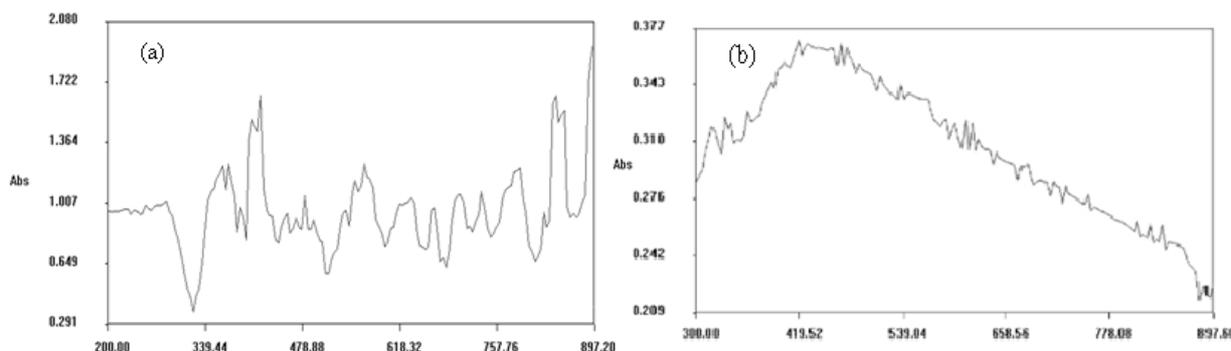


Figure 1. UV Spectrum of silver nanoparticles synthesized by (a) *A. niger* and (b) *A. terreus*

3.3. FT-IR spectrum analysis of synthesized silver nanoparticles

The synthesized silver nanoparticles were subjected to FT-IR analysis to detect the various characteristic functional group associated with it. The peaks obtained for nanoparticles synthesized from *A. niger* indicate the characteristics functional group present in the synthesized silver nanoparticles. It is inferred from Figure 2(a), that the samples have absorption peaks in the range of 3388.83 cm^{-1} , 2925.19 cm^{-1} , 1554.42 cm^{-1} , 1385.02 cm^{-1} , 1235.52 cm^{-1} , 1062.63 cm^{-1} , 828.14 cm^{-1} , and 563.89 cm^{-1} . The absorption peak at 563.89 cm^{-1} corresponds to metal-oxygen (silver stretching vibrations) vibrational mode. The peak at 1062.63 cm^{-1} is ascribed to the stretching vibration of C-N bond of aliphatic amines. The peak at 1235.52 cm^{-1} and 1385.02 cm^{-1} are ascribed to aromatic ethers and trimethyl group respectively. The peak at 1554.42 cm^{-1} is ascribed to the vibrational modes of secondary amine N-H bend or primary amine N-H bend. The peak at 2925 cm^{-1} is ascribed to methylene C-H stretch. The peak at 3388 cm^{-1} is ascribed to O-H group of tertiary alcohol.

The peaks obtained for nanoparticles synthesized from *A. terreus* indicate the characteristics functional group present in the synthesized silver nanoparticles. It is inferred from Figure 2(b) that the samples have absorption peaks in the range of 3688.56 cm^{-1} , 3546.76 cm^{-1} , 2949.57 cm^{-1} , 2104.79 cm^{-1} , 1643.11 cm^{-1} , 1552.44 cm^{-1} , 1398.89 cm^{-1} , 1366 cm^{-1} , 1054.85 cm^{-1} , 827.93 cm^{-1} and 564.16 cm^{-1} . The absorption peak at 564 cm^{-1} corresponds to metal-oxygen (silver stretching vibrations) vibrational mode. The peak at 1052 cm^{-1} is ascribed to the stretching vibration of C-O bond of the primary alcohol. The peak at 1398 cm^{-1} is ascribed to carboxylate group. The peaks at 1552 cm^{-1} and 1644 cm^{-1} are ascribed to the vibrational modes of secondary amine N-H bend or primary amine N-H bend and alkyl C=C stretch, amide group. The peak at 205 cm^{-1} is ascribed to alkyne stretch. The peak at 3547 cm^{-1} is ascribed to O-H group of carboxylic acids. The presence of these functional groups makes the synthesized silver nanoparticles are as potential antimicrobial agent. These silver nanoparticles can be coated on cotton fibres to make fungal resistant fabric material.

