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Original article

Synthesis of eco-friendly silver nanoparticles using *Allium* sp. and their antimicrobial potential on selected vaginal bacteria

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ABSTRACT

Allium cepa and garlic *Allium sativa* plants were used to evaluate their potential synthesis of silver nanoparticles and their antibacterial effect on *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Transmission electron microscopy (SEM) was used to distinguish the morphology of the nanoparticles attained from plant extracts. Energy dispersive X-ray (EDX) spectrometer established the existence of elemental sign of the silver and homogenous allocation of silver nanoparticles. Diffraction by using X ray (XRD) analysis for the formed AgNPs revealed spherical plus cubical shapes structure with different planes ranged between 111 and 311 planes. The antibacterial action of AgNPs against vaginal pathogens, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* was recognized. Our work showed a rapid, eco-safety and suitable method for the synthesis of AgNPs from *Allium cepa* and garlic *Allium sativa* extracts and can be used in biomedical applications.

1. Introduction

Nanoparticles are classified as being materials in which at least one dimension of the material is less than 100 nm in diameter. Nanoparticle investigation is currently an area of "passionate scientific research" due to a wide variety of potential applications in biomedical, optical and electronic fields. Nanoparticles are becoming an area of research interest due to their unique properties, such as having increased electrical conductivity, ductility, toughness, and formability of ceramics, increasing the hardness and strength of metals and alloys, and by increasing the luminescent efficiency of semiconductors (Rittner, Abraham, 1998). The use of metallic nanoparticles in the field of catalysis, optoelectronics, pinpointing biological troubles and exhibit devices uncovered many significant findings. Among the Nobel metals, silver (Ag) is the metal of preference in the field of biological systems, living organisms and medicine (Parashar et al., 2009). There are diverse methods for nanoparticles formation. In which biological methods are considered as safe and economically sound for the nano material fabrication as an alternative to conventional physical and chemical methods. Current nanotechnology developments have led to nanomedicine, a new field which includes many diagnostic and therapeutic applications involving nanomaterials and Nano devices (Kagan et al., 2005). Synthesis of nanoparticles using plant extract supplies progression more than chemical and physical method as it is cost helpful, environment safety, simply scaled up for great range production and in this process there is no requirement to use high pressure, power, temperature and poisonous chemicals (Antariksh et al., 2012). Different types of nanomaterials like copper, zinc, titanium (Retchkiman-Schabes et al., 2006), magnesium, gold (Gu et al., 2003), alginate (Ahmad et al., 2005) and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficiency against bacteria, viruses and other eukaryotic microorganisms. However, there is still need for economic, commercially viable as well environmentally clean synthesis Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process (Jose et al., 2005; lok et al., 2007). The most important application

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of silver and silver nanoparticles in medical industry is topical ointments to prevent infection against burn and open wounds (Ip et al., 2006).

Nanobiotechnology has enhanced the production of minor AgNPs with little toxic effect to human and more effectiveness alongside bacteria. Furthermore, nanoparticles are alternative to antibiotics viewing better action against multidrug opposing bacteria and consequently, plant derived nanoparticles proved better to other methods (Song and Kim, 2008). The method of the AgNPs antibacterial action is efficiently explained in conditions of their interaction with cell membranes of bacteria by troubling its permeability and respiratory role (Vankar and Shukla, 2012; Ghosh et al. 2012). There are several reports on the synthesis and use of *Helianthus annus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolor*, *Zea mays*, *Azadirachta indica* (Shankar et al., 2004), *Medicago sativa* (Li et al., 2007), *Aloe vera*, *Diopyros kaki* (Song and Kim, 2008), *Mangnolia kobus*, *Coriandrum* sp. (Narayanan and Sakthivel, 2008) *Carica papaya* (Jain et al., 2009); leaf extract of weed (Parthenium sp.) and *Ipomoea aquatic*, *Enhydra fluctuans* and *Ludwigia adscendens* (Roy and Barik, 2010) nanoparticles in pharmaceutical and biological applications were made. The method of the AgNPs antibacterial action is efficiently explained in conditions of their interaction with cell membranes of bacteria by troubling its permeability and respiratory role (Vankar and Shukla, 2012; Ghosh et al. 2012).

