



COMPARATIVE STUDY ON BIOSYNTHESIS OF VALUABLE ANTIMICROBIAL AND ANTITUMOR NANO-SILVER USING FRESH WATER GREEN AND BLUE-GREEN MICROALGAE

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ABSTRACT

Two levels Plackett-Burman experimental design was employed to screen the main factors that significantly affect the synthesis of bioactive silver nanoparticles. *Scenedesmus obliquus* ethanol extract, *Scenedesmus obliquus* dried algal powder and *Spirulina platensis* aqueous extract were the most favorable reducing and capping agents. Also the high pH value (10), rpm (150), high concentration of AgNO₃ (4 mM) and dark conditions had the highest significant influence on biosynthesis of active nanoparticles. Characterizations of the formed AgNPs were approved by UV-Visible Spectroscopy, Transmission Electron Microscopy and Fourier Transform Infrared spectroscopy. The nanoparticles were varied in sizes and shape that ranged between 2.83 and 27.17nm, depending on the organism used and the method of biosynthesis. Valuable inhibitory effects were showed by all bio-synthesized nanoparticles against four tested Gram-positive and Gram-negative bacteria, except that formed by *Spirulina platensis* powder. Moreover, the nanoparticles exhibited wide range of antifungal activities. AgNPs prepared from ethanol extract of *Spirulina platensis* was the most active against HepG2 and MCF-7 cell lines, with IC₅₀ values 62.1 and 56.2 ug/mL, respectively. The obtained results, indicated the advantages of AgNPs from microalgae and possible industrial applications as a source of antifungal, antibacterial, and antitumor formulation.

Keywords: Plackett-Burman design, Biosynthesis, AgNPs, Microalgae, Antimicrobial, Antitumor

INTRODUCTION

Modern sciences deal with nanotechnology as one of the most important and active area of research. According to (Dahl et al., 2007) silver nanoparticles in range from 1 to 100 nm, usually have interesting with valuable applications in nanotechnology. Synthesis of silver nano-particles had received attentiveness due to their outstanding properties and different applications in water treatment (Con and Loan, 2011). Also silver nano-particles showed possible applications in catalysis as mentioned by (Paul et al., 2014) and plasmonics (Khlebtsov and Dykman, 2010), optoelectronics (Muruganandam et al., 2014), pharmaceutical applications (Ravichandran, 2009) and biological sensor (Venkatesan and Santhanalakshmi, 2014). AgNPs with many advantages of, compatibility and eco-friendliness for different pharmaceutical and medical applications can be synthesized from different biological materials like plant extract according to (Sinha and Paul, 2014), also (Sinha et al., 2015) and (Kannan et al., 2013) respectively indicated that fresh water algae and algae from marine sources can be used for the synthesis of AgNPs, without addition of any harmful chemicals for the synthesis process. Fresh water microalgae extracts exposed high amounts of terpenoids, tannins, saponins, steroid, carbohydrate and proteins that can serve as effective metal reducing and capping agent's, to supply a robust coating on the metal nanoparticles in a single step (Sinha et al., 2015). The resistance of many microbial diseases to synthetic drugs gradually increased, therefore an urgent need to develop new antitumor, antifungal and antibacterial agents. Biosynthesis of nanoparticles using algae from marine sources has been extensively investigated for their activities as an antimicrobial agent (Kumar et al., 2013; Prasad et al., 2013). On the other hand, reports on fresh water algae are lacking. Therefore, the main object of our research is the application of a statistical method to evaluate main factors affecting the green-synthesis of biologically active AgNPs from *Scenedesmus obliquus* and *Spirulina platensis* fresh water microalgae and to investigate the effect of different methods of extraction on the size, shape and biological activity of the biosynthesized nanoparticles.

MATERIALS AND METHODS

Algal materials used for biosynthesis of AgNPs

Green alga (*Scenedesmus obliquus*) and blue green alga (*Spirulina platensis*) were obtained from the Algae Development Unit of National Research Center, Cairo, Egypt. Algal materials were prepared according to (Sinha et al., 2015). Algae washed using distilled water and shade-dried for 5 days also oven dried at 60° C until constant weight was obtained. Then ground into fine powder using electric mixer and stored at 4° C.

Plackett-Burman design (PBD)

Plackett-Burman experimental design (Abd El Aty et al., 2016& 2018) was used to evaluate the relative importance of various factors for biological synthesis of AgNPs. Eleven variables (*S. obliquus* ethanol extract, *S. obliquus* aqueous extract, *S. obliquus* dried algal powder, *S. platensis* dried algal powder, *S. platensis* ethanol extract, *S. platensis* aqueous extract, Temperature, AgNO₃ concentration, RPM, pH value, Incubation conditions light (1)/ dark (0) and Incubation period) were selected for the study, each variable was represented at two levels, high concentration (+1) and low concentration (-1) as shown in Table 1. Tested factors represented in twelve trials Plackett-Burman experimental design with the response (AgNPs biological activity against *E.coli*). All experiments were carried out in duplicate and the average activity was taken as the response. Plackett-Burman experimental design is based on the first order linear model (Plackett and Burman, 1946):

$$Y=B_0 + \sum BiXi \dots\dots\dots \text{Eq. (1)}$$

Y is the response (Inhibition zone diameter, IZD), B0 is the model intercept and Bi is the variables estimates. The effect of each variable was determined by the following equation,

$$E (Xi) = 2(\sum Mi+ - Mi -) \dots\dots\dots \text{Eq. (2)}$$

E (Xi) is the effect of the tested variable. Mi+ and Mi- represent IZD (mm) from the trials where the variable (Xi) measured was present at high and low concentrations, respectively and N is the number of trials in Eq. (2). Statistical analysis of PBD is performed by using Design-Expert® 8 software from StatEase, Inc.