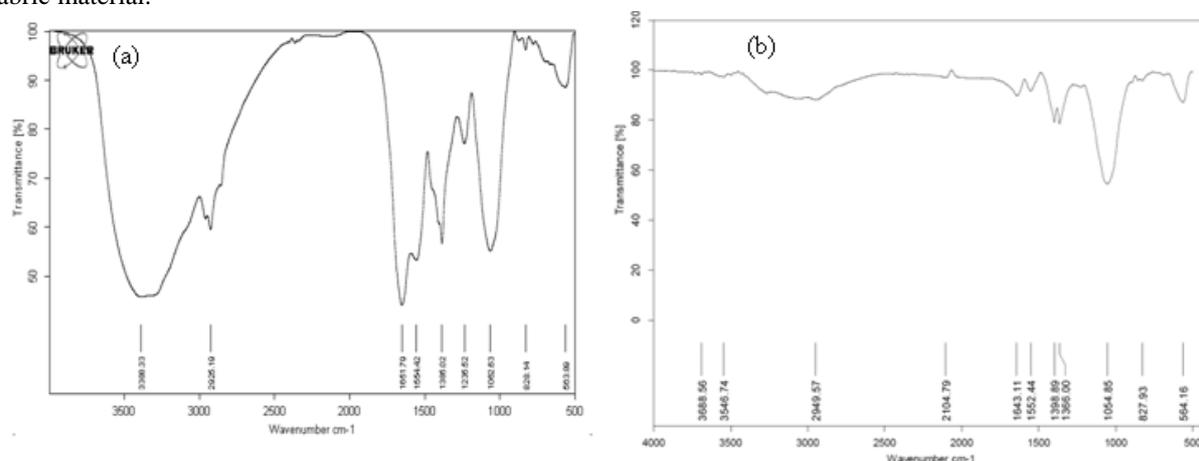


Figure 2. FT-IR spectrum of silver nanoparticles synthesized by (a) *A. niger* and (b) *A. terreus*

3.4. XRD analysis of synthesized silver nanoparticles

X-ray diffraction patterns of silver nanoparticles synthesized from *A. niger* and *A. terreus* is shown in the Figure 3 (a) and (b). Sharp and strong diffraction peaks were observed in both the Figure 3(a) and (b). The 2θ maximum value was obtained at 32° . The shift in the 2θ peak values of silver nanoparticles may be due to the presence of protein molecule from fungal culture filtrate along with the nanoparticles.

