Plant Archives Vol. 19, Supplement 2, 2019 pp. 874- 879 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES BIOSYNTHESIZED BY OLIVE OIL

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Abstract

Silver nanoparticles (AgNPS) were synthesized using olive oil as reducing and stabilizing agent. AgNPS prepared in non-aqueous solution, where the start material was dissolved in acetone as a solvent and to be miscible with olive oil. After heating at 110 C, the color turned into brown colloidal. AgNPS characterized by UV–visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), atomic force microscopy (AFM), dynamic light scattering (DLS) and scanning electron microscopy (SEM). All techniques showed the formation of spherical AgNPS of metallic sliver in crystalline phase with narrow particle size distribution. Estimation of minimum inhibitory concentration of AgNPS and The antibacterial activity of Silver nanoparticles was evaluated by agar well diffusion method . Several kinds of bacteria were studied like *Proteus mirabilis, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Vibrio cholera, Vibrio paraheamolyticus, Klebsiella pneumonia,* and *Salmonella enteritidicus*, where AgNPS showed broad spectrum antibacterial activity.

Keywords: Antibacterial, Silver nanoparticles, AFM, XRD, DLS, olive oil

Introduction

Nanotechnology is an emerging field of science in today's world, which greatly benefit humans. The aim of nanotechnology is producing and make use of nanosized substance calculating 1-100nm (Jemal *et al.*, 2017). Nanomaterials is more attractive for application differ fields due to of their unique features, used to delivering drug, imaging diagnosis, detection of macromolecules or pathogens, etc. (Salata, 2014). Silver is vastly utilized in the preparation of several, antimicrobial agents through the past few years due to possess the antimicrobial activity, stability and catalytic features. Silver nanoparticle is synthesized by different method including biological, physical and chemical (Xia *et al.,* 2016; Klapiszewski *et al*., 2015; Swamy, 2015a; Sharma, 2009). The biological for the synthesis of silver nano-particles has most interesting areas of research with increasing growth and importance in medicine, this synthetic approach is related with the growing need of developing clean, non-toxic, eco-friendly, cheap as well as to when use physical and chemical methods to avoid the toxic substances. Plant mediated nanoparticles were largely utilized because it (Cruz *et al*., 2010; Nakkala *et al*., 2017). The rate of reduction of metal ion using plants is much faster than microorganisms. The size and the shape of the nanoparticles synthesized using plants will depend on the relative rates of these processes that can be controlled through the adjustment of the reaction parameters as the pH (Gardea *et al*., 2003). In recent years, various leaves extracts of plants such as *Azadirachta indica*, *Aloe vera* and *Ocimum sanctum*, etc. have been used for synthesized of silver nanoparticles (Shankar *et al*., 2004; Chandran *et al*., 2006; Philip and Unni, 2011). Montedoro *et al.* are classified the aromatic compounds of olive oil, aliphatic and aromatic hydrocarbons, aliphatic and triterpenic alcohols, aldehydes, ketones, ethers, esters, and furan and thiophene derivatives, the olive oil is differing in chemical composition depending on the extraction technology Kiritsakis, 1998; Gökçebağ *et al*., 2013; Ozkaya *et al*., 2004). In these paper amid to green synthesis of silver nanoparticles by olive oil and evaluate the antibacterial activity.

Material and Methods

Preparation of Silver Nitrate Solution

Silver nitrate purchased from Fluka company was weighed about 0.169 gm and added to 100 ml of acetone purchased from GCC. with continuous stirring until saturation to prepare 0.1 N of silver nitrate.

Preparation of Silver Nanoparticles

Olive oil of 50 mL was added to 50 mL of silver nitrate solution in acetone, and then boiled until yellow color observed at 100 ºC the temperature is settled at 110 ºC for 30 min. At the same time, acetone was evaporated leaving dark brown suspension of AgNPS. when adding cold acetone causing precipitation of AgNPS. The brown suspension was cooled and separated by using the centrifuged many times to remove the surplus amount of olive oil in each time adding acetone. The precipitate was dried at 80 C for night then the powder of AgNPS was used for characterization and other tests.