Pseudomonas aeruginosa is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues. It causes urinary tract infections, respiratory system infections (Neu, 1983). *Staphylococcus aureus* is a facultative anaerobic and it is frequently part of the skin flora and causes a variety of suppurative (pus-forming) infections and toxinoses in humans (Kluytmans et al., 1997). *S. aureus* causes superficial skin lesions such as boils, styes and more serious infections such as osteomyelities and endocarditis (Timasz, 1994). The carotenoid pigment staphyloxanthin is responsible for *S. aureus* characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system.

In this study, we used aqueous plant extract of *Allium cepa* and *Allium sativum* for the formation of ecofriendly silver nanoparticles and study their antiseptic activity against some Gram positive and Gram negative vaginal bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

2. Materials and methods

2.1. Sample Collection

All chemicals used in this experiment were of the highest purity and obtained from Sigma (Alexandria, Egypt) and Merck (Cairo, Egypt). Onion (*Allium cepa*) as well as garlic (*Allium sativa*) were used for the green synthesis of silver nanoparticles collected from local market of Alexandria in the month of July, 2014. Silver nitrate ($AgNO_3$) of analytical grade were used for synthesis of silver nanoparticles. A stock of 1 mM was prepared and stored in a brown bottle to avoid light disintegration of silver nitrate.

2.2. Extraction of onion and garlic

Onion and garlic were washed with clean sterile distilled water and allowed to air dry for one hour. The outer covering of the onion and garlic were manually peeled off. The onion bulbs and garlic bulbils being separated were washed and extracted in the following way: Exactly 200 g of each fresh onion and garlic were blended into fine powder and soaked in 100 mls of distilled water for 24 h. The pulps obtained were left in a clean, sterile glass container and shaken vigorously to allow for proper extraction and it was filtered using a sterile muslin cloth after which the extract was obtained and stored at 4 °C until required for the synthesis of Ag-Nanoparticles.

2.3. Biological synthesis of silver nanoparticles

Silver nitrate was used as precursor for synthesis of silver nanoparticles. 5 ml of 1 mM silver nitrate aqueous solution was added to each 100 ml of clear plant extract. The flasks were put into shaker (150 rpm) at 30 °C and reaction was carried out for a period of 72 h. In this process, *A. cepa* (onion) and *A. sativa* (garlic) extracts act as the reducing and stabilizing agents. Silver nanoparticles were obtained gradually by the erosion and chemical degradation of plant extract. The brown colors formation indicate that the AgNPs were synthesized from the plant extracts and they were centrifuged at 5000 rpm (Hettich EBA20S Portable Centrifuge) for 10 min in order to obtain the pellet which is used for further study.

2.4. UV–visible spectroscopy analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV–Vis spectrum of the reaction medium at 2 h after diluting a small aliquot of the sample into distilled water. UV–Vis spectra of these aliquots were monitored as a function of time of reaction on UV–Vis spectrophotometer operated at a resolution of 1 nm. The colors change in the reaction mixtures (metal ion solution + *A. cepa* (onion) or *A. sativa* (garlic) extracts) were recorded through visual observation which showed the bioreduction of silver ions in aqueous solution. UV–Vis spectral analysis was done using UV–VIS spectrophotometer V-460 (Jenway).

2.5. X-ray Diffraction (XRD) & SE

XRD analyses for crystalline metallic silver nanoparticles were examined as described by Vidhu et al. (2011). The bioreduced silver nitrate solution was drop-coated onto glass substrate for XRD analysis. On the other hand, the suspension of nanosilver particles was centrifuged at 10,000 rpm at 4 °C for 10 min to obtain a pellet of pure nanoparticle for XRD analysis. X-ray Diffraction (XRD) measurements were carried out on a Philips-X'Pert MPD X-ray diffractometer. The pattern was recorded by Cu-Kα radiation, with λ of 1.5406 Å and a nickel monochromator filtering the wave at a tube voltage of 40 kV and tube current of 30 mA. The scanning was done in the region of 2θ, from 20°to 80°, at 0.02°/min and the time constant was 2 s. The mean particle diameter of AgNPs was calculated from the XRD pattern, according to the line width of the maximum intensity reflection peak. The size of the nanoparticles was calculated through the [Scherrer equation]: $D = (K\lambda)/(\beta_{cor} \cos \theta)$, with $\beta_{cor} = (\beta^2 \text{ sample} - \beta^2 \text{ ref})^{1/2}$, where D is the average crystal size, K is the Scherrer coefficient (0.89), λ is the X-ray wavelength (λ = 1.5406 Å), 2θ is Bragg's angle, β cor is the corrected full width at half maximum (FWHM) in radians, and β sample and β ref are the FWHM of the reference and sample peaks, respectively.