Biosynthesis of silver nanoparticles

According to Plackett–Burman design the green synthesis of AgNPs was carried out using three different methods.

Biosynthesis of AgNPs by the algal powder

The reaction mixture contained 1 g of *S. obliquus* and *S. platensis* dry matter and 50 mL of 4mM AgNO₃ aqueous solution, and incubated at 30°C for 24h with the continuous stirring condition. The change in color was envisaged as the evidence of bioreduction (Meena et al., 2007; Abdel-Raouf et al., 2018).

Biosynthesis of AgNPs by the algal aqueous extract

About 5 g of dried *S. obliquus* and *S. platensis* separately with 50 mL of sterilized distilled water were boiled for 15 min at 60° C. and filtered with Whatman no. 1 filter paper. Equal volumes of the filtrate and 4 mM AgNO₃ aqueous solution were mixed in an Erlenmeyer flask and incubated at 30°C for 24h. (Sinha et al., 2015).

Biosynthesis of AgNPs by the algal ethanol extract

The extraction was achieved using the solvent ethanol according to Abdel-Raouf et al. (2018) with some modifications. About 5g of the dried finally powdered algal biomass was added to 100mL solvent in a 250 mL Erlenmeyer flask. The extraction was executed on a sonication water bath for 30 min. After them, all algal extracts were filtered and the solvents were evaporated at 50°C. 50 mL of 4mM aqueous AgNO₃ solution were added to dried sample and kept at 30°C for 24h.

Characterization studies of biosynthesized silver nanoparticles

The formation of AgNPs was confirmed by different analysis.

Ultraviolet–visible spectral analysis

The absorption spectrum of aliquots (2 mL) prepared by different method from both algae was scanned using UV-Visible spectrophotometer in the range of 200–800 nm. The sharp peak given by UV visible spectrum has indicated the formation of AgNPs at the absorption range 400 - 450 nm (Abd El Aty and Ammar, 2016).

Table 1 The Plackett–Burman design with eleven variables.

Variable code	Variable	Level	
		Low(-1)	High(+1)
A	So.EE(mL)	0	5
B	So.AE (mL)	0	5
C	So.DAP (g)	0	1
D	Sp.DAP (g)	0	1
E	Sp.EE(mL)	0	5
F	Sp.AE(mL)	0	5
G	Temperature (°C)	28	35
H	AgNO ₃ concentration (mM)	1	4
J	pH value	5	10
K	Incubation conditions light (1)/dark(0)	0	1
L	RPM	0	150

So.EE, *S. obliquus* ethanol extract; So.AE, *S. obliquus* aqueous extract; So.DAP, *S. obliquus* dried algal powder. Sp.DAP, *S. platensis* dried algal powder; Sp.EE, *S. platensis* ethanol extract; Sp.AE, *S. platensis* aqueous extract. RPM, round /min.

Transmission Electron Microscopy (TEM)

The size and shape of nanoparticles were characterized by TEM (JEOL-2100) according to Shaheen and Abd El Aty, (2018).

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transform infrared (FTIR) has been used to identify the probable biomolecules responsible for reduction, capping and effective stabilization of the nanoparticles using (FTIR-8300, Shimadzu, Japan), at a resolution of 2 cm over a

wave number ranging from 400 to 4000 -cm. finally the characteristic peaks were recorded.

Application studies of biosynthesized silver nanoparticles. Screening of antibacterial activity

The biosynthesized AgNps were screened *in-vitro* against different pathogenic strains of Gram-positive and Gram-negative bacteria by the agar well diffusion technique (Shaheen and Abd El Aty, 2018). 200 µl of six nanoparticles colloidal solutions were evaluated against Gram-positive (*Staphylococcus aureus* ATCC29213 and *Bacillus subtilis* ATCC6633) and Gram-negative bacteria (*Salmonella enterica* ATCC25566 and *Escherichia coli* ATCC25922). Wells of about 10 mm in diameter were made in the solidified agar media inoculated with pathogen and filled with the nanoparticle colloids, and then they were left over night at 4 °C to allow the release of nanoparticles into the medium. After the incubation for 24 h at 30 °C the inhibition zones were measured in (mm) as Mean ± SD.

Screening of antifungal activity

The pathogenic (*Candida albicans* ATCC10321, *Candida tropicalis* ATCC750) and (*Aspergillus niger* NRC53, *Fusarium solani* NRC15) were analyzed by well diffusion method. Approximately 10 mm diameter of wells was made on the inoculated (1× 10⁶ spores^{-ml}) potato dextrose agar (PDA) plate with the help of sterilized cork borer. 200 µl of synthesized particles were inoculated to the wells and left over night at 4 °C to allow the release of nanoparticles into the medium. The plates were incubated in incubator for 72 h at 28 °C and the zones of inhibition were discussed as mentioned before (Abdel Wahab et al., 2018).

Screening of cytotoxicity activity

Cell culture

HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma) were maintained in RPMI medium supplemented with 10% fetal bovine serum and incubated at 37 °C in 5 %CO₂ and 95% humidity. Cells were sub-cultured using trypsin 0.15 %. All cell lines were purchased from Vacsera (Giza, Egypt) (Abd El Aty et al., 2020).

