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Research Article
Antibacterial Activity of Silver Nanoparticles Using *Ulva fasciata* Extracts as Reducing Agent and Sodium Dodecyl Sulfate as Stabilizer

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Abstract

Background and Objective: Silver nanoparticles were synthesized via chemical reduction and using stabilizers agents. This study aimed to compare between biosynthesis of silver nanoparticles (AgNPs) by marine alga *Ulva fasciata* (*U. fasciata*) as reducing and stabilizing agents with biosynthesis of AgNPs by marine alga *U. fasciata* as reducing agents and sodium dodecyl sulfate as stabilizer against bacterial activities. **Materials and Methods:** In this study macro green alga (*Ulva fasciata*) aqueous extracts using as reducing agents to reduce Ag ions to Ag^0 and sodium dodecyl sulfate (SDS) as stabilizer. Ultra violet-visible spectroscopy, transmission electron microscope (TEM), scanning electron microscopy (SEM), fourier transform infrared spectroscopy, energy dispersive X-ray spectroscopy (EDX). Zeta potential and zeta sizer were used to characterize green synthesis silver nanoparticles (AgNPs). **Results:** Biosynthesis of AgNPs showed broad surface plasmon resonance peak at 446, 428 and 426 nm with 1 h and 1 and 2 weeks that reflects the stability of nanoparticles until 2 weeks. Transmission electron microscopy study proved that the shape is spherical and average size was ranged from 9-20 nm. Fourier transform infrared spectroscopy analysis (FTIR) indicated evidence that pretence of amide band of protein as possible reducing agents. Zeta potential was at -30, zeta sizer indicated that the size range around 53.5 nm. Biosynthesis AgNPs had antibacterial activities against *Escherichia coli* ATTCC 8739, *Proteus mirabilis* ATTCC 9240, *Micrococcus leutus* and *Kocuria varians.* **Conclusion:** The biosynthesis of silver nanoparticles using *U. fasciata* aqueous extracts as reducing agents and sodium dodecyl sulfate (SDS) as stabilizer had highest inhibition activity against both Gram-negative and Gram-positive pathogenic bacteria more than silver nanoparticles biosynthesis by *U. fasciata* aqueous extracts as reducing and stabilizing agents and also $AgNO_3$ as a bulk.

Key words: *Ulva fasciata*, silver nanoparticles, pathogenic bacteria, biosynthesis, sodium dodecyl sulfate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Silver nanoparticles are highly effective against bacteria, fungi and cancer cells[1-3] and also used as antiviral, anti-inflammatory, anti-angiogenesis, anti-platelet and anti-permeability activity[4]. Nanoparticles have advantageous in food packing, cosmetics, paints and textile industry[5,6]. Silver nanoparticles had many applications such as conductive inks, cosmetics, microelectronics, adhesives, coating for solar energy, intercalation materials for electrical batteries, diagnostics, silver nanocoated medical devices, optical receptors and therapeutics[7-13]. Many methods of silver nanoparticles synthesis have been reported such as grinding, thermal decomposition of silver bulk, beam electron irradiation, vapor condensation and chemical reduction of silver ions[14-17]. Chemical synthesis of silver ions is demand reducing agents and surfactant as stabilizer in bulk solutions[18]. Many reducing agents have effective in synthesis silver nanoparticles such as hydrazine, citrate ions, triethanolamine, ethylene glycol and sugars[19-24]. Stabilizers as polyvinyl pyrrolidone, fatty acids and sodium dodecyl sulfate (SDS)[18,21,25]. From the above reports, synthesis of nanoparticales needs high pressure, temperature, toxic chemicals and also very high expensive[26-28]. Green syntheses of silver nanoparticles using plants have ecofriendly methods without using of expensive cost and toxic chemicals[29]. Seaweeds have valuable contents such as polysaccharides (laminarians, alginates and fucans) that using of synthesis of pharmaceutical and nutraceutical compounds[30]. Seaweed mediated reduction of silver into Ag nanoparticles using *Ulva lactuca*[30]. *Ulva fasciata* has potent therapeutic value and it is used for synthesis of silver nanoparticles[31]. Silver nanoparticles synthesis by *Ulva fasciata* have toxic against cancer[1,31,32]. Silver nanoparticles synthesis by *Ulva fasciata* had very high antibacterial activity against the common pathogens bacteria of human and fish[33].

