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Full Length Research Paper

Biosynthesis of Silver Nanoparticles by *Spirulina platensis & Nostoc sp.*

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Synthesis of nanoparticles that have environmentally acceptable solvent systems and eco-friendly reducing agents is of great importance. The aim of this work was to synthesis of silver nanoparticles (AgNPs) using *Spirulina platensis* and *Nostoc* sp. at room temperature. UV–visible spectrometry study revealed surface plasmon resonance at 402 nm. The transmission electron micrographs of nanoparticles in an aqueous solution showed the production of AgNPs (average size of most particles~11.5 nm by *Spirulina platensis* and 20.3 nm by *Nostoc* sp.). The Fourier Transmittance Infrared spectrum (FT-IR) confirms the presence of biocomponent in the algae which was responsible for the nanoparticles synthesis. The antibacterial activity silver nanoparticles against both gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidedymis*) and gram negative bacteria (*Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa*) by well diffusion method showed excellent antibacterial activity against all tested bacterial. This green process gives the greater potential biomedical applications of silver nanoparticles.

Keywords: Biogenic nanoparticles, silver nanoparticles, algae, Antimicrobial Activity of Silver nanoparticles

INTRODUCTION

Biosynthesis of nanoparticles using microorganisms as an emerging bionanotechnology has received considerable attention due to a growing need to develop environment friendly technologies in materials synthesis (Rai *et al.*, 2011). Particles with a size up to 100 nm are usually referred to as nanoparticles (Willems and van dem Wildenberg, 2005) and (Simi and Abraham, 2007). In the 21st century, nanotechnology is the newly emerging multidisciplinary research area with synthesis of nanosized materials (Amin *et al.*, 2012). Nanotechnology is the

manipulation and production of materials ranging in size from 1 to 100 nanometer scale (Mohanpuria *et al*., 2008). Nanoparticles exhibit completely new or improved properties, based on specific characteristics, such as grain size, distribution and morphology, if compared with larger particles of the bulk material they were made of Silver has long been recognized as having an inhibitory effect on many bacterial strains and microorganisms commonly present in medical and industrial processes (Jiang *et al.*, 2004). The most widely used and known applications of silver and Silver Nanoparticles (AgNPs) are in the medical industry. These include topical ointments and creams containing silver to prevent the infection of burns and open wounds (Becker, 1999). Other widely used applications are medical devices and implants prepared with silver-impregnated polymers (Silver, 2003). The nanoparticles can play a topmost role in the field of nanomedicines such as health care and medicine diagnostic and screening purposes, drug delivery systems, antisense and gene therapy applications, and tissue engineering and expectations of nanorobots configuration (Kubik *et al.,* 2005). However, up to date, most microorganisms that have been reportedly used for synthesis of silver nanoparticles are pathogenic to either plants and/or humans (Ahmed *et al.*, 2003). So over the years, researchers have turned to nonpathogenic microorganisms (Mehta and Gaur, 2005). *Spirulina platensis* and *Nostoc* sp. are two blue–green microalgae (cyanobacteria), is an important representative of these microorganisms.

2- MATERIALS AND METHODS

2-1- Preparation of Algal Biomass: A strain of *Spirulina platensi*s was obtained from algae unit of National Research Center was used. The condition of cultivation *S. platensis* cells in Zarrouk's medium. (Zarrouk, 1966) The culture was carried out at 30 ± 2^{O} C at pH 10 in light/dark conditions (16/8hrs) and 3000 lux with shaking of culture manually thrice a day. The monitoring of *S. platensis* growth was measured spectrochemically at 640 nm each three days for 21 days (Thirumala, 2012). Also a strain of *Nostoc* sp. from Plant and Microbiology Department, Faculty of Science, Al-Azhar University (Girl Branch) was used and cultured a in BG-11 (Blue-Green algae) medium for cyanobacteria (Rippka *et al*., 1979).

The growth potential of alga was maintained through regular sub culturing techniques, under laboratory conditions at 25^{O}C, in a 16/8 h light/dark cycle, Biomass production of the cyanobacterium colony cultures was measured with wet weight (Zongjie *et al*., 2011).

2-2-Biosynthesis of Silver Nanoparticles by Cyanobacteria

For this, cyanobacteria from mid exponential phase of its growth were collected. *S. platensis* cells harvested after 5-6 days cultivation while *Nostoc* sp. cells harvested within15-24 day's cultivation and then washed twice in distilled water. The wet biomass of *S. platensis* and *Nostoc* sp. (1g) was re suspended in 500ml Erlenmeyer flask with 100ml of 1mM, 2mM and 3mM aqueous silver nitrate by using deionized water (pH7) at 25^{O}C for 24hours (Kalabegishvili *et al*., 2012). In another method the algae culture were procured at the mid exponential phase and subjected to centrifugation at 5 000 rpm for 5 min, the supernatant added to 1mM silver nitrate solution (Devina Merin *et al*., 2010) in different concentrations 10% , 20% and 30%.

2-3- Estimation and Characterization of Silver Nanoparticles

1- UV-vis Spectrometry: The UV-visual spectra of the samples were recorded by spectrophotometer (Nicolet evolution 100, Cambridge) with digital data acquisition, wavelength range 220– 600 nm.

2- Fourier Transformer Infra Red (FT-IR): The Infrared spectrophotometer (IR) used AKX0901119012A0607, genesis series Nicolet IS-10 F, The instrument is in Egyptian petroleum institute (EPRI).

3- Dynamic Light Scattering:

The size and size distribution of particles in the colloids were measured using a Nano ZS zetasizer system (Malvern Instruments). Measurement parameters were as follows: a laser wavelength of 633 nm (He–Ne), a scattering angle of 173◦ (fixed—without changing possibility), a measurement temperature of 25^{O}C, a medium viscosity of 0.8872mPa·s and a medium refractive index of 1.330, and material refractive index of 1.59. Before DLS measurement, the colloid was passed through a 0.2 m polyvinylidene fluoride (PVDF) membrane. The sample was loaded into quartz microcuvette.