Cell viability assay

After 24 h of seeding 10000 cells per well in case of HepG2 and MCF-7 cell lines (in 96 well plates), the medium was changed to complete medium containing a final concentration of the extracts of 100 µg/mL in triplicates. The cells were treated for 48 h. 100 µg/mL doxorubicin was used as positive control and 0.5 % DMSO was used as negative control. Cell viability was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay as described by Mosmann et al. (1983). The equation used for calculation of percentage cytotoxicity: (1- (av(x) / (av(NC)))) * 100

Where Av: average, X: absorbance of sample well measured at 595 nm with reference 690 nm, NC: absorbance of negative control measured at 595 nm with reference 690.

Determination of cytotoxicity activity IC₅₀ values

In case of most active nano-silver possessing high cytotoxicity on different cancer cell lines, different concentrations were prepared dose response study. The results were used to calculate the IC₅₀ values of each AgNps using probit analysis and utilizing the SPSS computer program (SPSS for windows, statistical analysis software package / version 9 / 1989 SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Plackett–Burman design (PBD) for biosynthesis of silver nanoparticles

The experimental design Plackett–Burman was applied to determine the most effective variables for obtaining biologically active silver nanoparticles. The response (R1) measured as inhibition zone diameter (IZD) in mm against the pathogenic Gram negative bacteria *E.coli*. Results shown in Figure 1 revealed that the variables (Sp.AE, AgNO₃ concentration, So.EE, pH value and So.DAP) were significantly influence the synthesis of biologically active AgNPs. Results showed variations in the inhibition zone diameter (IZD) ranging from 11 to 27 mm as shown in Table 2. This variation in the biological activity (IZD) of AgNPs emphasizes the effect of different variables on the size, shape and biological activity of nanoparticles. Analysis of ANOVA explained that the Model F-value (59.02) is significant. The "Pred R-Squared" of 0.8972 is in reasonable agreement with the "Adj R-Squared" of 0.9597 (Table 3).

The first order model equation developed by PB design showed the dependence of nanosilver activity on the studied variables:

$$R1 \text{ (IZD, mm)} = +12.06 + 3.11 * A + 1.06 * C - 2.38 * D - 3.57 * E + 7.38 * F - 2.02 * G$$

+5.47*H +2.72*J -2.41*K..... Eq. (3)
 Out of PB results we found that *S. obliquus* ethanol extract (So.EE) , *S. obliquus* dried algal powder (So.DAP) and *S. platensis* aqueous extract (Sp.AE) were the most favorable reducing agents. Also the high pH value (10), rpm (150), high concentration of AgNO₃ (4 mM) and dark conditions had the highest significant influence on biosynthesis of active nanoparticles, as shown in trial 3 **Table 2**. According to results obtained from PB experiment different methods of biosynthesis should be applied to obtain the most active nanosilver. Results showed that, when the algal powder and extracts were mixed with AgNO₃ solution and incubated in dark the colour was turned into pale to dark brown colour which indicates the formation of silver nanoparticles, with intensity increasing during the period of incubation (**Figures 2&3**). This colour difference was due to the reduction of silver ions (**Swamy et al., 2014**). Metallic nanoparticles scatter and absorb light at certain wavelengths due to a phenomena known as surface plasmon resonances (**Noguez et al., 2007**).

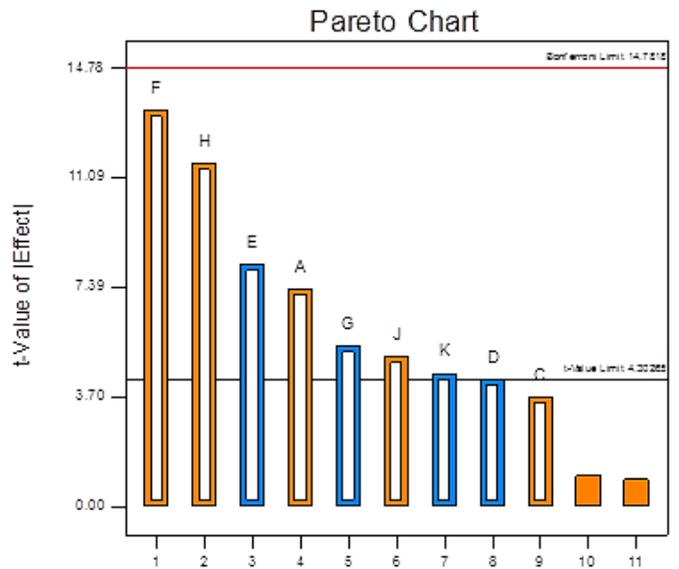


Figure 1 Pareto chart of eleven-factor effects on biological synthesis of AgNPs and its biological activity (IZD).

Table 2 Plackett-Burman experimental design with the response (Inhibition zone diameter) IZD- mm.

Trials	Reducing source				Metal and Reaction conditions							Biological activity of AgNPs against <i>E.coli</i> (IZD- mm)
	Factor1 A: So.EE	Factor 2 B: So.AE	Factor 3 C: So.DAP	Factor 4 D: Sp.DAP	Factor 5 E: Sp.EE	Factor 6 F: Sp.AE	Factor7 G: Temp.	Factor 8 H: AgNO ₃	Factor 9 J: pH value	Factor10 K: Dark/light	Factor11 L: RPM	
1	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	15±0.211
2	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	11±0.497
3	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	27±2.486
4	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	24±1.119
5	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	14±3.605
6	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	16±0.746
7	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	15±0.052
8	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	13±7.086
9	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	19±1.367
10	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	17±1.119
11	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	13±1.492
12	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	21±1.740

+1 and -1 explained the high and low levels of variables according to Table (1). Values = Mean ±SD.