Characterization of the synthesized silver nanoparticles

The UV-Vis spectrophotometer model LF 4030 Scienco (Korea) was used to determine AgNPS absorption spectra from 200-800 nm in water and hexane. Atomic force microscope model AA 3000, angstrom advanced inc. (Korea) was utilized to observe the texture of AgNPS. The dynamic light scattering (DLS) model SALD-210 (USA) was used for determination the size distribution of AgNPS. FTIR analysis IR affinity instrument, manufactured by shimadzu (Japan) was used to investigate AgNPS. The crystalline structure of the particles was determined by XRD instrument model DX-2700 SSC, where the source is Cu Kα1 radiation (λ= 1.540562 Aº) and operate at 30 mA current and 40 kV voltages. The surface nature of AgNPS was analyzed by

using Scanning Electron Microscope (SEM) model Inspect 550, Netherland, operated at 25 kV.

Antibacterial activity of silver nanoparticles

The antibacterial activity of silver nanoparticles was tested against eight bacteria *Proteus mirabilis*, *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Vibrio cholera*, *Vibrio paraheamolyticus* and *Salmonella enteritidicus* by agar well diffusion method (Jena *et al*., 2016), the bacterial strains were cultured on Muller Hinton agar. Agar plates were prepared and used a sterile gel borer for making wells. 100µl silver nanoparticles solution was added to each well. Petri dishes were incubated at 37 °C for 24 h. The inhibition zones of bacteria were observed and measured in millimeters.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

A rang of dilution of AgNPS were prepared, the dilutions of silver nanoparticles ranging from (100-0.78) µg/ml prepared by mixed the each one of silver concentration with 5 ml of Muller Hinton broth. Inoculated with bacteria and adjusted with 0.5 Macfarland standard then incubated at 37 ºC overnight. The MIC were represented the lowest concentration with no growth. Then subculture by spread in Petri dishes were incubated and Final results were recorded as MBC were represented the lowest concentration with no growth.

Results and Discussion

Characterization of the synthesized silver nanoparticles

The color change of solution due to reduction of silver ion to AgNPS, final color liked tea color. According to the UV VIS spectra, the absorption is over measurement and unclear so the solution is diluted four folded in cyclohexane.Fig.1 indicates to UV-Visible spectroscopy absorption spectra for the synthesized silver nanoparticles from Olive oil after dilution in cyclohexane before purification (red curve). The maximum absorption appeared at 400 nm related to surface plasmon resonance (SPR). After purification of AgNPS from excess oil and re-dispersed in water (blue curve), the SPR is shifted to 432 nm. The difference in SPR absorption is due to charge transfer between AgNPS and Olive oil molecules in cyclohexane (García−Macedo *et al*., 2009). The UV-visible spectra range 400- 460 nm indicates surface Plasmon ringing of AgNPS, wide peak could be related to the poly dispersed nature of silver Nano particles with spherical shape (Swamy *et al*., 2015 b; Netala *et al*., 2015; Mallikarjuna *et al*., 2011; Jyoti, 2016). In our result, the absorption peak relatively narrow and that means AgNPS were spherical particles and very symmetric in size.