4- Transmission Electron Microscopy (TEM):

TEM was performed using JEOL-JEM 2100 Electron microscope operating at 200KV. TEM studies were done at magnification 1.5 x and resolution up to 0.143 nm. Samples were prepared by placing a drop of solution with the silver nanoparticles on carbon-coated TEM grids. The films on the TEM grids were allowed to dry at room

temperature before analysis.

2-4- Determination of antibacterial activity by well diffusion method

The AgNPs synthesized from cyanobacteria was tested for its antibacterial activity against pathogenic bacteria such as *Staphlococus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* by standard well diffusion method in Mullor Hinton Agar (MHA) plates. Pure cultures of bacterial pathogens were grown in Nutrient broth at 37^{O}C for 18-24 hours. Wells were made on the Mullor- Hinton agar plates using a gel puncture and the plates were inoculated by swabbing the bacterial pathogens to create a confluent lawn of bacterial growth. Then 40 µL, 60 µL, 80 µL and 100 µL of the biosynthesized AgNPs solution were poured on to corresponding well using a micropipette. As control, 40 µL, 60 µL, 80 µL and 100 µL of 1 mM AgNO3 solution were poured on to control well. After incubation at 37^{O}C for 24 hours, diameter of zone of inhibition in millimeter around each well was measured (Thomas *et al*., 2012).

3- RESULTS AND DISCUSSION

3-1- Characterization of AgNPs by UV-Visible Spectrophotometer

In this study, extracellular synthesis of AgNPs has been shown from filamentous *Spirulina platensis* and *Nostoc* sp. It is well known that AgNPs exhibit a yellowish-brown color in aqueous solution, due to the excitation of surface Plasmon vibrations in AgNPs. Reduction of the silver ion to AgNPs during exposure to the *Spirulina platensis* and *Nostoc* sp. biomasses could be followed by a color change as in Figure 1 and 2, and, thus, UV-vis spectroscopy. Figure (3 and 4) shows the UV-Vis spectrum of the nano silver formation and the change in the color of the reaction mixture to yellow then dark brown, indicating the biotransformation of ionic silver to reduced silver, and the subsequent formation of AgNPs in an aqueous medium. A grayish black silver precipitate observed macroscopically.

It is observed that band occurs at 400nm and 402 nm for *Spirulina platensis* and *Nostoc* sp. subsequently due to plasma resonance of silver nanoparticles. So it was evident that metabolites excreted by algal culture exposed to silver could reduce silver ion. On other hands, the reduction of silver ions did not occur in absence of algal cells. In agreement of this study, Mubarak Ali *et al.* (2011) reports the extracellular biosynthesis of silver nanoparticles using marine cyanobacterium *Oscillatoria wille* NTDM01which reduces silver ions and stabilize silver naoparticles by a secreted protein. The silver nitrate solution incubated with washed marine cyanobacterium changed to yellow color indicating the formation of silver nanoparticles.

A grayish- black silver nanoparticle precipitate on cyanobacterium was observed in experiment macroscopically. They observed a characteristic protein shell at 265nm in Ultraviolet spectrum.

Figure (5) shows the increasing the concentration of silver nitrate solution with1g *S. platensis* wet biomass causes the biotransformation of ionic silver to reduced silver. It was observed a clear peak with maximum absorbance at 400nm with1mM of silver nitrate solution and week peaks with 2 mM and 3Mm of silver nitrate solution with absorbance around 400nm.

Also by increasing the concentration of silver nitrate solution with 1g *Nostoc* sp. wet biomass causes the biotransformation of ionic silver to reduced silver, and the subsequent formation of SNPs in an aqueous medium. UV vis spectrophotometer reveals that 2mM of silver nitrate with *Nostoc* sp. give the maximum absorbance at 427nm compared with silver nitrate concentrations 1mM and 3mM give 402 and 415 nm subsequently as in Figure(6).

Beside that the**,** using of 1mM of silver nitrate with10% supernatant of *S. platensis* in Zarrouk's medium give a peak with maximum absorbance at 400nm and 20% and 30% supernatant of *S. platensis* give unclear peak around 400 nm at figure (7).On other hand, using of 1mM of silver nitrate with10% supernatant of *Nostoc* sp. in BG11 gives maximum absorbance at 440nm an 20, 30 % supernatant of *Nostoc* sp. 384nm and 371nm subsequently Figure (8). In that concern, **Devina Merin *et al.* (2010)** observed a characteristic plasma resonance peak at 420 nm at 24 hrs. The plasma bands are broad with an absorption tail in the longer wave length, which could be in principle due to size distribution of nanoparticles. The reduction of silver ion occurs through electron shuttle or through reducing agents released into solution by algal culture.

3-2- Fourier transformer Infra Red (FT-IR):

FTIR was used to identify the biomolecules in *S. platensis* and *Nostoc* sp. responsible for the silver ions reduction and stabilization of reduced silver ions (figure 9 and 10). The FT-IR spectrum of the green AgNPs from *Spirulina platensis* shows strong absorption peaks at 3727.85,3442,2926.05,1628.56,1385.34 ,1275.44 and 671.41 cm^{-1} which represents the various functional group like OH stretching of alcohols or phenols, N-H group(amino acids),C-O of carboxylic anion, saturated C-O group and N-O stretching respectively (Figure 9) The absorption peak at 3442 cm^{-1} indicates the presence of N-H group (amino acids).The disappearance of secondary metabolites after the bio reduction of silver nanoparticles was also confirmed by FTIR spectroscopy and is thought to result