Table 3 Plackett-Burman design analysis.

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob>F
Model	2026.88	9	225.32	59.02	0.0165 significant
A- So.EE	201.55	1	202.54	52.96	0.0178
C- So.DAP	49.84	1	49.84	12.54	0.0665
D- Sp.DAP	67.65	1	69.64	16.29	0.0505
E- Sp.EE	250.17	1	251.17	65.90	0.0145
F- Sp.AE	669.50	1	670.51	177.60	0.0055
G- Temperature	108.81	1	108.81	28.27	0.0324
H- AgNO ₃	502.11	1	503.11	133.01	0.0073
J- pH value	94.77	1	96.77	24.52	0.0369
K- Incubation conditions	75.50	1	75.50	19.11	0.0462
Residual	7.52	2	2.95		
Cor Total	2035.49	11			

Std. Dev.= 1.96 , Mean= 14.06, R-Squared= 0.9863, Adj R-Squared=0.9597, Pred R-Squared= 0.8972, (factors L- Incubation period & B- So.AE were removed where , Prob > |t|=0.1000 and 0.4720, respectively)

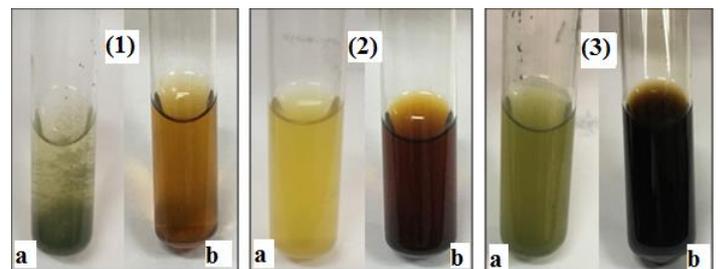


Figure 2 Biosynthesis of silver nanoparticles by *Scenedesmus obliquus* with different methods, (1) Dried algal powder, (2) Aqueous extract, (3) Ethanol extract, before (a) and after (b) exposure to silver nitrate solution for 24 h.

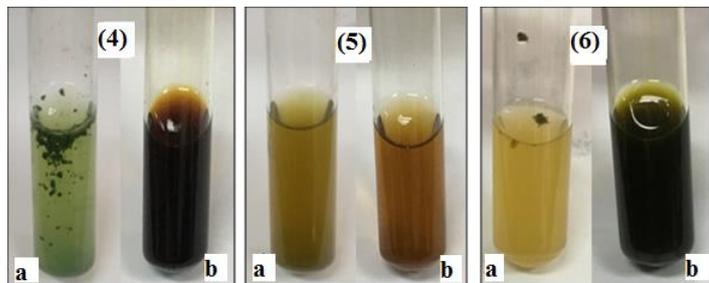


Figure 3 Biosynthesis of silver nanoparticles by *Spirulina platensis* with different methods, (4) Dried algal powder, (5) Aqueous extract, (6) Ethanol extract, before (a) and after (b) exposure to silver nitrate solution for 24 h.

Characterization of silver nanoparticles

UV-Visible Spectroscopy

Formation of colloidal silver nanoparticles can be followed by changes of UV-Vis absorption. The UV-vis absorption spectrum of *S. obliquus* powder and extracts showed a definite peak determined at 421 and 427 nm for aqueous and ethanol extracts respectively, indicating the formation of AgNPs. Similar results were observed by **Sinha et al. (2015)** who revealed absorption peak of AgNPs at 445nm using aqueous extract of green alga *Pithophora oedogonia*. Where a weak peak observed at 398nm with algal powder showed that not all metals have been reduced **Figure 4**. Synthesized AgNPs from *S. platensis* powder showed absorbance peak at 362 and abroad beak from 384 to 499 nm with algal aqueous extract, but it showed a sharp distinct beak at 418 nm with the ethanol extract **Figure 5**. According to **Ravindra and Rajasab, (2014)** the obtained peaks emphasized a surface plasmon resonance (SPR) that has already been formed for different metal nanoparticles with size in range from 2 to 100 nm.

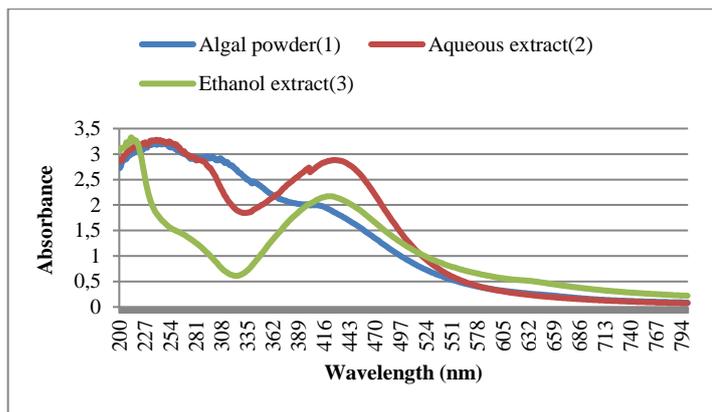


Figure 4 UV absorbance of AgNPs biosynthesized by *Scenedesmus obliquus* powder (1), aqueous extract(2) and ethanol extract(3).