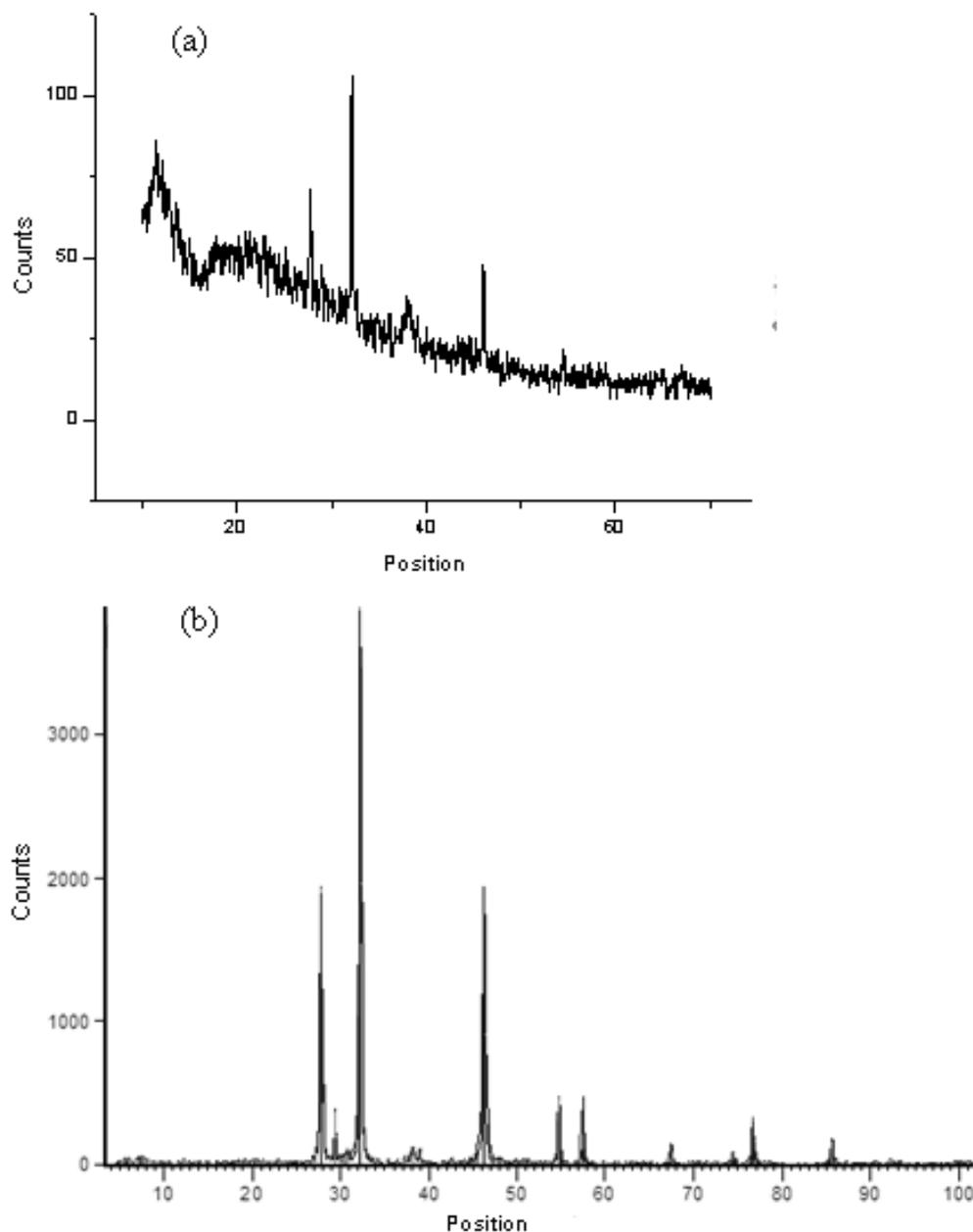


Figure 3. XRD pattern of silver nanoparticles synthesized by (a) *A. niger* and (b) *A. terreus*

3.5. Structural characterization of silver nanoparticles

Scanning Electron Microscope was used to deduce the particle size and morphology of the synthesized silver nanoparticles. It can be concluded from Figure 4 (a) and (b) that the nanoparticles obtained from *A. niger* were compactly arranged and their sizes range is in between 26.5 - 42.3 nm, whereas the nanoparticles obtained from *A. terreus* were compactly arranged, almost spherical in shape and their sizes range is in between 60 - 120 nm.