FTIR spectroscopy analysis of oil olive and biosynthesized silver nanoparticles by oil olive are illustrated in Fig.2. The main characteristic absorption bands in oil olive were appeared at wave numbers. The comparative study of two FTIR spectra revealed that some bands undergo a shift to higher or lower frequencies. The results showed a shift in the band at 3008 cm^{-1} to 3010.88cm^{-1}which was attributed to =C−H (cis) stretchbonds. A shift in the band at (2924.09 and 2856.58 to 2920.23 and 2856.58 cm^{-1} respectively) was attributed to the stretching vibration of aliphatic C−H methylene stretch asymmetric and symmetric stretching band. The stretching vibration of carbonyl group (C=O) was appeared at 1745.58 cm^{-1} and shifted to 1743.65 cm^{-1}, furthermore results disappear band 1656.85 $cm^{-1}$is related to C=C cis bond, while appear band 1562.34cm^{-1} may be related to conjugation of C=C. The C−H methylene scissoring appeared at 1458.18 cm^{-1} and shifted to 1462.04cm^{-1}.The band 1369.46cm^{-1} is related to bending of =C−H (cis) benbonds shifted to 1415.75, band 1369cm^{-1} is related to $C-H_3$ ben shifted to 1377.17 and band 1232.51 cm^{-1} is related to C−O shifted to 1240.23 cm^{-1}. The band 1161.15 and 1103.28 cm^{-1} is related to C−O stretch shifted to 1166.93 and 1111.00cm^{-1}, respectively. Appear band 1097.50 cm^{-1} is related to C−O stretch. The band 723.31 cm^{-1} is related to =C−H (cis) shifted to721.38cm^{-1}.

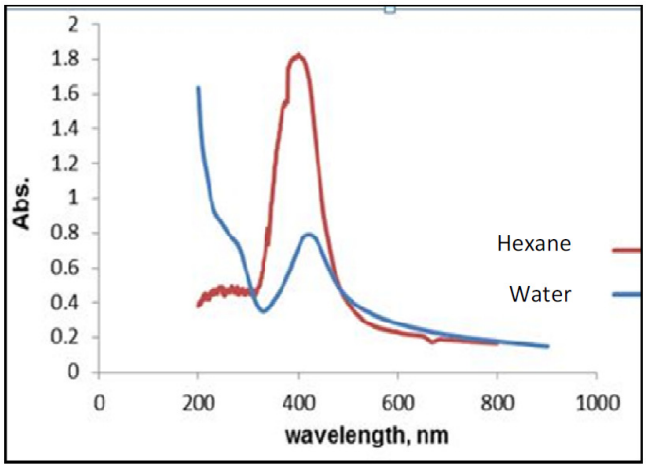

Fig. 1 : UV-Visiblespectroscopy of silver Nano particles

The absorbance bands at 1377, 1233, 1159 and 1118 cm^{-1} were broad and shifted, may be related to ester group and monounsaturated and polyunsaturated acyl groups, which reduce metal ions (Ahmed at al 2018). The researcher show that appear new peaks and decrease in the intensity of 2922, 2853, 1743 and 1160 cm^{-1} which related to -CH2, -CH, C=O, C=C and C-O groups in esters (monounsaturated and polyunsaturated acyl groups), where responsible for reduction and stabilizer agent of synthesized gold nanoparticles (Kumar *et al*., 2018). In previous study, the utilized the palm oil in reducing and stabilizer of gold nanoparticles, that related to carbonyl capped of gold nanoparticles thereby electron transfer from carboxylic group to gold nanoparticles, causing a clog of the motion of the molecules inside the particular area with a decrease in entropy (Sadrolhosseini *et al*., 2017).

XRD pattern it is clear that silver nanoparticles were synthesized by oil olive were crystalline nature, shown in Fig. 3, the result of XRD pattern shows at 2θ of 38.35º and 44.29º these are corresponding to (111) and (200) planes for silver, respectively. XRD is used to determine the chemical composition and the crystalline nature of material (Ponarulselvam *et al*., 2012). XRD shows that AgNPS were formed in one phase of cubic center crystal of silver metal without any formation of silver oxides. Olive oil has interesting feature to synthesized AgNPS to stabilized nanoparticle and keeping AgNPS out of oxidation as a capping agent. By XRD calculation, the average particle size (D) was determined by using Scherrer's equation, and formula of ($D = 0.94\lambda / B \cos\theta$), where 0.9 is the shape constant, λ (in the case of CuKa1) = 1.54060 Å, which is the

wavelength of x-ray beam, B: the full width at half maximum intensity of the peak and θ: diffraction angle. This equation is an indication of particle size related to the broadening of diffraction peak. When the peak is broad, the particle size is a function related it with inverse proportion. D value calculated for AgNPS according to the highest intensity peak and it was equal to 25 nm.