2.6. Scanning electron microscopy (SEM)

Examination of silver nanoparticles analysis was done in Electron Microscope Unit in the Faculty of science at the Alexandria University (Alexandria, Egypt). Thin films of the silver nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min

2.7. Collection of pathogens

The test organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from high vaginal swab (HVS) samples from patients with urinary tract infection was collected from the Microbiology Laboratory Unit of Alexandria University Teaching Hospital. For isolation of two different types of Human Vaginal Gram positive and Gram negative Bacteria On different media

(1) **Isolation on MacConkey's Agar Medium**:
Positive *Pseudomonas aeruginosa*, this indicates Gram negative bacteria.
Negative *Staphylococcus aureus*, this indicates Gram Positive bacteria.

- **Isolation on Blood Agar Base Medium:**
Positive *Pseudomonas aeruginosa*.
Positive *Staphylococcus aureus* (Golden-yellow).

(2) **Antibiogram on Muller-Hinton Agar Medium:**
- All 2 types of vaginal bacteria gave antibiotic sensitivity

2.8. Test organism confirmation

Few tests were carried out to reconfirm the test organisms including gram staining, catalase test, coagulase test, oxidase test and motility test. The pure cultures were sub cultured on nutrient Agar slants and preserved in the refrigerator at 4 °C until required for the study.

2.9. Preparation of disc

The sterile discs approximately 5 mm in diameter was placed on Mueller Hinton agar (MHA) plates treated with garlic and onion nanoparticles. The disc was then placed over the swabbed MHA plates and incubated at 37 °C for overnight to study the antimicrobial activity.

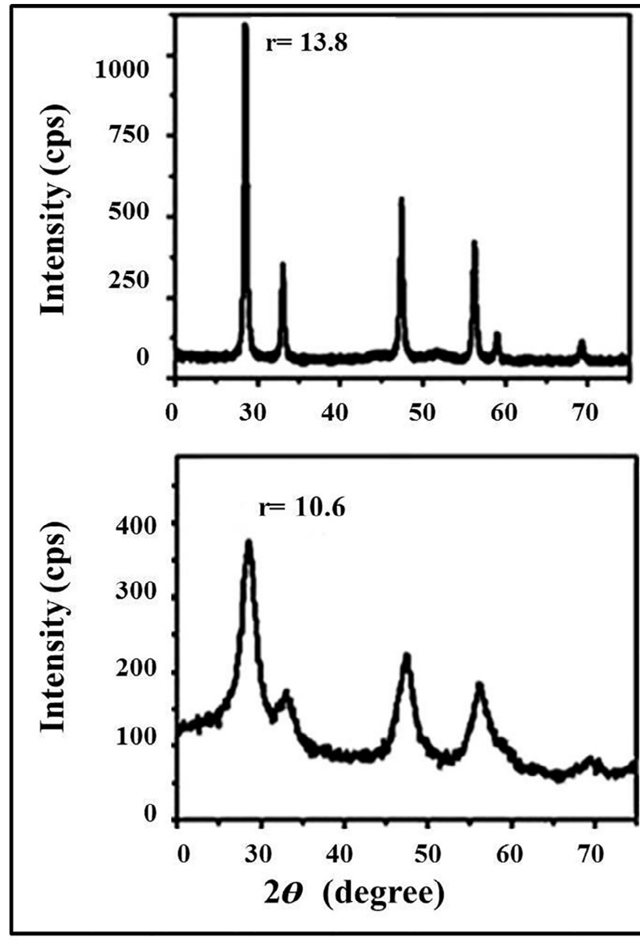

Fig. 2. XRD pattern of biosynthesized Ag-NPs in aqueous solution of *Allium cepa* and *Allium sativa.*