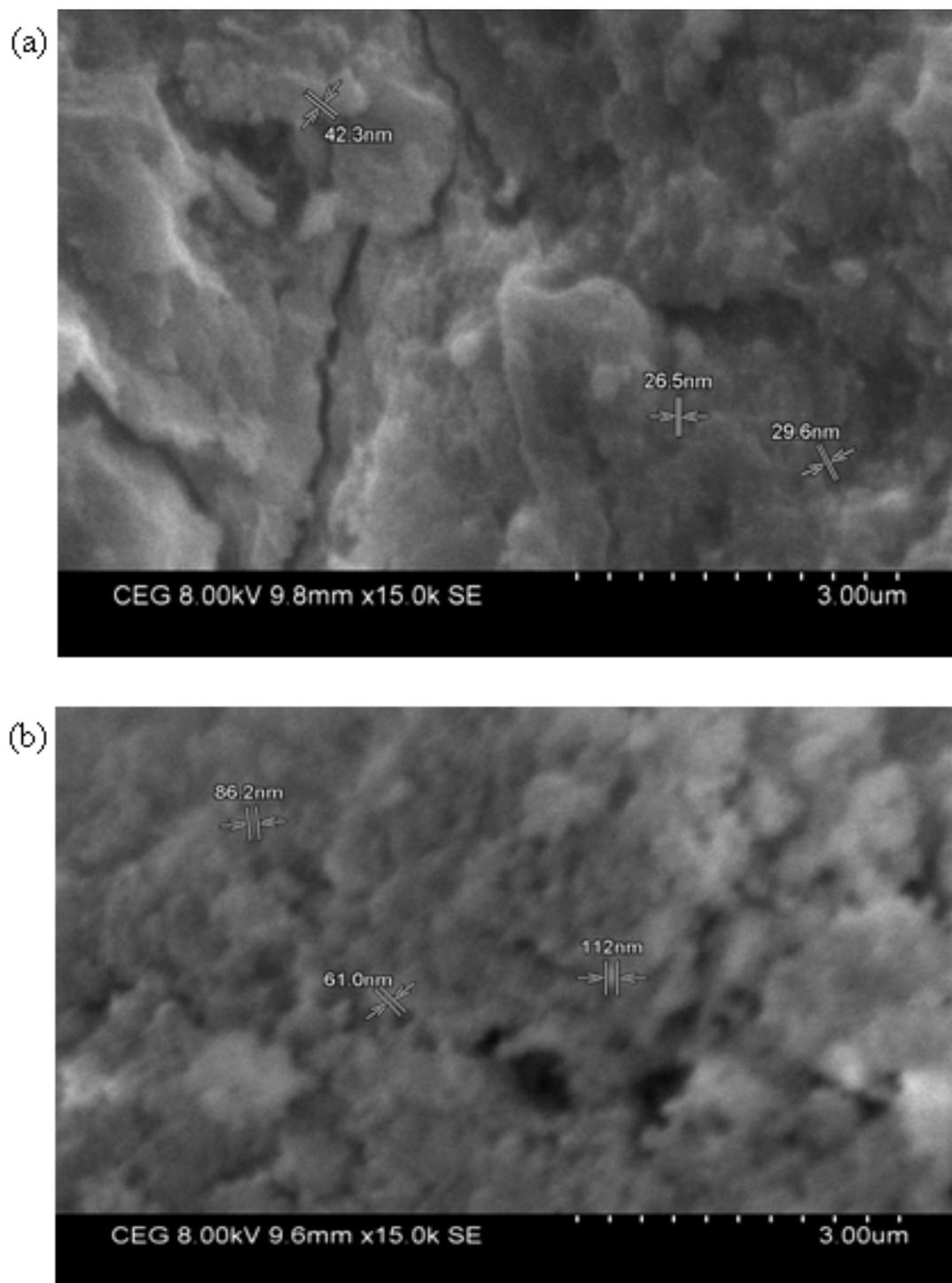


Figure 4. SEM image of silver nanoparticles synthesized by (a) *A. niger* (b) *A. terreus*

3.6. EDX analysis of synthesized silver nanoparticles

The energy dispersive spectroscopic analysis is done to get an indication of the amount of silver nanoparticles present in our sample. Purity of the nanoparticles can also be investigated using EDX analysis. Presence of silver in the nanoparticles was confirmed from the spectrum peak shown in the Figure 5 (a) and (b). Strong signals of potassium and calcium can be seen in both the figures, as these salts are the media components used for culture growth. Weak signals of oxygen and carbon can be observed which may be due to the proteins present along with nanoparticles.