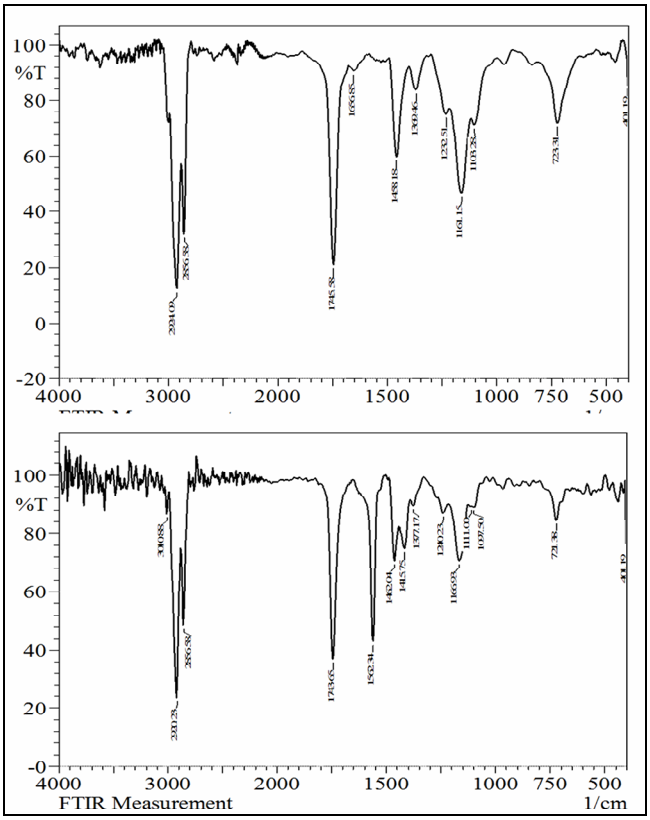

Fig. 2: FTIR analysis of biosynthesized silver nanoparticles by oil olive

Surface topography of AgNPS was showed in figure 4, where SEM image appeared in three scales 5, 1 and 0.5 µm. SEM image showed the synthesized AgNPS by Olive oil with fine texture and spherical nanoparticles. This procedure is very interested to prepare AgNPS with high stability in size and can be dispersed in water or hexane. This is due to the rigid surrounding of Olive molecule around AgNPS. The measured size of AgNPS was 38-36 nm and approach to size calculated by XRD peaks broadening which was 25 nm.