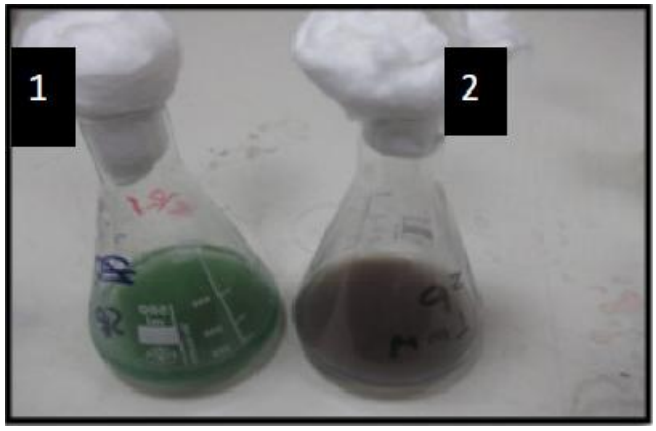

Figure (1): a) the conversion of silver nitrate to nano silver by *Spirulina platensis* biomass. The picture shows the growth of *Spirulina platensis* in Zarouk's medium (1) and the color changes (2).

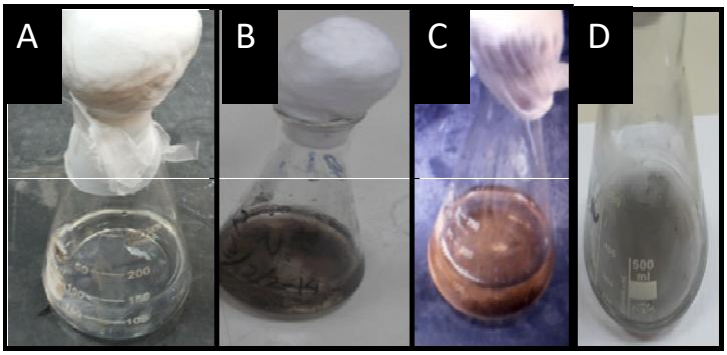

Figure (2): a) silver nitrate solution as negative control. b) *Nostoc* sp. biomass in BG11 medium. c) The picture shows the color changes of silver nitrate solution by *Nostoc sp.* in biomass. d) The complete reduction of ionic silver (Ag+) and grayish black precipitation of AgNPs.

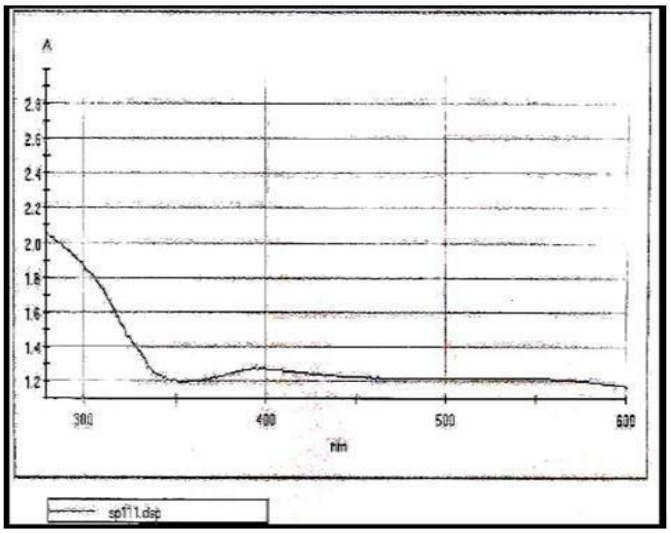

Figure (3): UV-Vis spectrum recorded after the reaction of 1mM silver nitrate solution with1g *Spirulina platensis* wet biomass at pH 7 and 25 ^{O}C and formation of AgNPs.

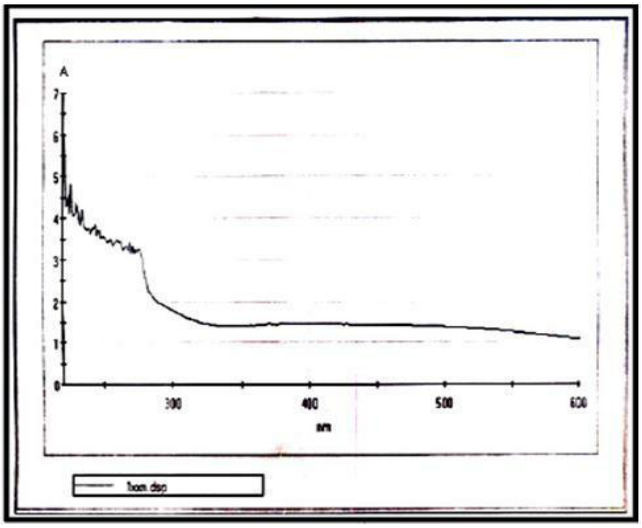

Figure (4): UV-Vis spectrum recorded after the reaction of 1mM silver nitrate solution with 1g *Nostoc* sp. wet biomass at pH 7 and 25 ^{O}C and formation of AgNPs.

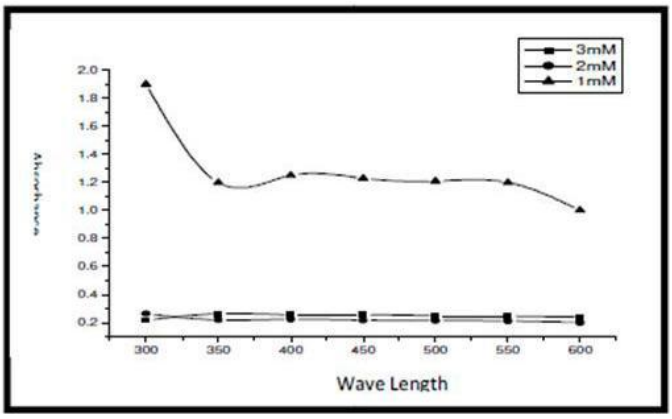

Figure (5): UV-Vis spectrum recorded after the reaction of 1mM, 2mM and 3mM silver nitrate solution with1g *S. platensis* wet biomass at pH 7 and 25 ^{O}C and formation of AgNPs.

