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Determination the antibacterial activity of Ag-nanoparticlas produce biologicaly form different algae spp.

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ABSTRACT

Invented a new antimicrobial agent became a necessary ovreworld withand significantly raising a bacterial resistance for wide rang of antibiotic. Recently, studiesindicate that different nano metals cause serious injuries to microorganisms due to oxidation of the plasma membrane, protein denaturation and other effects. Different algae species have the ability to produce anAg-nanoparticles, which has a bacteriocidel effect against many species. Biosynthesis of Ag-nanoparticles from three algae species*(Westiellopsis sp.,Chroococcus minor* and *Oscillatoria sancta)*against four types of bacteria isolated form Baghdad hospitals investigated in this study.Colloidal Ag-nanoparticles were characterized by AFM,and UV-Visible spectrometer. Different concentration of Ag-Nps (25, 35, 45 and 55) µg/µl prepared in liquid medium from*Westiellopsis sp.*, which has best biosynthesis of Ag-NPs, so it used against (MRSA, *E.coli*, *Salmonella*and*Pseudomonas aeruginosa).* Results showed that effect of inhibition by Ag-Nps increased by increasing the concentration of it, and the heighest effect observed to the concentration (55µg/µl)*.Pseudomonas aeruginosa* has a siginfcant inhibition effect with 18 mm in diameter, then *Salmonella*with 16 mm. E.coli has 13 mm inhibition zone, while MRSA only 10.6 mm. This study showed that the Ag-nanoparticles synthesis by algae specieseffective to inhibit the different bacterial isolates. It expected to have remarkable applications in pharmaceutical and biomedical fields.

KEYWORDS: *Westiellopsis sp.*,*Chroococcus minor*, *Oscillatoria sancta*,Biosynthesis of Ag-nanoparticles, AFM, UV-Visible spectrometer.

INTRODUCTION

The productiveof nano material in a specific formatincluding size, shape and application getting an intensive attention recently in nanotechnology field.There is a simple example for the biological synthesis a nano material using the living cells such as bacterial or algal cells (1),which is depend on simply introduce the ions of any metal on the serfuce of the biological cell then the surface enzyme anaylsied the metal inside the cells to convert it to nano material (2). Production of AgNps involved in many application such as treatment, therapeutic and catlaytsits. Now a day, the antimicrobial activity of the Ag- nps become a widely accepted due to the ability of bacterial cells of raised resistance for different antibiotics (3). Algae are a metablolically active cells which is great source for the useful metabolits. The primary or secondary metabolies might have a theraputice activites,most of algea reduies showed a significant antimicrobial behiaver (4). Now a day many researches have reported that the ability of many algae species to synthesis nano particles (5). Marin algae showed great ability to produce Ag-Nps which has an intensive micrbioial effect against wide range of pathogenic bacteria (6).The aim of this study is biosynthesis of Ag-NPS and charicheterized size and shape of Ag-nano material from different algae species and invsitgate the anitmicrobial property againt bacteria.

MATERIAL AND METHODS

Algae isolation and identification:

Oscillatoria sancta,Chroococcusminor and*Westiellopsis sp.*, were isolated from AL- Mustansiriya University Gardens according to Patterson Method [7]. The algae identified by using an optical microscope (Olympus) according to [8]. Algae samples were cultured using modified chu-10 medium for algae growth (Table 1).

Table 1: The concentration of the components of modified chu- 10 medium

concentration g/l	Salt B-micronutrient	concentration g/l	salt A-macronutrient
1.00	EDTA . Na_2	5.8	Sodium Meta Silicate
2.86	H_3BO_3	57.56	$Ca(NO_3)_2.4H_2O$
1.81	$MnCl_2.4H_2O$	10	K_2HPO_4
0.222	$ZnSO_4.7H_2O$	25	$MgSO_4.7H_2O$
0.390	Na Mo $O_4.5H_2O$	4.36	EDTA .Na_2
0.079	$CuSO_4.5H_2O$	3.15	$FeCl_3.6H_2O$
0.0494	$Co(NO_3)_2.6H_2O$	20	Na_2CO_3

Serial dilution and streaking techniques were used for the isolation and purification of algae, which cultivation constant laboratory condition (temp 25° C , 200 Micro Einstein / m^2 intensity of illumination/ Sec for a period of 6 to 18 hours Lighting: darkness) [9]. The culture kept in the above condition for two weeks, all prepared algae culture centrifuged using an ordinary centrifuge 3000 rpm for 15 minutes. The sediment, dry at 40° C for 48 hours [10].