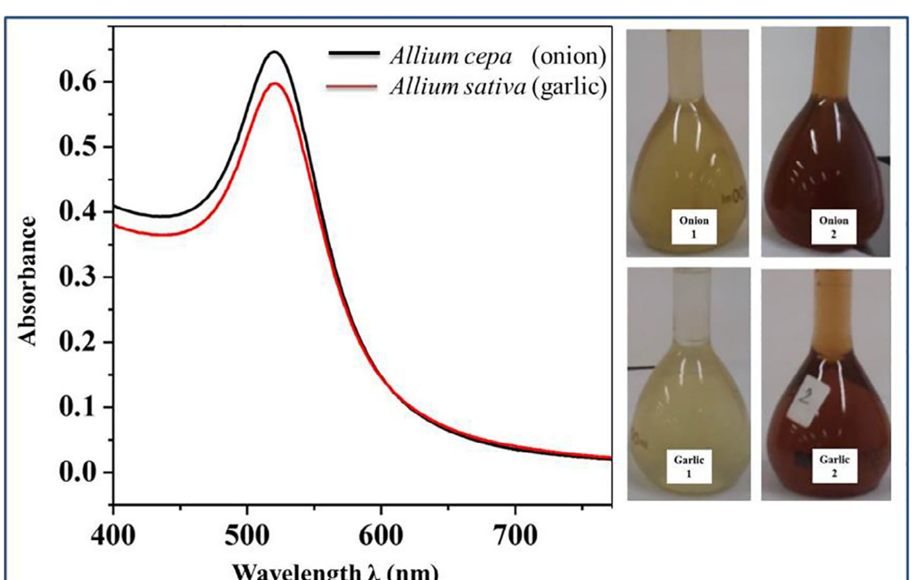

Fig. 1. UV–Vis spectra showing absorption recorded as a function of 1 mM $AgNO_3$ with *aqueous leaf extract of Allium cepa* and *Allium sativa*. Color changes of aqueous plant extract with time.

2.10. Antibacterial activity of plant based silver nanoparticles against pathogen

The antibacterial assays were done on vaginal bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* by standard disc diffusion method. Fresh overnight cultures of inoculums (100 μl) of each culture were spread on to Mueller – Hinton Agar (MHA) plates. Sterile paper discs of 5 mm diameter containing silver nanoparticles were placed in each plate. The Antibiotic sensitivity discs which was used in the Antibiogram test were, Cefoperazone (CFP 75 μg), (OXOID); Ciprofloxacin (CIP 5 μg), (OXOID) and Imipenem (IPM 10 μg), (OXOID). The plates containing the bacterial and AgNPs were stand for 1 h to allow diffusion to take place and then incubated at 37 °C for 24 h, and then examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a metre ruler, and the mean value for each organism was recorded and expressed in millimeters.

3. Results

Reduction of Ag^+ ion into silver particles during exposure to the plant extracts from onion and garlic supernatants could be followed by color changes from pale yellow color to a brownish color on completion of the reaction with Ag^+ ions. This process indicated the complete reduction of Ag^+ ions by the reducing agent released in the extract.

The absorption spectra of AgNPs formed, shows the creation of AgNPs with almost 100% plant reduction of Ag ions as supported by qualitative testing of supernatant after the decontamination of silver nanoparticles by heat (Fig. 1). A strong peaks were observed for the silver nanoparticles, prepared using garlic and onion respectively (Fig. 1).

A typical XRD (Fig. 2) pattern for Ag-NPs has been shown for phase formation and exhibits diffraction peaks (Braggs reflections), which were indexed on the basis of face-centered cubic structure. The diffraction peaks for both *Allium cepa* and *Allium sativa* were (1 1 1), (2 0 0), (2 2 0), (3 1 1), (2 2 2) and (4 0 0) corresponding to 28.14°, 34.39°, 44.61°, 57.58° and 71.13° angles respectively. This is confirmed that synthesized Ag-NPs were of crystalline nature with face-centered cubic structure. Also, broadening of Braggs peaks provide additional indication of the formation of silver in Nano size.