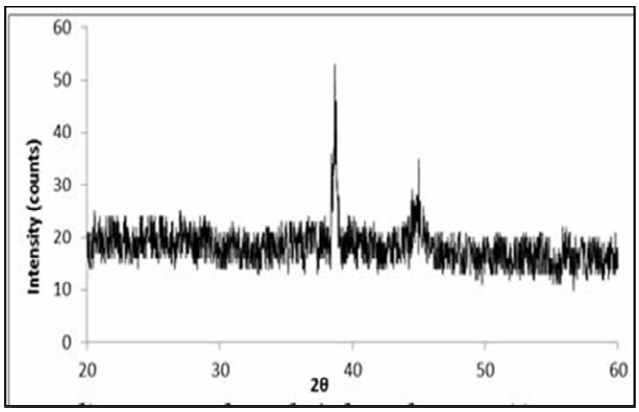

Fig. 3: XRD of silver nanoparticles

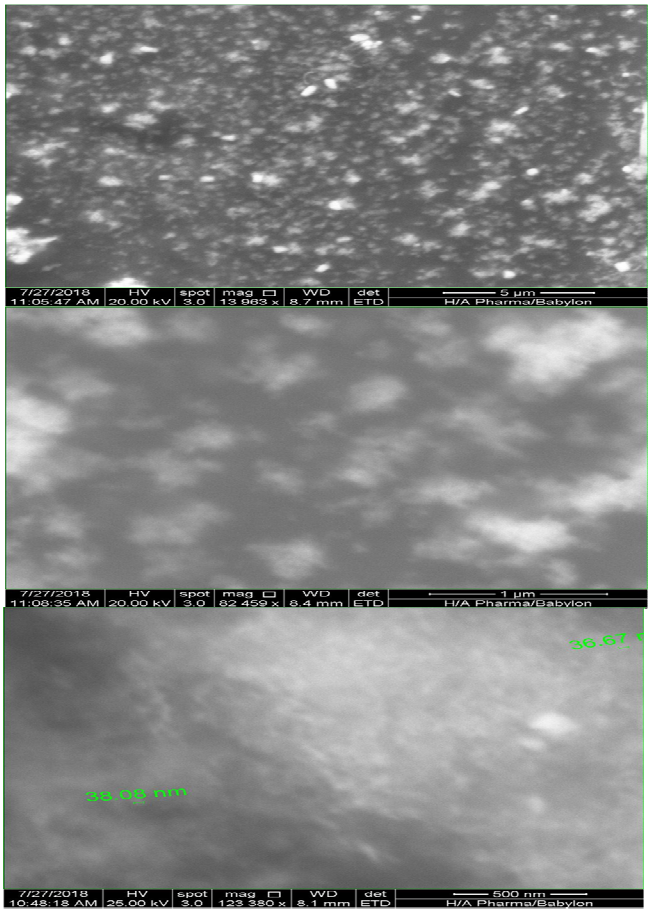

Fig. 4 : SEM image of synthesized AgNPS by Olive oil in three scales 5, 1 and 0.5 µm

The AFM images of silver nanoparticles were shown in Fig. 4 (a), the evolutions of topography of surface of the silver nanoparticles synthesized by Olive oil. AgNPS synthesized by this procedure is the main reason to produce a very fine nanoparticles with a high roughness and average size of 54 nm. This result is good in comparison with previous study, where it was found to be in the range 75 nm using stem derived callus extract of bitter apple (*Citrullus colocynthis*) (Satyavani *et al.*, 2011). Also, in Fig.4 (b) showed the granularity distribution of AgNPS that prepared by Olive oil. AgNPS was in the range of 25-90 nm and most popular size was 50 nm indication of successive preparation of AgNPS in a distribution less than 100 nm.

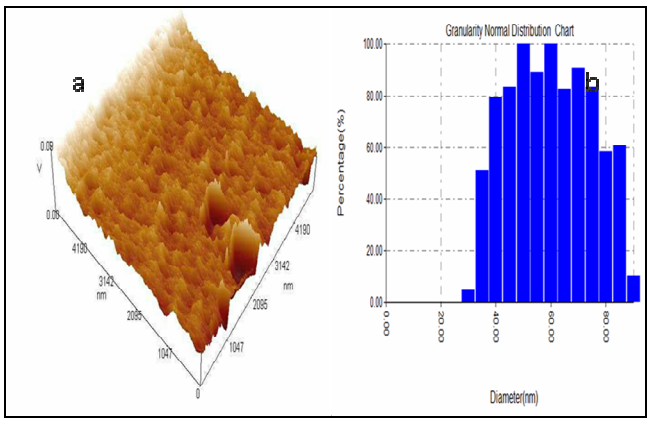

Fig. 5 : Three-dimensional image of silver Nano particles was synthesized by Olive oil (a) and granularity report (b).

The dynamic light scattering (DLS) was applied to reveal the size distribution of silver nanoparticles**.** The results obtained by DLS is the average particle size was determined by DLS, it was found equal 34 nm. In previous study, it was found 5-20 nm as revealed in the size distribution graph by green synthesis of silver nanoparticles using *Terminalia arjuna* extract (Ahmed et al, 2015). The main result of DLS measurement is very narrow distribution of AgNPS that synthesized by Olive oil. This size is very close to the size than observed in SEM image.