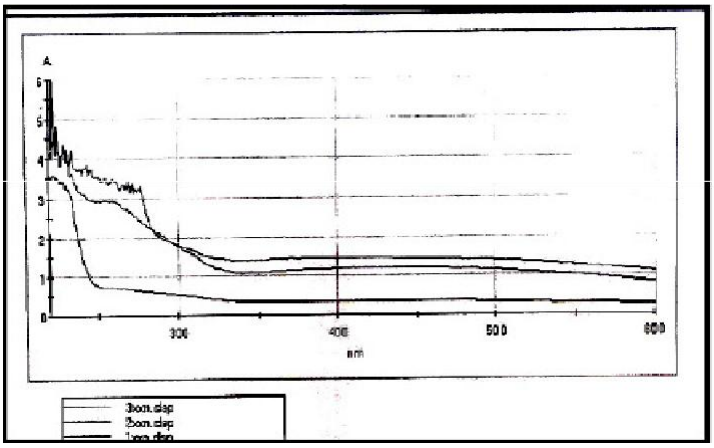

Figure (6): UV-Vis spectrum recorded after the reaction of 1mM, 2mM and 3mM silver nitrate solution with1g *Nostoc* sp. wet biomass at pH 7 and 25 ^{O}C and formation of AgNPs.

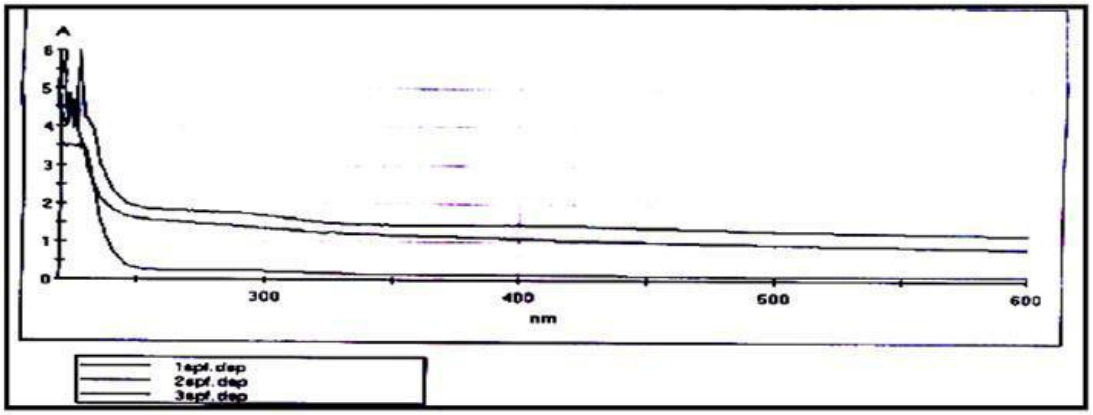

Figure (7): UV-Vis spectrum recorded after the reaction of 1mM silver nitrate solution with supernatant of *S.platensis* at pH 7 and 25 °C and formation of AgNPs after 24hrs.

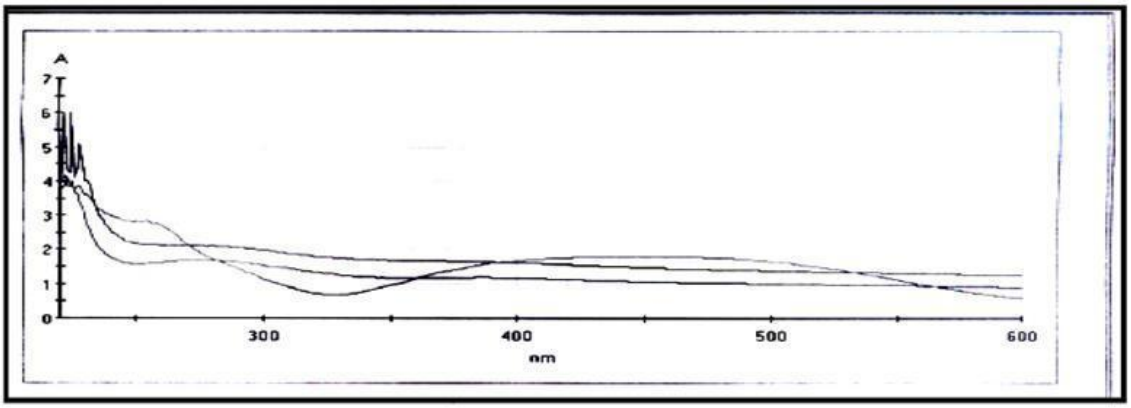

Figure (8): UV-Vis spectrum recorded after the reaction of 1mM silver nitrate solution with supernatant of *Nostoc sp.* at pH 7 and 25 °C and formation of Ag NPs after 24hrs.