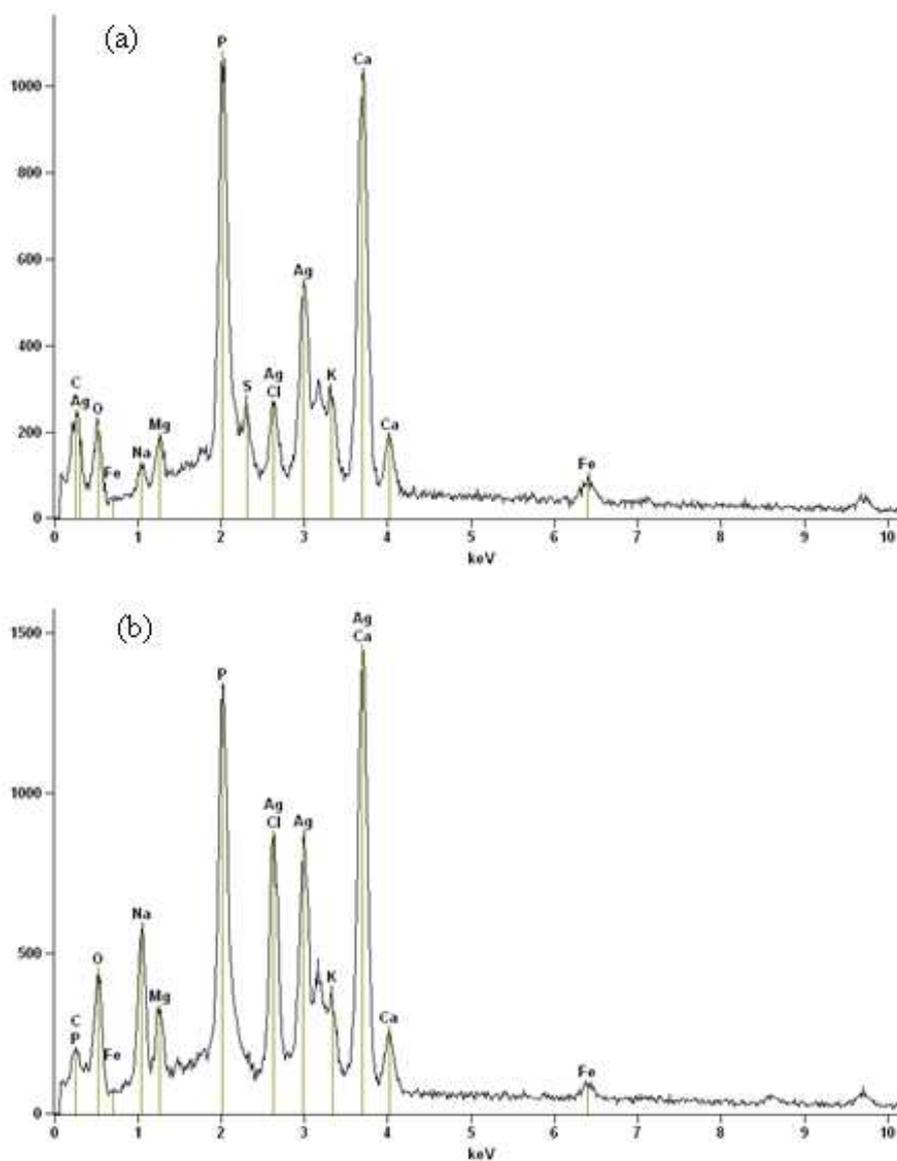


Figure 5. EDX analysis of silver nanoparticles synthesized by (a) *A. niger* (b) *A. terreus*