Bacterial strains:

All bacteriaisolated from different sourcesofsome Baghdad hospitals. All isolates grown at 37^{o}C for 24 hours, then loop-full of inoculated broth were streaking at nutrient agar (Oxoid), all petri- dishes incubated at 37^{o}C for 24 hours, then bacterial isolates characterized by cultural, microscopical and chemical tests (11). All species are being confirmed diagnosis using viteck system (Al-Habibiya hospital).

Biosynthesis of colloidal Ag nano:

Biosynthesis of silver nanoparticles from threealgal species done,1g of culture of microalgal strains growing in chue- 10 media harvested by centrifuge to collect pellets which washed with sterile distilled water to remove excess media. Cells filtrate to obtain 5 ml biomass then treated with 95ml of $AgNO_3$ solution (1 mM) from(sigma- Aldrich),the final solution incubated at room temperature for 24 hours. The color changes during incubation periodfrom fine yellow to dark brown indicatesbiosynthesis of nanoparticle (12).

UV–VIS Spectroscopic analysis:

To figure out the formation of Ag-Nps, the UV–VIS absorption spectra of the prepared colloidal solutions recorded using a spectrophotometer against deionized water without any addition as blank (ref. no.Dadosh, 2009). The bioreduction of Ag-NPs solutions was monitored by periodic sampling of aliquots (1 ml) of aqueous component after 20 times dilution and measuring the UV–VIS spectrum of the solution at 24 hrs . UV–VIS spectra of these aliquots were monitored as a function of time of reaction on a (Schimadzu- 1601) spectrophotometer in 200–700 nm wavelength. The system operated at resolution of 1 nm (13) .

Atomic Force Microscopy (Afm):

A thin film of Ag Nps was prepared on a glass slide by dropping 100 µl of Ag-Nps sysnthesis on it, the prepared slide was allowed to dry for 5 min at room temperature. The slides then scanned using the AFM apparatus (14).

Preparation of Ag-NPs suspension:

In 250 ml screw cup bottle, 1g of Ag-Nps purchesd form sigma dissolved in 100 ml of D.w. to prepare 1% of Ag-Nps suspension as stock solution. The suspension sterilized using the autoclave (121 ºC for 15 min). Different concentrations (25, 35,45 and 55) µg/µl of Ag-NPs are prepared and used in all experiments (15).

Antibacterial activity of silver nanoparticles:

Well agar diffusion method was performed to investigate the antimicrobial activity of silver nanoparticles according to (16). Few colonies from overnight culture of every type of tested bacteriatransferred to1 ml of normal saline to prepare the bacterial suspension and adjusted to 0.5 McFarland turbidity that is equal to 1.5×10^7 CFU/ml. The bacterial suspension was inoculated on nutrient agar plates using a sterile cotton swab. Nine mm

in diameter wells have been punched on nutrient agar medium and swabbed with bacteria using cotton swabs. 100Ml of dried $AgNO_3$ in 100 µl of D.w. was prepared, 25, 35, 45 and 55µl of dispersed solution was put in the well to get concentration of (25, 35, 45 and 55) µg/ µl, respectively. 1 mM of $AgNo_3$ was used as a control. The diameter of inhibition zone around wells was measured in millimeter. The cut off value of AgNps against both bacteria was 20µg/µl.

RESULTS AND DISCUSSION

The formation of silver nanoparticles confirmed through visual assessment. The reaction mixture turned to dark brown color from fine yellow color within 24 hr. indicated the synthesis of silver nanoparticles from three different species of algal isolates (*Westiellopsis sp.*, *Chroococcus*minor and *Oscillatoriasancta*). (Figure-1). Biomass of algae will convert the sliver nitrate in dark form a green to a dark brown color with intensity changed by increasing the incubation period. The production of the Sliver-Np was very significant by *Westiellopsis sp.* other than other algal species.