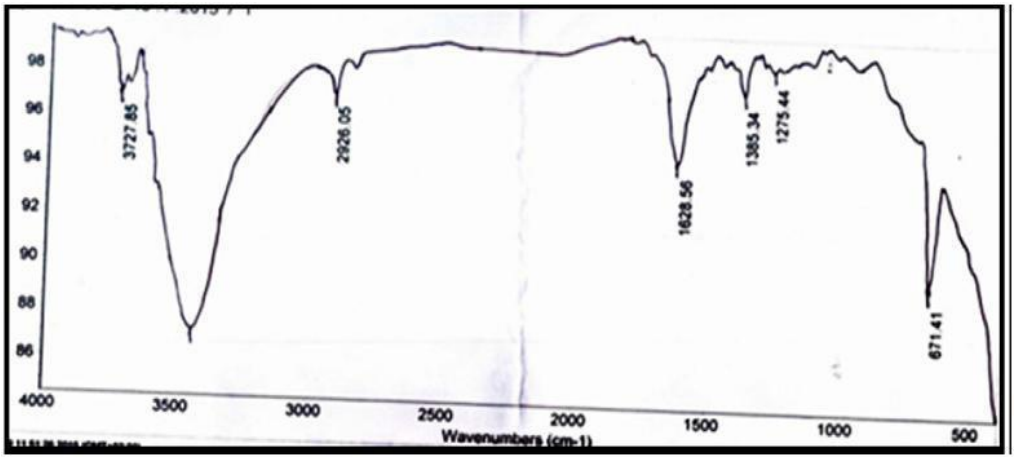

Figure (9) FTIR of *Spirulina platensis* shows the presence of protein shell for the reduction of silver ions**.**

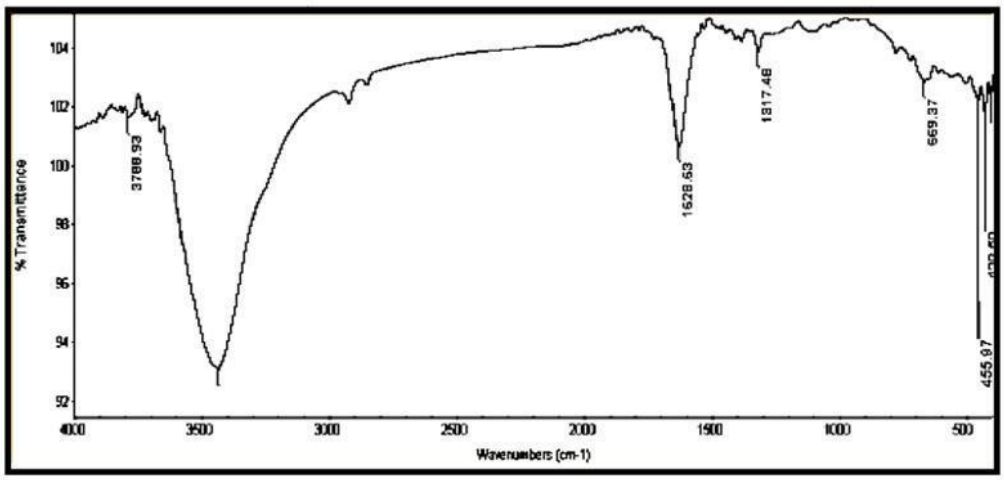

Figure (10) FTIR analysis of *Nostoc* sp.shows the presence of protein shell for the reduction of silver ions**.**

from the reduction of Ag ions by the polyols, which themselves are oxidized to unsaturated carbonyl groups with a peak at 1663 cm^{-1} . Also The FT-IR spectrum of the green AgNPs from *Nostoc* sp. showed strong absorption peaks at 3788, 3430, 2924,2857, 1628.63,1548,1317.48, 669 and 455 cm^{-1}**.**The bands seen at 3788, 2924 were assigned to the stretching vibrations of primary and secondary amines respectively. The corresponding bending vibrations were seen at 1628.63 and 1548cm^{-1}. The band observed at 1317.48 can be assigned to C-N stretching vibration of amines (Figure 10).

In Agreement of this study, Mubarak Ali (2011) confirmed the presence of a protein shell which is responsible for the nanoparticles biosynthesis. The presence of protein as the stabilizing agent surrounded the silver nanoparticles. The protein molecule made up of different functional group in amino acid sequences such as amino, carboxyl, sulfate groups present in the cyanobacterial protein favor the formation of extremely small-sized silver nanoparticulates with narrow particle size distribution and hydroxyl and sulfonic groups are beneficial to synthesis of silver nanoparticle with a slightly larger particle size in a weak reducing environment. The presence of silver nanoparticles inside the cytoplasm, Ag+ is presumably reduced to Ag_o Because AgNO3, a toxic reagent, was used in metabolic processes, it ultimately killed the cells. During the death of cyanobacteria, nanoparticles of silver produced inside the cells were release through the cell membrane into solution, as indicated by the precipitation of silver nanoparticles around the cells. The dead cyanobacteria also released organics (protein, and other biochemical) that caused further precipitation of silver from solution outside the cells. The protein molecules act as reducing agent for silver nanoparticles. The protein molecule made up of different functional group in amino acid sequences such as amino, carboxyl, sulfate groups present in the cyanobacterial protein favor the formation of extremely small-sized silver nanoparticulates with narrow particle size distribution and hydroxyl and sulfonic groups are beneficial to synthesis of silver nanoparticle with a slightly larger particle size in a weak reducing environment. The silver ions were reduced in the presence of nitrate reductase, leading to the formation of a stable silver hydrosol 10-25 nm in diameter and stabilized by the capping peptide (Mukherjee *et al.*, 2001).

3-3-Dynamic Light Scattering

Dynamic light scattering (also known as photon correlation spectroscopy or quasi-elastic light scattering) is a technique in physics that can be used to determine the size distribution profile of small particles in suspension or polymers in solution. When light hits small particles, the light scatters in all directions (Rayleigh scattering) as long as the particles are small compared to the wavelength (below 250 nm). If the light source is a laser, and thus is monochromatic and coherent, the scattering intensity fluctuates over time. The larger the particle, the slower the Brownian motion will be. DLS monitors the Brownian motion with light scattering. Smaller particles cause the intensity to fluctuate more rapidly than large particles. Small particles move more rapidly so correlation decreases more quickly (Berne and Pecora, 2000). Data in Table (1) indicate the size distribution or population of silver nanoparticle synthesized by S. platensis using Dynamic Light Scattering Method (Malvern instrument). The radius of silver nanoparticle (AgNPs) is 29.36 nm for 20.9% of the sample.Data in Table (2) indicate the size distribution of