The size and shape of the silver nanoparticles were examined clearly under Electron Microscope at 80 kV of operation voltage. SEM images of biologically synthesized typical silver nanoparticles were obtained from *Allium cepa* and *Allium sativa* extracts, although the exact shape of the nanoparticles was not clearly predicted. Higher magnification of the images showed that the particles are dispersed and roughly spherical. The particle size (Fig. 3) of the nanoparticles calculated showed that the particles were with sizes of 28.41–56.82 nm for *Allium cepa* and about 22.73–60.61 nm for *Allium sativa*. The average size of the nanoparticles was found to be 42.14 nm (Fig. 3).

The antimicrobial activity of nanoparticles from onion and garlic was tabulated in Table 1and shown in Fig. 4. The onion particles showed higher activity against the pathogenic. The activity was limited against *Staphylococcus* sp., after that the *Pseudomonas* sp., the garlic particles were active against *Pseudomonas* sp., and *Staphylococcus* sp. These onion particles also showed the activity against gram positive and gram negative organism. Both the Allium sp. was good antimicrobial agent.

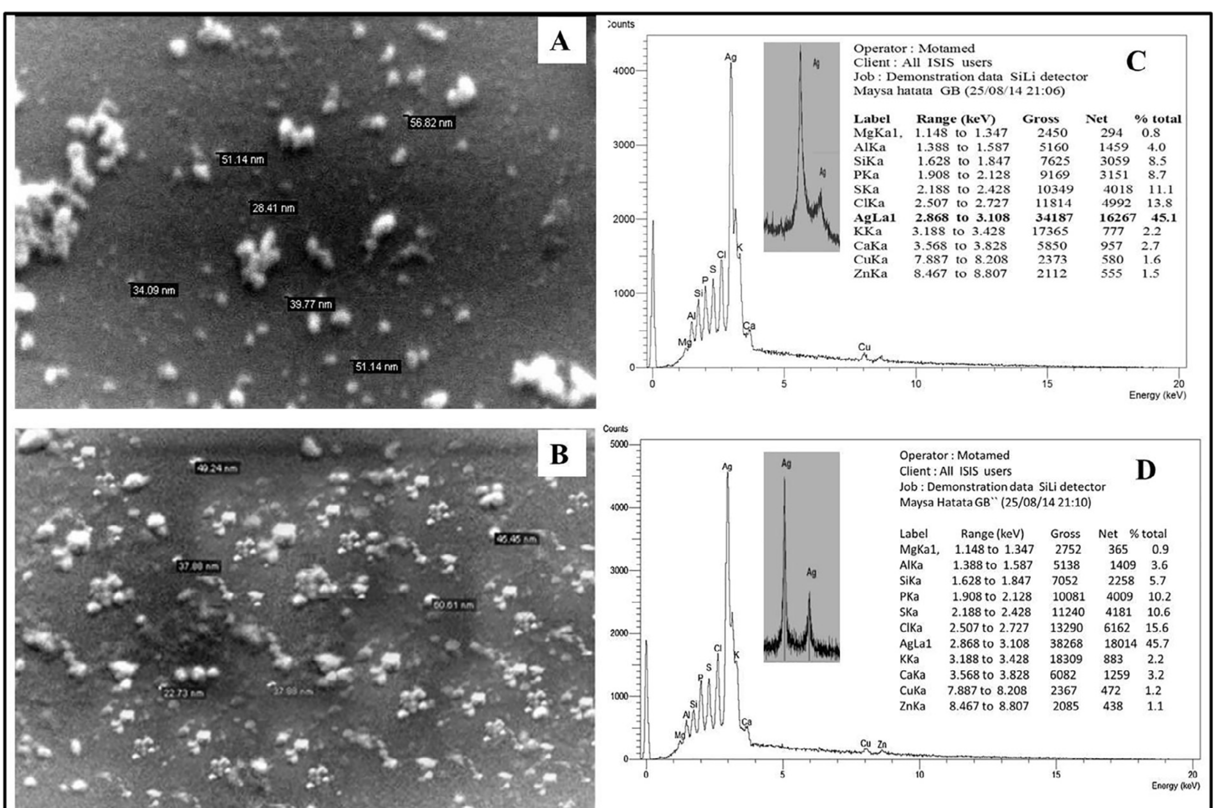

Fig. 3. SEM images of AgNPs by plant extract of (A) *Allium cepa* and (C) *Allium sativa*. Analysis of Energy dispersive X-ray (EDX) spectrometer of the particles formed by leaves extract of (B) *Allium cepa* and (D) *Allium sativa*.