The appearance of dark brown color may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$(1). Cyanobacteria commonly use nitrate as the major source of nitrogen for three different purposes including growth, generation of metabolic energy, and redox balancing (2).The real mechanism of the production of Ag Nps. still not cleared however, researchs hypotheised that due to needs of Ag ions in many of the Krebs cylcle during metablic activity of algae, and capping of microalgal proteins metabolites and reduction of silver ions may lead to the formation of silver nanoparticles in the solution (17).

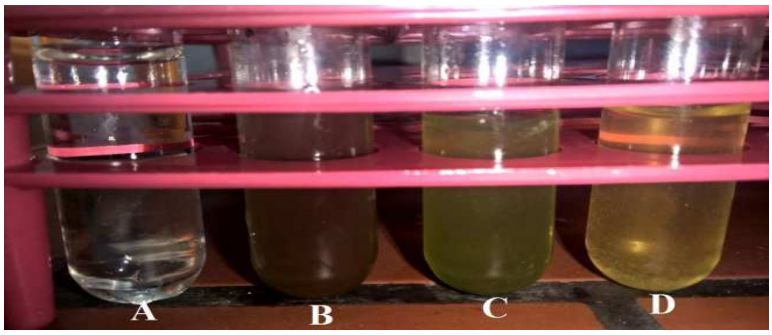


Fig. 1: (A) control(1 mM of$AgNo_3$),(B)1 mM of $AgNo_3$ after 24hrs of mixed with *Westiellopsis sp.* (C)1 mM of $AgNo_3$ after 24hrs of incubated with *Chroococcus*(D) 1 mM of $AgNo_3$ after 24hrs of incubation with *Oscillatoria sancta*

Characterization of Ag Nanoparticles:

UV–Vis spectrum of reaction mixture (1 mM of $AgNO_3$)after 24hrs of mixed with three algal isolates comparing with controlat different wavelengths ranging from 200 to 900 nm showed strong absorption peak with centering at 450 nm to *Westiellopsis* sp.(Figure-2) indicated the formation of Ag-NPs. This absorption is close to that seen for silver nanoparticles formed by different methods [18]. The wide absorption peak may be induced by the wide size distribution of Ag nanoparticles.

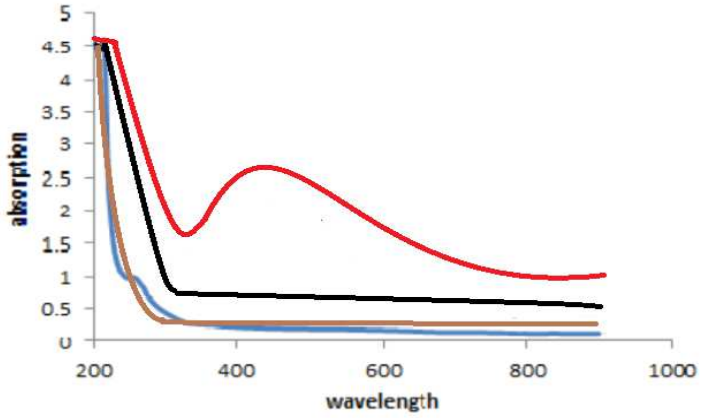


Fig. 2: (brown line) 1 mM of$AgNO_3$-control,(red line)1 mM of $AgNO_3$after 24hrs of mixed with *Westiellopsis* sp., (blue line)1 mM of $AgNO_3$after 24hrs of incubated with *Chroococcus* sp., (black line) 1 mM of $AgNO_3$after 24hrs of incubation with *Oscillatoria* sp.

The characterization of Ag-Nps synthesis by *Westiellopsis*sp.was investigate using atomic force microscope (AFM). The expriments carrid out to measure the dimensions of the particls (long, top and size) of synthesis Ag-Nps (Figure-3). while(Figure-4) showed Ag-NPS were biosynthesized in different sizes by three isolates of*Westiellopsis.*The size was mesured by using AFM the diameter starting from (1 to 85.8) nm and the average ofAg-Nps diameter was (35.5 nm).