Table (1): Size Distribution of AgNPs measured by DlS technique for *S.platensis*

Mean Number (%)	Size of AgNPs(r.nm)
1	18.92
6.6	21.91
15.8	25.37
20.9	29.39*
19.1	34.03
14.2	39.41
9.2	45.64
5.5	52.85
3.2	61.21
1.8	70.89
1.1	82.09
0.7	95.07
0.4	110.1
0.2	127.5
0.1	147.7

(*) Mainly the Radius of silver nanoparticle around 29.39nm

Table (2): Size Distribution of AgNPs measured by DLS technique for *Nostoc sp.*

Mean Number (%)	Size of AgNPs(r.nm)
5.2	12.18
18.1	14.105
26.5	16.335*
23.1	18.92
14.5	21.91
7.2	25.375
3	29.385
1.1	34.03
0.4	39.41
0.2	45.64
0.1	52.85
0.1	61.2
0.1	70.9
0.1	82.1

(*) Mainly the Radius of silver nanoparticle around 16.335nm

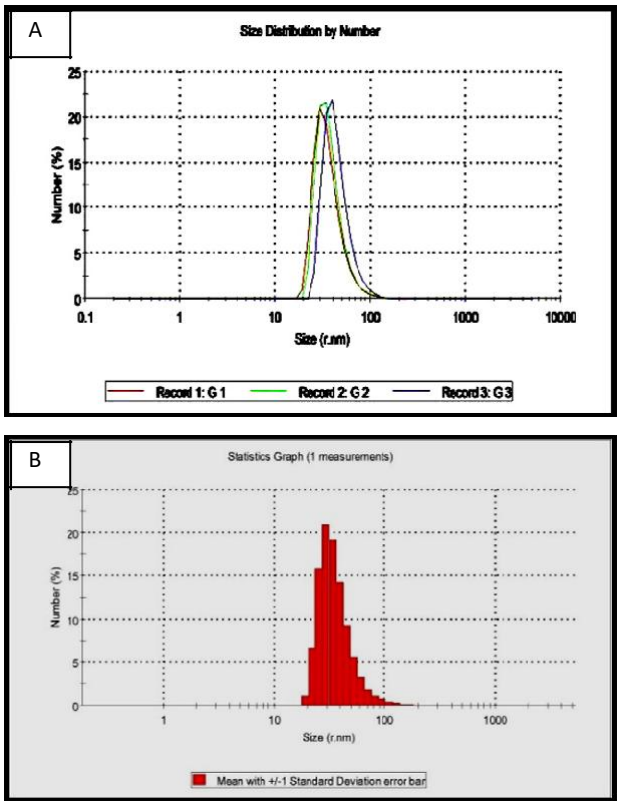

Figure 11: a) Size distribution curve of AgNPS by DLS method repeated three times for the sample b) Static graph for size distribution of AgNPs by DLS method.

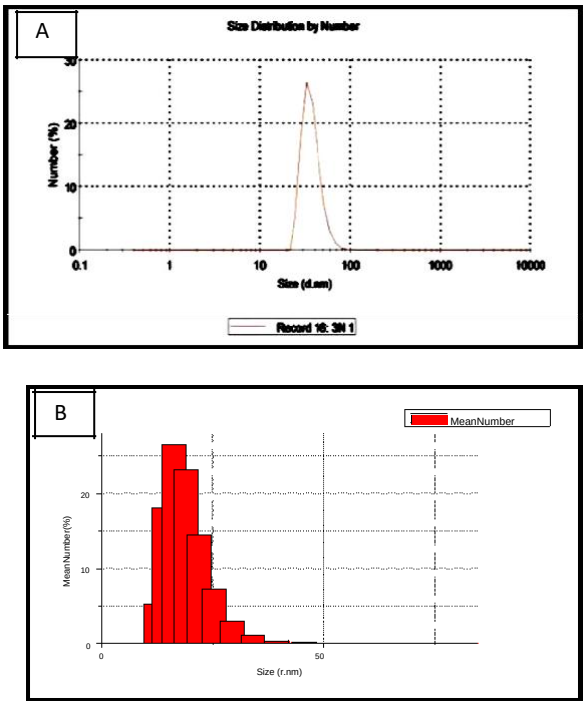

Figure (12): a) Size distribution curve of AgNPs by DLS method for the sample. b) Static graph for size distribution of AgNPs by DLS method

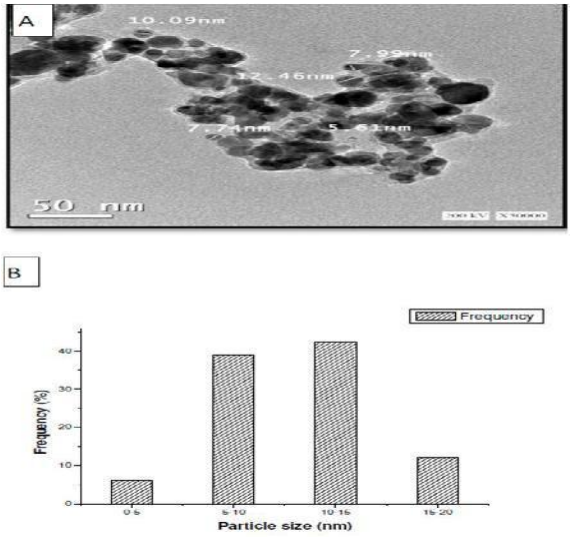

Figure 13 A: Particle size distribution histogram of AgNPs determined from TEM image.
B: TEM image of developed AgNPs by *S. platensis*