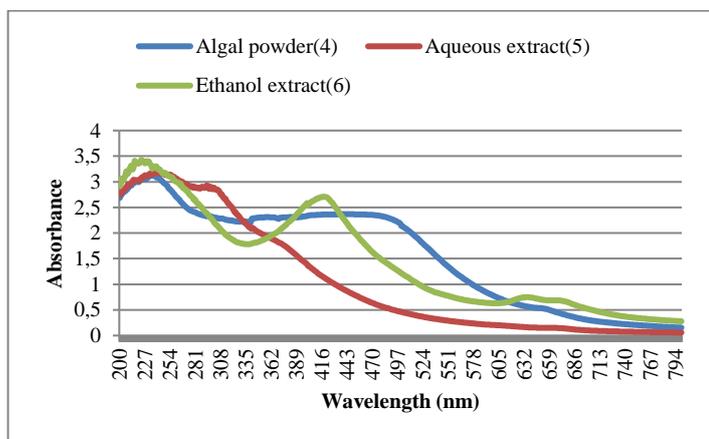


Figure 5 UV absorbance of AgNPs biosynthesized by *Spirulina platensis* powder (4), aqueous extract (5) and ethanol extract (6).

Transmission electron microscopy (TEM) of AgNPs

Transmission electron microscopy of AgNPs synthesized by *S. obliquus* and *S. platensis* provided information on morphology and size of the AgNPs (**Patel et al., 2015**). TEM micrograph showed that the shapes and sizes varied significantly

among the species used and also the technique of biosynthesis (**Figures 6&7**). TEM images of AgNPs synthesized by powder, aqueous and ethanol extracts of *S. obliquus* were showed in **Figure 6 a, b, c**. Results have revealed that the particles are well dispersed without aggregation and have a spherical shape in three methods. Where, the particle size was varied from 2.83 to 22.66 nm with powder, in ranges from 7.75 to 18.05 nm with aqueous extract and AgNPs biosynthesized from ethanol extract were all shown to be smaller ranging from 3.67 to 9.52 nm diameter.

Results in **Figure 7a, b, c** showed that the shapes of the particles included spherical, irregular and some aggregations. The spherical shape was predominant in the case of *S. platensis* ethanol extract with average sizes of the particles from 3.68 to 7.32 nm, similar results also were discussed by **Patel et al. (2015)** who reported the formation of the smallest particles of 13nm diameter in the presence of C-phycoyanin isolated from *Spirulina*. Some irregular clusters of particles were formed by *S. platensis* powder, these results agree with nanoparticles formed by *Synechocystis* sp. and *Anabaena* sp. (**Morones et al., 2005**). TEM measurements of the AgNPs biosynthesized from *S. platensis* aqueous extract showed well dispersed nanoparticles with average size from 13.74 to 27.17 nm diameter with appearance of little irregular forms.

These results indicated that the powder, aqueous and ethanol extracts of both algae can change the size and shape of the silver nanoparticles, which indicated the effect of the bioactive constituents of the studied algae as reducing and controlling agents (**Alenazi, 2013**).

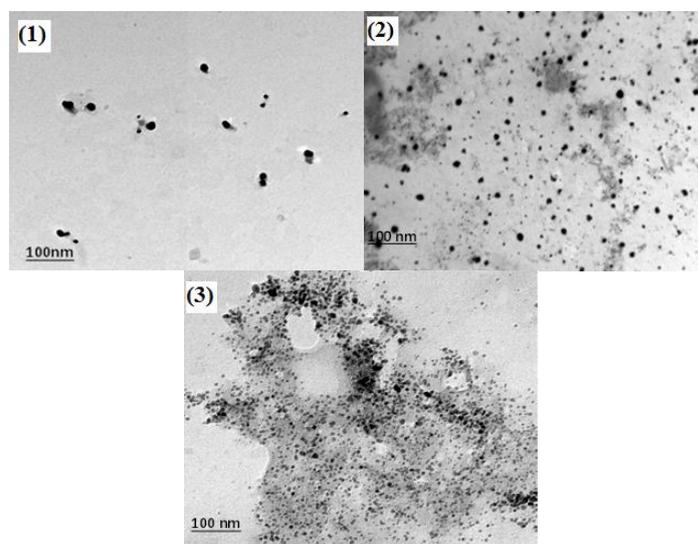


Figure 6 TEM of AgNPs biosynthesized by *Scenedesmus obliquus* powder (1), aqueous extract (2) and ethanol extract (3).

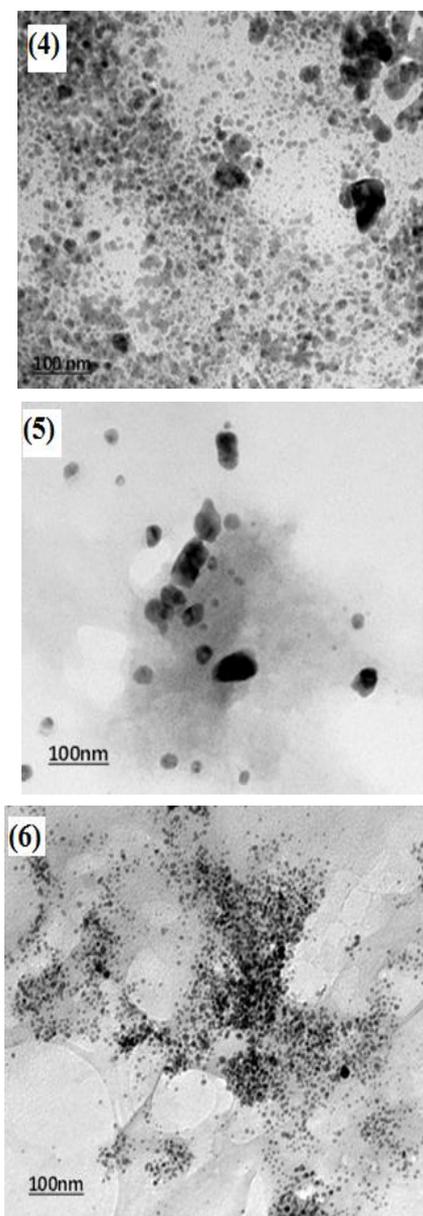


Figure 7 TEM of AgNPs biosynthesized by *Spirulina platensis* powder (4), aqueous extract (5) and ethanol extract(6).