Table 1
Biochemical characteristics of test organisms.

Probable isolate	Gram stain	Catalase	Coagulate	Oxidase	Motility
Staphylococcus aureus	Positive	+	+	–	–
Pseudomonas aeruginosa	Negative	–	–	–	+

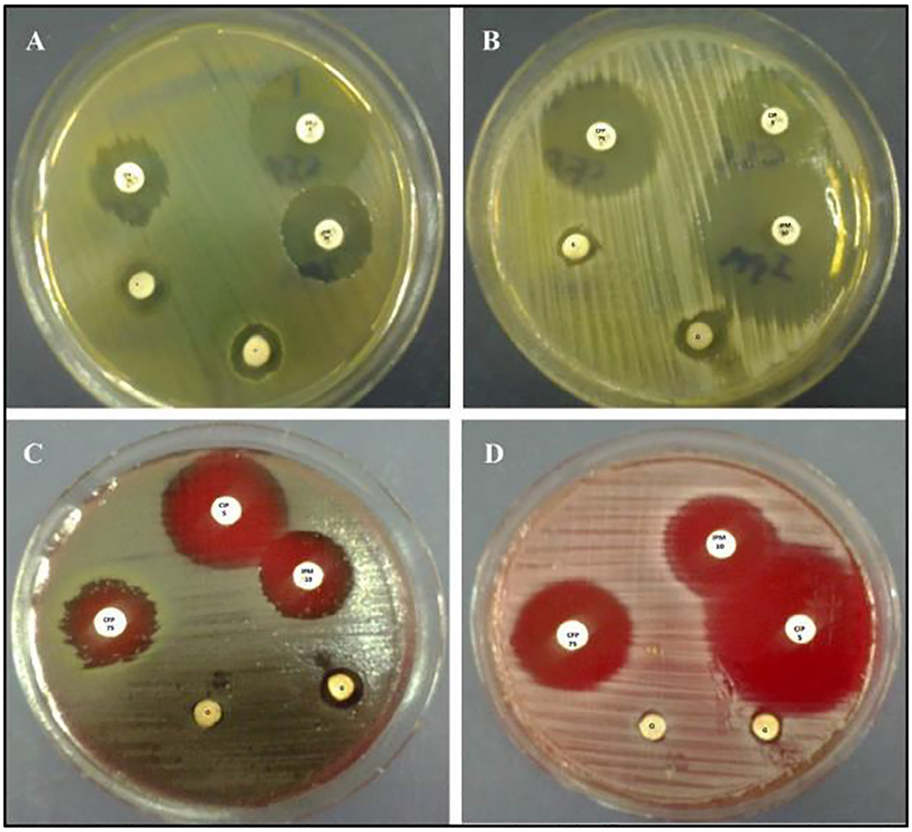

Fig. 4. Antimicrobial activities of synthesized Ag nanoparticles in aqueous extract of *Allium cepa* and *Allium cepa* and *Allium sativa* plants with $AgNO_3$ toward *Pseudomonas aeruginosa* (A and C) and *Staphylococcus aureus*(B and D). O = Onion; G = garlic.

4. Discussion

Silver nanoparticles display yellowish brown color solution due to the reduction of silver ions to silver nanoparticles during exposure to the plant extracts. The difference in the rate of bioreduction observed between studied plants may be assign to the differences in the activities of the enzymes present in *Allium cepa* and *Allium sativa* extracts. Green synthesis provides advancement over physical and chemical method as it is cost effective environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, temperature, energy and toxic chemical (Ravindra et al., 2012).