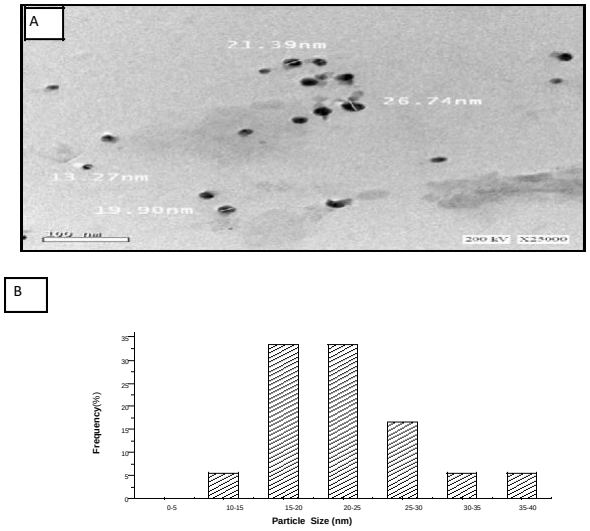

Figure (14) A: Particle size distribution histogram of AgNPs determined from TEM image.

B: TEM image of developed AgNPs by *Nostoc sp.*

silver nanoparticle synthesized by *Nostoc* sp. the radius of silver nanoparticle (AgNPs) is 16.335 nm for 26.5% of the sample. Measurements uncertainties were calculated as the standard deviation also the curve of size distribution demonstrate that diameter of AgNPs synthesized by *S. platensis* and *Nostoc* sp. is around 58.7 and 32.66 nm subsequently as in Figure (11and 12). Also (Rejeeth *et al.,* 2014) explained that a small number of large particles can contribute in the increase of DLS size resulting in recording average size 50nm of AgNPs.

Measurements uncertainties were calculated as the standard deviation also the curve of size distribution demonstrate that diameter of AgNPs synthesized by *S. platensis* and *Nostoc* sp. is around 58.7 and 32.66 nm

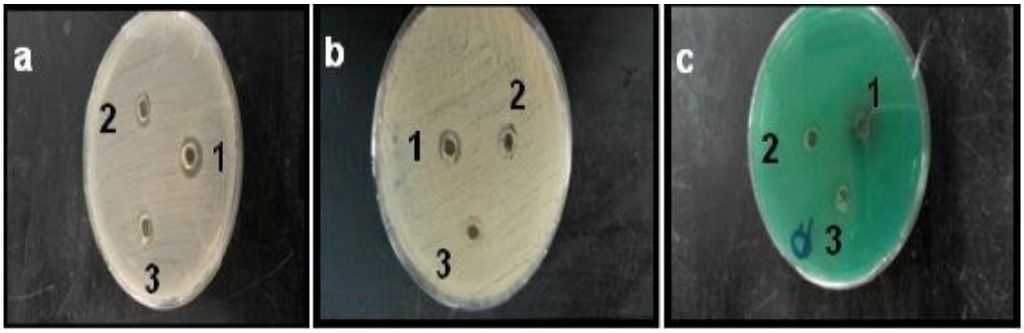

Figure 15: Antibacterial activity of AgNPs synthesized by *Spirulina platensis* and *Nostoc sp.* toward (a) *Staphylococcus aureus* (b) *Staphylococcus epidedymis* (c) *Pseudomonas aeruginosa*. Where: (1) 40µl AgNPs synthesized by *Nostoc* sp. (2) 40µl AgNPs synthesized by *Spirulina platensis* (3) 40µl of 1mM $AgNO_3$ as Control.

Table 3. Antibacterial activity of AgNPs synthesized by *Spirulina platensis* and *Nostoc* sp. compared to silver

	Zone of inhibition (mm)					
	40µl	***S. aureus***	**** S. epidedymis***	***P. aeruginosa***	****K. pneumonia***	****E. coli***
s Control		4	4	4	5	5
f 3	60 µl	4	9±1	4	6	5.5
	80 µl	6	10±1	6	7	6.5±2.1
	100 µl	11	11	11	10.6±0.57	6.5±2.1
AgNPs synthesized by *Spirulina platensis*	40µl	12.6±1.5	11±1	4	16±1	11.3±1.1
	60 µl	14±1	12±1	8	12.6±1.5	13.6±0.5
	80 µl	16±1	13±1	12.6±1.5	13.6±0.57	16±1
	100 µl	17.3±05	15±1	13.6±0.5	14.6±0.5	17.3±0.5
AgNPs synthesized *Nostoc*by sp.	40µl	13.6±0.5	11.6±0.57	4	15.6±0.57	16
	60 µl	15	13.5±0.7	7	17.6±1.52±	18.5±0.7
	80 µl	15.5±0.7	15	8	17.6±0.57	19.5±0.7
	100 µl	17±1	15.5±0.7	9	18.6±0.57	20

(*)Bacteria: *Staphylococcus aureus = S. aureus , Staphylococcus epidedymis = S. epidedymis, Pseudomonas aeruginosa = P. aeruginosa , Klebsiella pneumonia = K. pneumonia* and *Escherichia coli = E. coli*

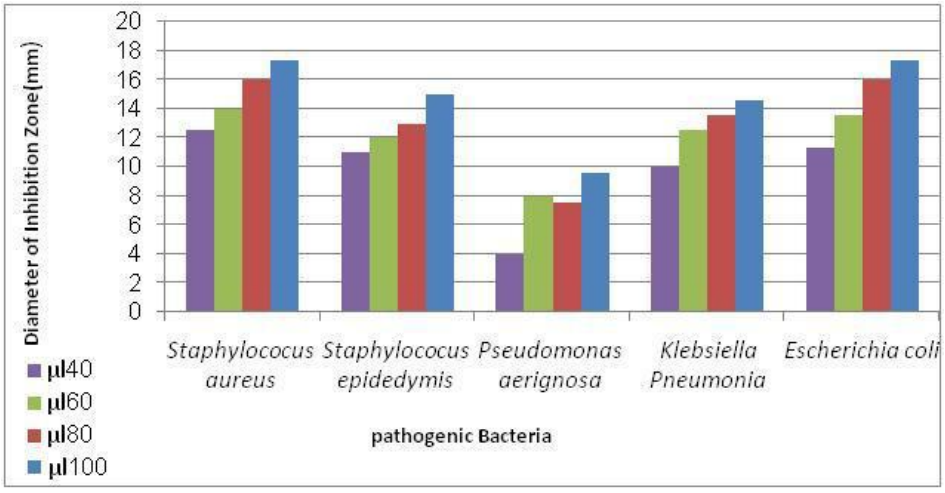

Figure 16: Diameter of zone of inhibition by AgNPs synthesized by *S. platensis* against pathogenic gram positive and gram negative bacteria.