FTIR analysis of AgNPs synthesized by *S. obliquus* and *S. platensis*

The functional groups which may be responsible for biosynthesis and nanoparticles stabilization, was detected by using Fourier Transforms Infrared Spectroscopy (FTIR). Results in **Figure 8a, b** showed bands in different regions of the spectrum. The certain common absorption band, at 3444 and 3436 cm^{-1} were characteristically vibrational (N-H) frequencies of amide I and amide II of protein. Vibrational peaks at 2117 and 2927 cm^{-1} were characteristically vibrational frequencies (O-H); hydroxyl groups (OH) are very abundant in polysaccharides which are mainly responsible for the metal reduction (Narayanan and Sakthivel, 2011).

Biological components are known to interact with the silver salt as well as the polysaccharides and proteinaceous matters were mediated their reduction to nano-particles (Bar et al., 2009).

Obtained result was in agreement with Sanghi and Verma (2009) who showed that, the common band at about 1639 and 1635 cm^{-1} corresponding to carbonyl vibrations in aldehydes, ketones and carboxylic acids, indicating the reduction of

the silver ions is coupled to the oxidation of the hydroxyl groups in ethanol algal extract. As mentioned by Kannan et al. (2013), the vibrational frequencies for and t (C-O-C) and t (C-O) were observed in the spectra of the extracts at (1430, 1427) and (1270, 1267) cm^{-1} . An alkyl halide can be expected at 532 and 524 cm^{-1} .

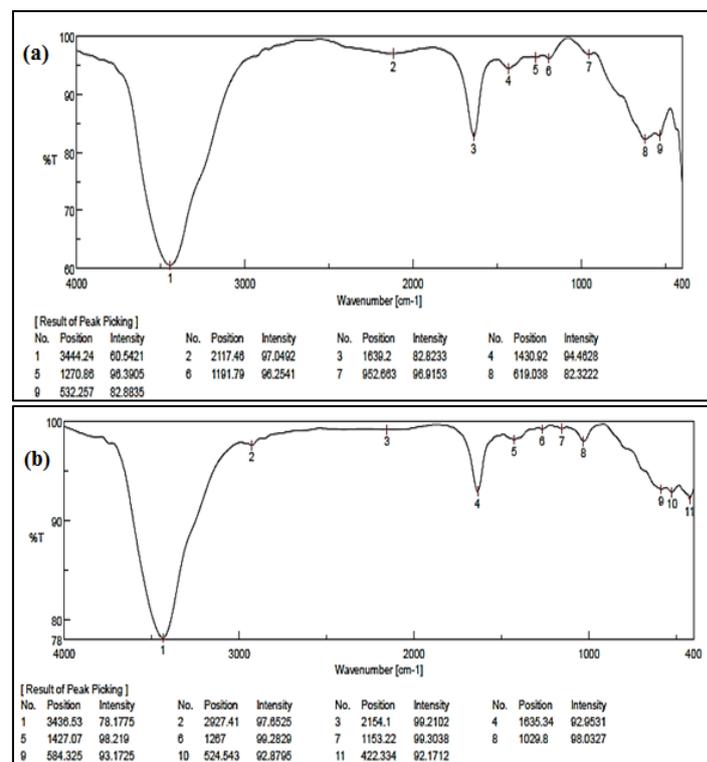


Figure 8 FTIR spectra of synthesized AgNPs using *Scenedesmus obliquus* (a) and *Spirulina platensis* (b) ranging from 4000–400 cm^{-1}

Analysis of antibacterial activity

The antibacterial activity of *S. obliquus* silver nanoparticles formed by different methods including; the algal powder (1), aqueous extract(2) and ethanol extract (3) was confirmed in all with different zones of inhibition (**Figure 9**). Results in **Table 4** indicated that the nanoparticles formed by method 1 and 3 were most effective against Gram-positive and Gram-negative bacteria (IZD from 20 to 26 mm) than that formed by method 2 (IZD ranging from 18 to 20mm). Silver nanoparticles biosynthesized by *S. obliquus* had low effects against Gram-negative bacteria. On the other hand, the silver nanoparticles biosynthesized by fresh water green alga *Pithophora oedogonia* (Mont.) killed the Gram-negative bacteria more rapidly than Gram-positive bacteria (Sinha et al., 2015).

Silver nanoparticles biosynthesized by *S. platensis* aqueous extract (5) and ethanol extract (6) showed inhibition zone against all studied Gram-positive and negative strains (17 - 25 mm), except that formed by algal powder (4) did not show any antibacterial activity against four studied bacterial strains. Also Patel et al. (2015) reported no antibacterial activity for nanoparticles synthesized by the cyanobacterium *Limnithrix* sp. 3721. As mentioned previously by Matsumura et al. (2003) who showed the inhibitory result of silver nanoparticles on bacteria is mainly due to the elevated affinity of silver towards sulfur and phosphorus-containing proteins on the bacterial cell membrane that affects the bacterial cell viability and inhibits the enzyme functions. Other studies of (Abd El Aty et al., 2020) and (Abd El Aty and zohair, 2020) prepared silver nanocomposite from fungal and bacterial sources with broad spectrum antimicrobial activity against Gram negative, Gram positive bacteria, pathogenic yeast and fungi.