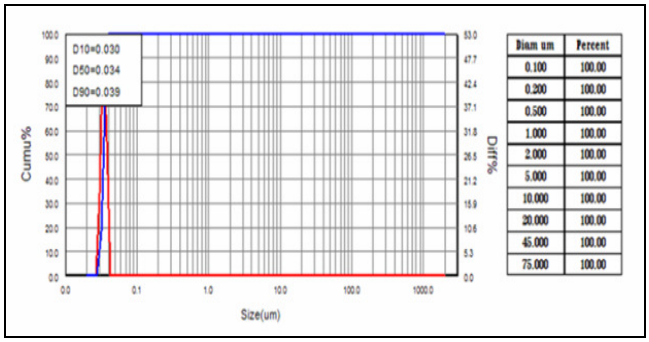

Fig. 6 : Distribution of silver Nano particles were synthesized by Olive oil.

Antibacterial activity of silver Nano particles and MIC and MBC

the results showed in the table1 and fig. 7, and found Inhibition zones of bacteria were observed that silver nano particle had inhibitory efficacy against to *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Vibrio cholera*, *Vibrio paraheamolyticus* and *Salmonella enteritidicus* with a diameter of inhibition zone were 30.33, 24.67, 30,25. 33, 28.67, 32.67, 34.67 and 30.67, respectively.

Table 1: Antibacterial activity of silver Nano particles

Bacterial isolate	Inhibition zone rate (mm)
Proteus mirabilis	30.33
Bacillus subtillus	24.67
Staphylococcus aureus	30
Escherichia coli	25.33
Klebsiella pneumonia	28.67
Vibrio cholera	32.67
Vibrio parheamolyticus	34.67
Salmonella enteritidis	30.67