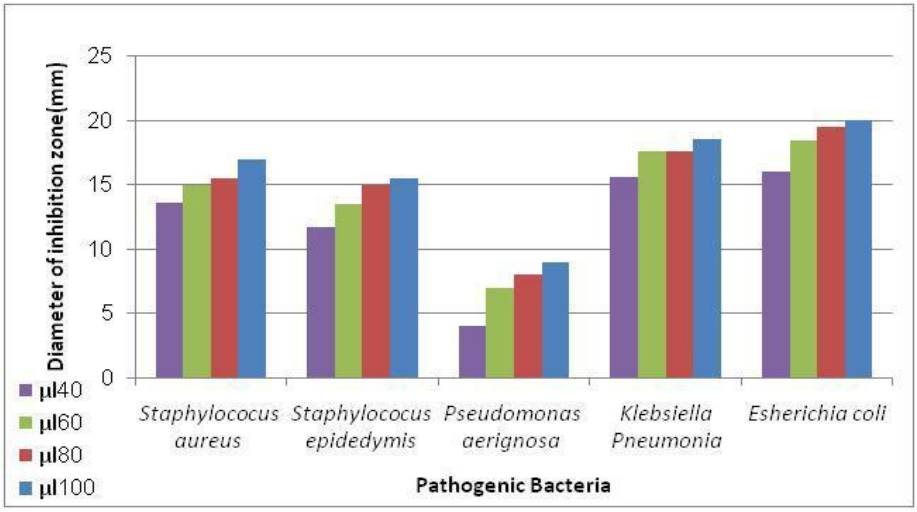

Figure 17: Diameter of zone of inhibition by AgNPs synthesized by *Nostoc* sp. against pathogenic gram positive and gram negative bacteria.

subsequently as in Figure (11 and 12). Also (Rejeeth *et al.,* 2014) explained that a small number of large particles can contribute in the increase of DLS size resulting in recording average size 50nm of AgNPs synthesized by *S. platensis*. Measurements parameters were as follows: dispersant RI = 1,330; viscosity = 0, 8872 cP; temperature= 25^{O}C.

3-4- Transmission Electron Microscopy (TEM)

TEM (Transmission Electron Microscope) is powerful method to determine the size of nanoparticles. From this figure13a, it is clear that the frequency peak comes at approximately 10–15 nm and particles, whose size ranges from 5-15 nm, account for about 81% of total particles observed**.** TEM analysis showed that most particles synthesized by *S. platensis* had a size of ~11.5 nm with spherical shape as in figure 13.

Figure 14a reveals spherical silver nanoparticles synthesized by *Nostoc* sp. with a size ~ 20.3 nm. Figure 14b shows 2 peaks come at approximately15-25nm that account for about 66.66% of total particles observed**.**

3-5- Antibacterial activity of AgNPs synthesized by Cyanobacteria:

The antibacterial activity of biosynthesized silver nanoparticles was performed against both gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidedymis* and gram negative bacteria (*Klebsiella pneumonia, Escherichia. coli and Pseudomonas aeruginosa*) by well diffusion method. The AgNPs synthesized from the isolate showed excellent antibacterial activity against all tested bacterial strains at volume of 40 µL/well except for *Pseudomonas aeruginosa*. After 24 hours of incubation, zone of inhibition of 13.6 mm, 11.6 mm, 15 mm 16 mm and 4mm were observed for the AgNPs synthesized by *Nostoc* sp. against *Staphylococcus aureus, Staphylococcus epidedymis*, *Klebsiella pneumonia, Escherichia coli and Pseudomonas aeruginosa*. While zone of inhibition of the AgNPs synthesized by *Spirulina platensis*12.6mm, 11mm, 10mm, 11mm and 4mm against *Staphylococcus aureus, Staphylococcus epidedymis, Klebsiella pneumonia, E.coli* and *Pseudomonas aerugniosa* as in figure (15).

By increasing the volume of AgNPs synthesized by Nostoc sp. or *Spirulina platensis* to 100 µL/well the zone of inhibition increases for both gram positive and negative bacteria as in table (3). The week inhibition zone of AgNPs synthesized by *Spirulina platensis* compared to AgNPs synthesized by *Nostoc* sp. may be due the aggregation of silver nanoparticles synthesized by *Spirulina platensis* as in figure (16, 17). .However, Silver Nanoparticles has showed antibacterial activities more than silver. In agreement of this study, **Theivasanthi and Alagar (2011)** reported that Silver Nanoparticles have showed antibacterial activities more than silver.

CONCLUSION

This work indicates that live algal biomass may be a valuable, cost effective means for the fabrication of SNPs, indicating their potential in the future production of other valuable nanostructures in the emerging field of nanobiotechnology. Finally, an environmentally friendly method using *Spirulina platensis* and *Nostoc* sp. was proposed to synthesize SNPs. The AgNPs showed potential antibacterial activity against human pathogens like S. *aureus*, *S. epidedymis, K. pneumonia* and *E. coli.*

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