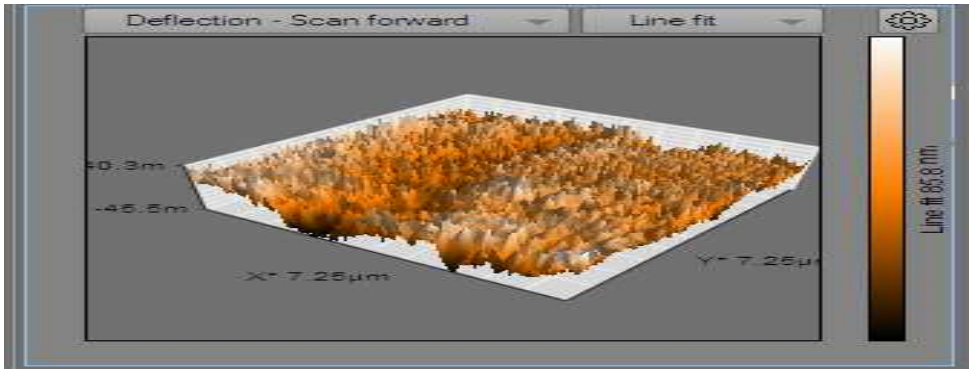


Fig. 3: AFM picture shows silver nanoparticles from*Welopsis* spp. with maximum top height (85.5 nm).

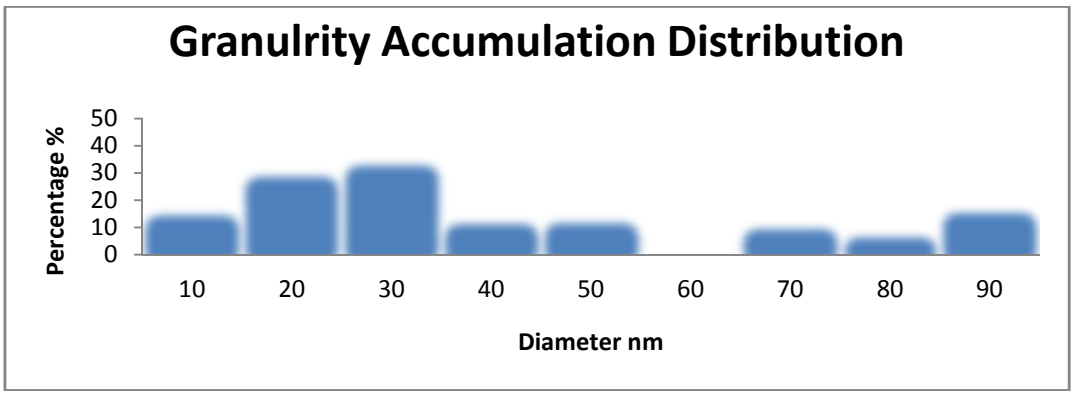
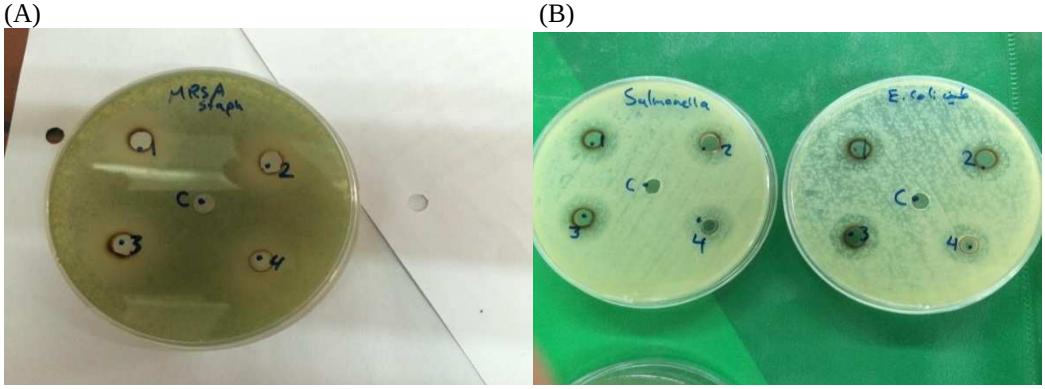


Fig. 4: Diameter, volume and accumulation of Ag-NPs

Antibacterial activityof Ag-NPs:

The production of the Sliver-Np was very significant by *Westiellopsis*spp. Therefore the biomass of the Ag-Nps produce by *Westiellopsissp.*, only test for the antibacterial activity against four types of bacteria (*E.coli*, S*almonella spp.*, MRSA and *P.aeruginosa)*(figure-5 and figure-6).