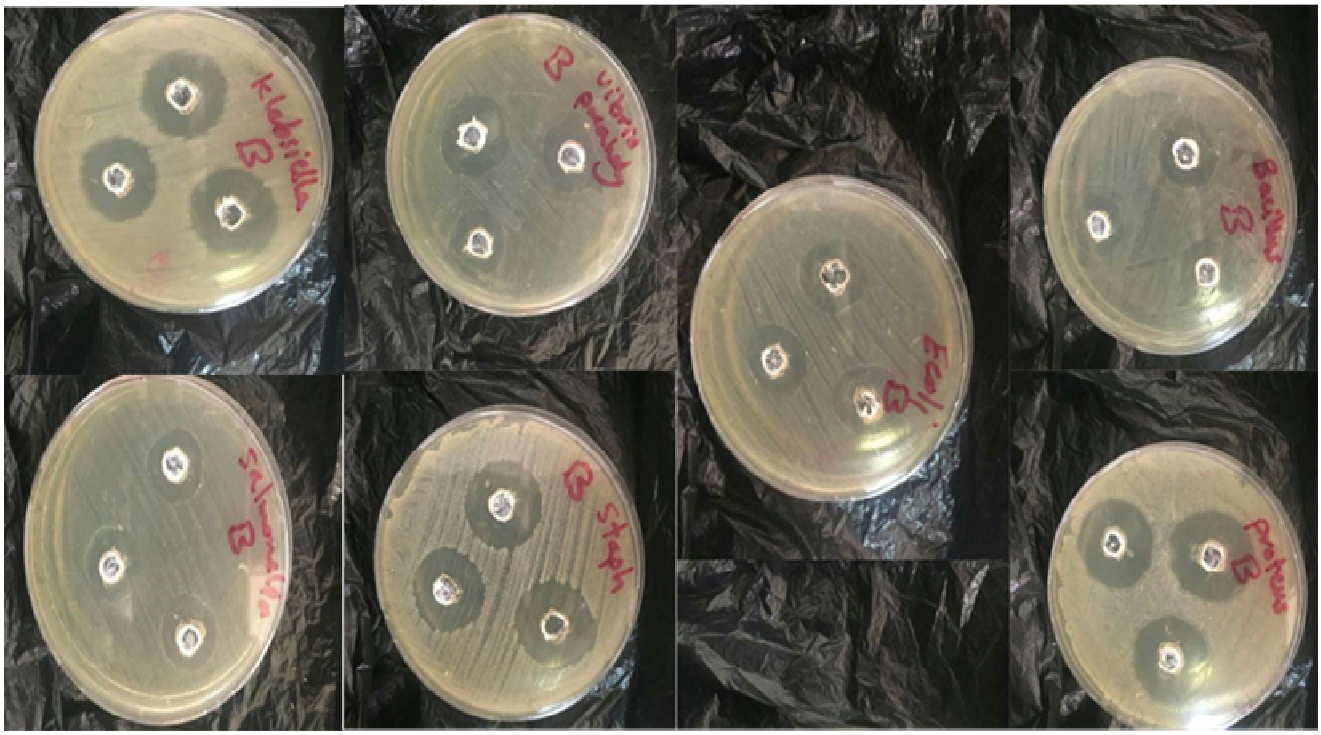

Fig. 7 : Antibacterial activity of silver Nano particles

Researchers suggested the possible mechanism of action of silver ions by penetrate the cell wall and further more damage DNA by reacts with the phosphorus residues and sulfur residues in proteins and result in cell death. Moreover, interfere with the replication (Kazachenko *et al.*, 2000; Rai *et al.*, 2009).

Agar dilution method was used to detect MIC of silver nanoparticles. Silver nanoparticles was diluted to obtain several concentration ranged between 100 µg/ml and 0.4 µg/ml. The results of this study showed that the MIC of silver nanoparticles was 6.25- 25 µg/ml, while MBC was 12.5–50 µg/ml, as shown in fig. 8.

The bactericidal properties of silver nanoparticles are related to the electrostatic reaction between negatively charge of bacterial cell wall and positively charge of nanoparticles metal ions (Perma *et al.*, 2017). The factor effecting on bactericidal properties is shape; size, surface area, and stability of silver nanoparticles. The size of silver nanoparticles is play critical roles in the antimicrobial activity, small size have and have high surface reactivity (Zafar *et al.*, 2016; Su *et al.*, 2017; Gliga *et al.*, 2014).

The previous study show that MIC of silver nanoparticles against Salmonella is 25 μgml^{-1} with sized of silver nanoparticles 28 nm while 12.5 μgml^{-1} with 8 nm. The MIC and MBC of silver nanoparticles against *Salmonella*

enterica [ATCC 13076] were 3.5 and 6.9 μgml^{-1}, respectively. Furthermore, study MBC of silver nanoparticles (100 nm of size) was approximately 1×10^4 μgml^{-1} (Smekalova *et al*., 2016; Singh *et al*., 2016; Flores *et al*., 2013).

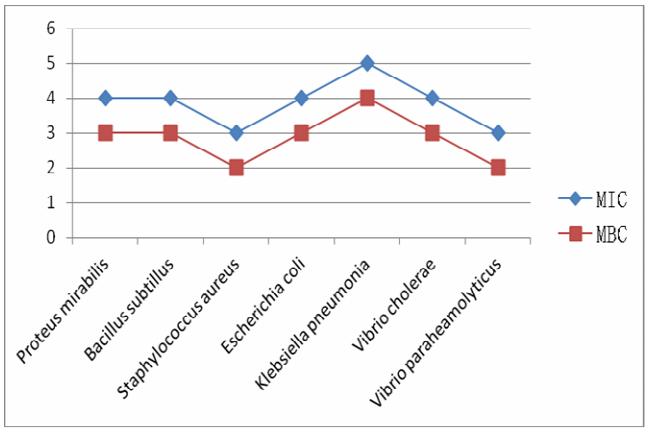

Fig. 8: MIC and MBC of silver nanoparticles

Conclusion

Several kinds of bacteria were studied like *Proteus mirabilis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholera*, *Vibrio paraheamolyticus*, *Klebsiella pneumonia*, and *Salmonella enteritidicus*, where AgNPS showed broad spectrum antibacterial activity. also All techniques showed the formation of spherical AgNPS of metallic sliver in crystalline phase with narrow particle size distribution.

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