The EDS analysis obtained in the present study confirmed the presence of silver nanoparticles of *Allium cepa* and *Allium sativa* and mostly showed strong signal energy peaks for silver atoms in the range 2–4 keV. Our results are consistent with earlier study, Gardea-Torresdey et al., 2002 obtained formation of individual spherical-shaped silver nanoparticles in the range 2.5–4 keV by using Alfalfa. Moreover, Vijayakumar et al., 2013 studied the formation of Ag nanoparticles in the range 2–4 keV by using *Artemisia nilagirica*.

The different synthesized silver nanoparticles are formed due to the achievement of leaf extract of *Allium cepa* and *Allium sativa* extracts which act as good bio-reductants for $AgNO_3$ in the process of Ag nanoparticles biosynthesis.

The high surface to volume ratio of silver nanoparticles increases their contact with microorganisms, promoting the dissolution of silver ions, thereby improving biocidal effectiveness. The ability of silver nanoparticles to release silver ions is a key to their antimicrobial activity (Dhrutika et al., 2013).

The XRD outline accordingly obviously displayed that the silver nanoparticles formed by the reduction of Ag^{+} ions by *Allium cepa* and *Allium sativa* extracts are crystal-like in nature and these results are consistent with Huang et al. (2007). The presence of structural peaks in XRD patterns and average crystalline size around 30 nm clearly illustrates that AgNPs synthesized by our green method were nanocrystalline in nature.

Silver nanoparticles exhibited antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* as it showed a clear inhibition zone. It could be concluded that, silver nanoparticles have been demonstrated to exhibit antimicrobial properties against bacteria with close attachment of the nanoparticles themselves with the microbial cell. Our results are consistent with the previous study done by Jose et al., 2005, who found that the antimicrobial activity being nanoparticles size dependent. The enhanced antibacterial effects of silver nanoparticles is characterized and also stated that once inside the cell, nanoparticles would interfere with the bacterial growth signaling pathway of putative peptides substrate critical for cell viability and division and the nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (Duran et al., 2005; Shrivastava et al., 2007).

The major mechanism through which silver nanoparticles manifested antibacterial properties was by anchoring to and penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues (Shrivastava et al., 2007).

Recently studies have demonstrated that specially formulated metal oxide nanoparticles have good antimicrobial activity. Its compounds have strong inhibitory and bactericidal effects as well as broad spectrum of antimicrobial activities of bacteria (Saha et al., 2011).

The antimicrobial nature of nanoparticles is the most exploited nature of nanoparticles in the medical field (Geethalakshmi and Sarada, 2010). The nanoparticles have unique biological and chemical properties which are make them excellent candidates for many purposes in the medical field and pharmaceuticals (Jain and Aggarwal, 2012). Antibacterial compounds have been used in the formulation of dental resin composites and ion exchanges fibbers and in coating of medical devices (Oza et al., 2012). The result of this work indicates that the synthesized Ag nanoparticles from onions and garlic have antibacterial properties. When the Ag-nanoparticles were tested on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the widest zones of inhibition were obtained with *P. aeruginosa*. These differences in the zones of inhibition may be directly related to the susceptibility of each test organisms to the onions and garlic Ag nanoparticles. The factors responsible for this high susceptibility of *P. aeruginosa* to the Ag-nanoparticles are not exactly known but may be attributed to the effect of nanoparticles on the bacterial cell wall. Recently studies have demonstrated that specially formulated metal oxide nanoparticles have good antimicrobial activity. Its compounds have strong inhibitory and bactericidal effects as well as broad spectrum of antimicrobial activities of fungi, virus, and bacteria since ancient times (Saha et al., 2011). Also, these nanomaterials could be useful for awide range of applications, such as studying uptake of certain molecules and identifying the efflux mechanism (Seema Ameen et al., 2016).

5. Conclusion

The rapid biological agents in the form of plants have emerged as an efficient candidate for the synthesis of nanoparticles. This biosynthesis of nanoparticles is cost efficient, simpler to synthesize and exhibit broad spectrum biocidal activity toward several types of bacteria. Since *Allium cepa* (onion) and *Allium sativa* are easily available throughout the nation and also is used in every house for cooking, the active Nano compound from these can be prepared and used effectively in the field of diagnostic, antimicrobial and therapeutics.

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