Table 4 Antimicrobial activities of six (1-6) biosynthesized AgNPs.

AgNPs source	No. of samples	Inhibition zone diameter (IZD)* (mm)							
		Gram positive bacteria		Gram negative bacteria		Yeast		Fungi	
		<i>S. aureus</i> ATCC29213	<i>B. subtilis</i> ATCC6633	<i>E. coli</i> ATCC25922	<i>S. enterica</i> ATCC25566	<i>C. albicans</i> ATCC10321	<i>C. tropicalis</i> ATCC750	<i>A. niger</i> NRC53	<i>F. solani</i> NRC15
<i>S. obliquus</i>	1	24±1.41	26±0.70	20±0.09	22±0.70	26±2.41	22±0.71	23±1.41	18±0.70
	2	18±0.70	20±2.41	18±0.70	20±1.41	25±0.71	17±1.21	20±1.42	15±2.10
	3	25±0.71	25±0.71	20±0.70	22±0.71	24±1.41	21±0.70	21±3.10	16±1.42
<i>S. platensis</i>	4	00±00	00±00	00±00	00±00	22±2.10	15±0.72	17±0.70	15±0.71
	5	22±0.71	24±1.21	22±2.41	25±1.21	30±0.70	28±1.42	28±1.21	25±1.41
	6	17±2.10	20±1.41	17±0.71	18±2.41	25±0.70	25±0.70	20±2.10	17±1.42

*Inhibition Zone Diameter (IZD) expressed as Mean ± SD in (mm).

Analysis of antifungal activity

Antifungal activity of all AgNPs (1, 2, 3, 4, 5 and 6) synthesized by both algal strains was tested against two yeast strains and two filamentous fungi (Figure 10). Results in Table 4 showed that *S. obliquus* silver nanoparticles (1, 2, and 3) exhibited wide range of antifungal activities. Maximum zone of inhibition was found against *C. albicans* (24 - 26 mm) and followed by *A.niger* (20 - 23 mm). But they have moderate fungicidal activity against *F. solani*. Silver nanoparticles biosynthesized by *S. platensis* aqueous extract (5) and ethanol extract (6) showed antifungal effects better than that obtained from the algal powder (4) (Table 4). The well dispersed nanoparticles with average size ranging from 13.74 to 27.17 nm diameter formed by *S.platensis* aqueous extract (5) was the most effective against different fungal strains (25 - 30 mm). As mentioned previously by Dorau et al. (2004) who reported the antifungal potentialities mainly due to the inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane bound enzymes and lipids resulting in lyses of cell.

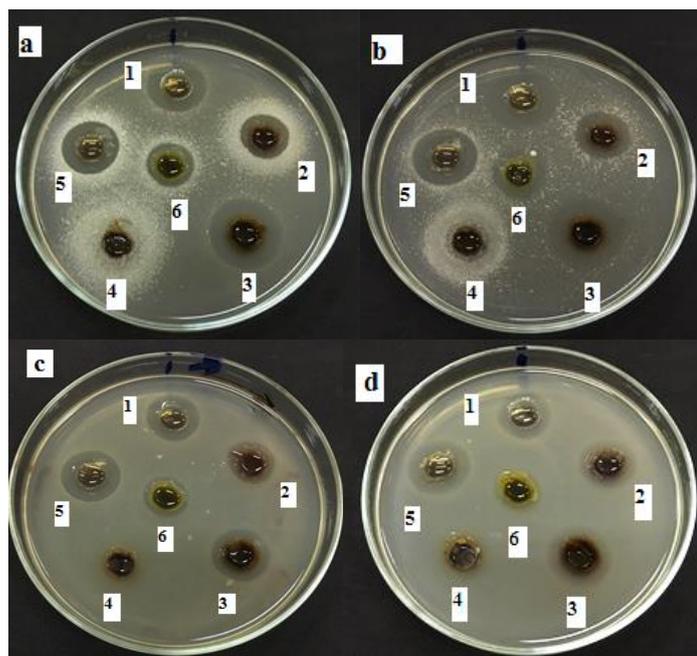


Figure 9 Antibacterial activity of *Scenedesmus obliquus* silver nanoparticles (1,2,3) and *Spirulina platensis* silver nanoparticles (4,5,6). Against Gram-positive *S. aureus* (a), *B. subtilis* (b) and Gram-negative *E. coli* (c), *S. enterica* (d).

Analysis of antitumor activity

Silver nanoparticles biosynthesized from green alga (*S. obliquus*) and blue green alga (*S. platensis*) were screened for their cytotoxic activities against Hepatocellular carcinoma cell line (HepG2) and breast cancer cell line (MCF-7) using the MTT assay (El-Menshawi et al., 2010). Initially, six different AgNPs were prepared using algal powder, aqueous and ethanol extracts. The results showed that the AgNPs prepared from ethanol extract of the blue green alga *S. platensis* was the most active, which possessed highly cytotoxic effect against HepG2 and MCF-7 cell lines (cytotoxicity= 81 % and 71% respectively) (Figure 11) and with IC₅₀ values 62.1 and 56.2 ug/mL respectively (Table 5). On the other hand, AgNPs biosynthesized by algal powder and aqueous extract showed weak cytotoxic effect in all two cell lines tested (Figure 11).