CONCLUSION

The biosynthesis of silver nanoparticles using *Aspergillus niger* and *Aspergillus terreus* is a cost effective and eco-friendly method. They can be used for large scale synthesis of silver nanoparticles. The presence of functional groups present on the silver nanoparticles was confirmed by FT-IR analysis with the peaks in the range of 560 - 3690 cm^{-1} . The different functional groups associated with silver nanoparticles synthesized from *A. niger* were found to be C-N, N-H, O-H, C-H. The functional groups associated with the silver nanoparticles synthesized from *A. terreus* were found to be C-N, N-H, O-H, C-H and C=O. Hence the synthesized silver nanoparticles can be used as an effective antimicrobial agent. Silver nanoparticles synthesized from *A. niger* were in the size range of 26.5 - 42.3 nm and found to be compactly arranged. Silver nanoparticles synthesized from *A. terreus* were in the size range of 60 - 100 nm and were found to be almost spherical in shape.

Acknowledgment

The authors would like to thank Anna University, Chennai and SRM University Chennai for their support for SEM, XRD, EDS and FT-IR Analysis.

REFERENCES

- [1] A Absar; S Satyajyoti; MI Khan; K Rajiv; M Sastry. *J. Biomed. Nanotechnmol.*, **2005**, 1, 47-53.
- [2] H Meruvu; M Vangalapati; SC Chippada; SR Bammidi. *Rasayan J. Chem.*, **2011**, 4, 217-222.

- [3] T Yumak; F Kuralay; M Muti; A Sinag; A Erdem; S Abaci. *Colloids Surf. B.*, **2011**, 86, 397-403.
- [4] HH Hamdeh; JC Ho; SA Oliver; RJ Willey. *J. Appl. Phys.*, **1997**, 81, 1851-1858.
- [5] A Kundu; S Anand; HC Verma. *Powder Technol.*, **2003**, 132, 131-136.
- [6] T Ahmad; IA Wani; IH Lone; A Ganguly; N. Manzoor; A Ahmad; J Ahmed; AS Al-Shihri. *Mater. Res. Bull.*, **2013**, 48, 12-20.
- [7] S Bid; SK Pradhan. *Mater. Chem. Phys.*, **2003**, 82, 27-37.
- [8] H Ehrhardt; SJ Campbell; M Hofmann. *Scr. Mater.*, **2003**, 48, 1141-1146.
- [9] SD Shenoya; PA Joy; MR Anantharaman; J. Magn. Magn. Mater., **2004**, 269, 217-226.
- [10] A Kundu; C Upadhyay; HC Verma. *Phys. Lett.*, **2003**, A311, 410-415.
- [11] K Tanaka; M Makita; Y Shimizugawa; K Hira; N Soga. *J. Phys. Chem. Solids*, **1998**, 59, 1611-1618.
- [12] I Mohai; J Szepvolgyi; I Bertoti; M Mohai; J Gubicza; T Ungar. *Solid State Ionics*, **2001**, 141-142, 163-168.
- [13] M Raffi. PhD thesis, Pakistan Institute of Engineering & Applied Sciences, Pakistan research repository, Islamabad. **2007**.
- [14] KN Thakkar; SS Mhatre; RY Parikh. *Nanomed-Nanotechnol.*, **2010**, 6, 257-262.
- [15] M Sastry; A Ahmad; I Khan; R Kumar. *Curr. Sci.*, **2003**, 85, 162-170.
- [16] S Zeinab; S Mojtaba; Z Farzad. *J. Clust. Sci.*, **2011**, 22, 661-665.
- [17] P Singh; R Balaji Raja. *Asian J. Exp. Biol. Sci.*, **2011**, 2, 600-605.
- [18] CB Kuber; SF Dsouza. *Colloids Surf. B.*, **2006**, 47, 160-164.
- [19] G Baskar; VB Pranav; M. Vasanth Kumar; T Dilliganesh. *Acta Metall. Sin.-Engl.*, **2014**, **27**, 569-572.
- [20] S Rashmi; V Preeti. *Bioresour. Technol.*, **2009**, 100, 501-504.
- [21] VB Pranav; T Dilliganesh; M Vasanth Kumar; M Chamundeeswari; G Baskar. *B. Mater. Sci.*, **2013**, 36, 1201-1205.