(A) (B)



(c)

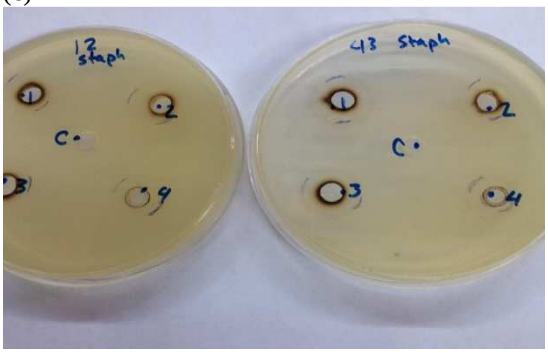


Fig. 5): Antibacterial effect of Ag- nano particlesagainst (a)*E.coli andSalmonella spp.* (b) MRSA (c) *P.aeruginosa*,measuring via agar diffusion method. C: control, 1: 25 µg/µL, 2: 35 µg/µL, 3: 45 µg/µL, 4: 55 µg/µL.

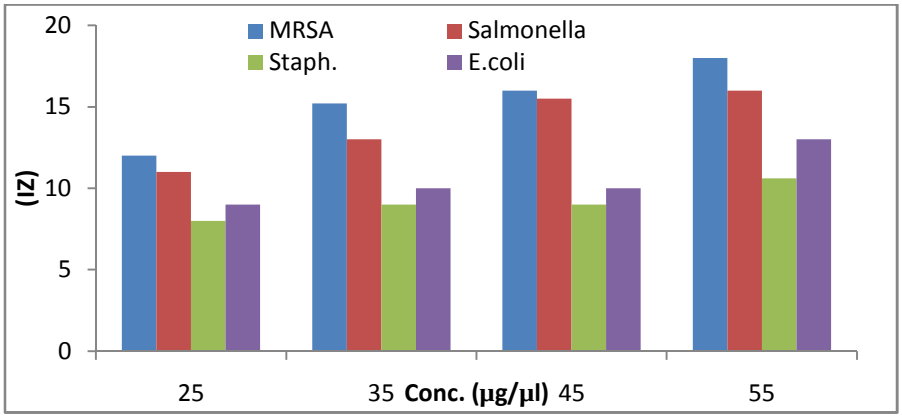


Fig. 6: Antimicrobial effect of Ag- nanoparticles against four types of bacteria (*E.coli, salmonella, MRSA, and P.aeruginosa).*

Pseudomonas aeruginosa has a siginfcant inhibition effect (18 mm) when treated with 55 µg/ µl of Ag-Nps, then *Salmonella* has a greatinhibition effect (16 mm) after treated with biosynthesis Ag- Nps.*E.coli* has (13 mm) inhibition zone, while MRSA only (10.6 mm).

The outstanding antimicrobial properties of Ag-NPs have led to the development of a wide variety of nanosilver products, including nanosilver-coated wound dressings, contraceptive devices, surgical instruments, and implants [18, 19].

Apart from these antimicrobial activities, Ag-NPs are also known to possess antifungal, anti-inflammatory, antiviral, anti-angiogenesis, and antiplatelet properties [20,21]. Additionally, more recent developments have seen Ag-NPs used in room spray, wallpaper gloves, laundry detergent, and wall paint formulations as well as in the textile industry for clothing manufacturing.

Conclusion:

The interest of invention an antimicrobial by a biological synthesis growing in last few decades with working of the pioneering work for many scientists.In this study *Westeillopsis*spp. was the main strain produce a Ag-Nps. It can be supposed that the algae cells could able to convert the Ag ions to Ag Nps using many enzymes inside the cells.To date, the mechanism in which the living cell converts the big molecules to a nano scales particalsstill not notably understand however, researches proposed that nano partcial could be screated as secondy meabloites resulting from Krebs cycle.

In this research a gram negative bacteria (*Pseudomonas aeruginosa*) has a siginfcant inhibition effect when treated with a Ag Nps sysnthysis by*Westeillopsis* Spp. with 18 mm in diameter, then*Salmonella* has a greatinhibition effect with 16 mm in diameter after treated with synthesis Ag Nps.It has been provde in this study that bacterial inactivation has been increase by increase the Ag Nps concentrations. The biosynthesized silver nanoparticles are expected to have remarkable applications in many important fields.

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