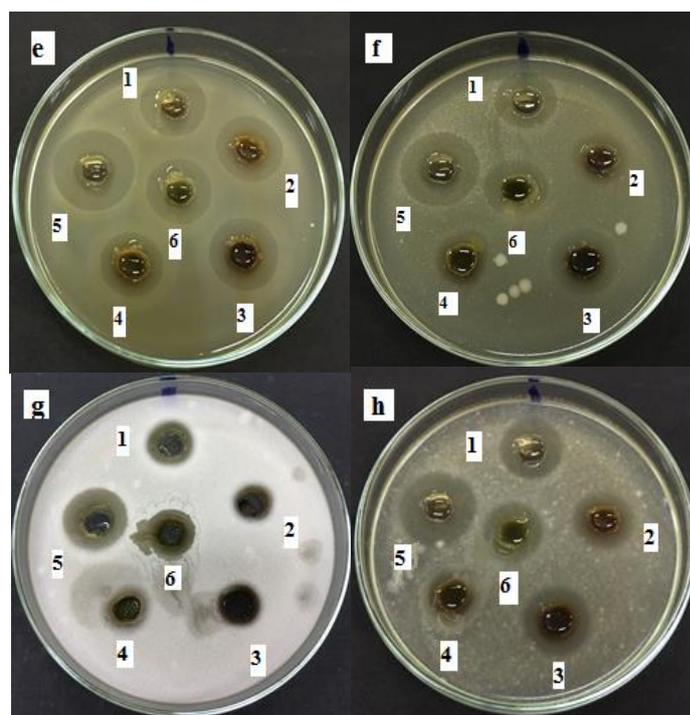


Figure 10 Antifungal activity of *Scenedesmus obliquus* silver nanoparticles (1,2,3) and *Spirulina platensis* silver nanoparticles (4,5,6). Against yeast *C. albicans* (e), *C. tropicalis* (f) and filamentous fungi *F. solani* (g), *A. niger* (h).

On the other hand, all *S.obliquus* silver nanoparticles prepared from (algal powder, aqueous and ethanol extracts) showed little or no cytotoxic effects on tested cancer cells. Our results indicated that, the biosynthesized AgNPs from the blue green alga *S. platensis* ethanol extract with spherical shape and average sizes in ranged from 3.68 to 7.32 nm, was a potential alternative therapeutic approach in certain types of cancer.

Table 5 IC₅₀ values of the most active biosynthesized AgNPs against two cancer cells

Entry	IC ₅₀ ug/ml	
	HepG2	MCF-7
AgNPs biosynthesized by <i>S. platensis</i> ethanol extract(6)	62.1±1.5	56.2±1.8

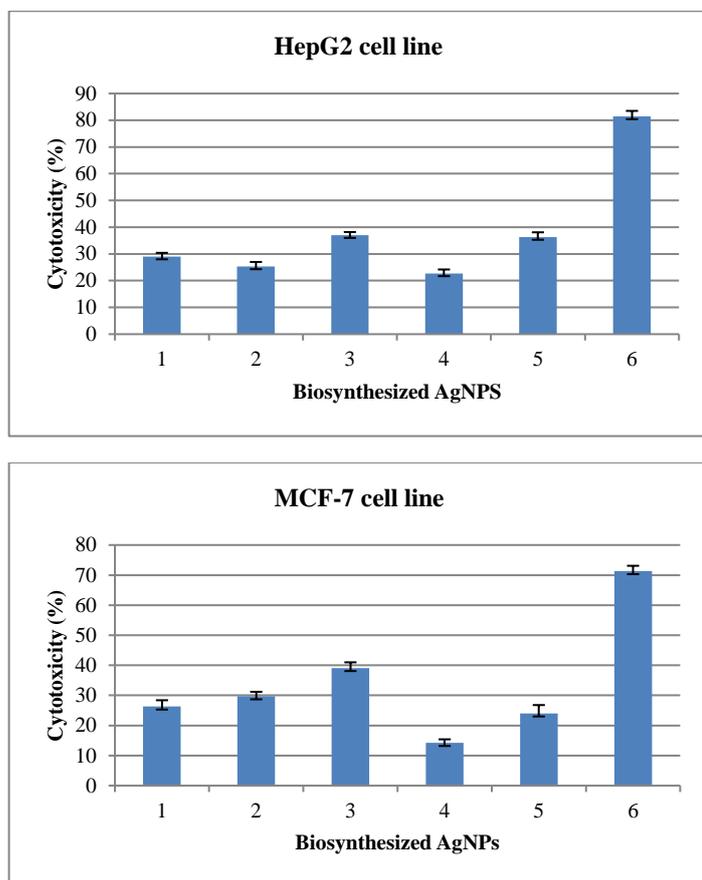


Figure 11 Cytotoxicity of silver nanoparticles biosynthesized from green algae *Scenedesmus obliquus* (1,2,3) and blue green algae *Spirulina platensis* (4,5,6) at 100 ug/mL

CONCLUSION

Among the noble metals silver is recommended in variable fields of medicine and biological system. PB experiment and the obtained data clearly indicate the dried powder and extracts of the algae *S. obliquus* and *S. platensis* can be used as an effective capping as well as the reducing agent for the synthesis of AgNPs. Analysis showed that nanoparticles (1-6) biosynthesized with different shapes and sizes depending on the species used and the method of biosynthesis. Six biosynthesized silver nanoparticles exhibited wide range of antimicrobial activity and high cytotoxic effect against HepG2 and MCF-7 cell lines especially for AgNPs prepared from ethanol extract of the blue green algae *S. platensis*.

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