The present study aimed to study the ability of *Ulva fasciata* aqueous extracts to reduce Ag^+ ions to Ag^0. Demonstrate the characterization of biosynthesis of AgNPs by UV-spectrophotometry, SEM, TEM, FTIR, zeta potential and zeta sizer. Determine the potential effects of biosynthesis of AgNPs with stabilizer (SDS) concentration and without stabilizer against pathogenic bacteria.

MATERIALS AND METHODS

This study was carried out in Microbial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat City,, Egypt at 2016.

***Ulva fasciata*:** Alga was collected from shallow water beside the shore of Abu-qur coast Alexandria Egypt and was identified according to Taylor[34].

Preparations of *Ulva fasciata* aqueous extracts: One gram of dry powder *Ulva fasciata* was added to100 mL DD water, boiled for 1 h then filtrated.

Biosynthesis of silver nanoparticles (AgNPs): According of methods of Devi *et al.*[35],10 mL of previous prepared extract was added slowly to 90 mL of freshly prepared 0.1 mM of silver nitrate ($AgNO_3$) Sigma Aldrich with stirring and heating at 40°C until the color change to red.

Biosynthesis of AgNPs with stabilizer (SDS): The synthesis of AgNPs with stabilizer (SDS) was carried out by adding different concentration of sodium dodecyl sulfate (1, 3, 6 and 9 mM) as stabilizer to fresh prepared AgNPs with stirring and heating at 40°C for 30 min.

Characterization of silver nanoparticles biosynthesized using *Ulva fasciata* silver ions: Silver (Ag^+) was reduced in aqueous extract of marine alga *U. fasciata* and formed silver nanoparticles (AgNPs). The biosynthesis of AgNPs were monitored using ultra violet-visible spectroscopy (Shimadzu UV-1601PC spectrophotometer, England) for wavelength 200-700 nm for 2 weeks to determine the stability. The size and shape of AgNPs were characterized by transmission electron microscope (TEM) (JEOL, JEM-2100, Japan). Surface morphology, size and distribution of AgNPs were monitored by scanning electron microscopy (SEM) JEOL JSM-6510/v, Japan. Energy dispersive X-ray EDX detector system (JEOL, JEM-2100, Japan) was used to determine the composition of elements of the nanoparticles sample. Fourier-transform infrared (FTIR) was used to investigate the biomolecules in *U. fasciata* aqueous extract that responsible for the reduction and capping of silver ions to form nanoparticles. Zeta potential value and size distribution of the nanoparticles were investigated using zeta potential analyzer (Malven Zeta size Nano-Zs90).

Pathogen selection: Microorganisms selected in this study are four human pathogen bacteria two of them are Gram negative bacteria *Escherichia coli* ATTCC 8739 and *Proteus mirabilis* ATTCC 9240, the others are Gram positive bacteria *Micrococcus leutus* and *Kocuria varians*.

Antibacterial activity of AgNPs

Paper disc diffusion assay: Antibacterial activity was investigated using the agar diffusion technique[36] in Petri dishes. Briefly 25 µL of AgNPs with different concentrations (25, 50, 75 and 100 µg mL^{-1}) were loaded on sterile and air dried filter paper (Whatman No.1 and 3 mm in diameter). The papers were placed on the surface of nutrient agar media and 10^5 CFU mL^{-1} of bacterial cells were spread on the surface of the nutrients agar plate. The plate were incubated at 30°C, after 28 h were investigated clear zone around the filter paper desk that indicated of antibacterial activity of AgNPs against *Escherichia coli* ATTCC 8739, *Proteus mirabilis* ATTCC 9240, *Micrococcus leutus* and *Kocuria varians*.

Morphological characters of *E. coli* ATTCC 8739: The change of morphological characters of *E. coli* ATTCC 8739 was determined by scanning electron microscopy (SEM) JEOL JSM-6510/v, Japan, before and after treatments with AgNPs. After 48 h, bacterial cells suspension centrifuged micro hematocrit centrifuges Hunan, China (Mainland) and washed with phosphate buffer and fixed with 2% glutaraldehyde and 1% osmium tetroxide. After fixation sample was washed with buffer solution and followed by dehydration. The dehydrated bacteria were dried immediately and coated with gold/palladium and then examined by SEM.

RESULTS AND DISCUSSION

Morphological characters of biosynthesis of silver nanoparticles: The color of the biosynthesis of silver nanoparticles was turned to yellowish brown while the control of algal extract was colorless .

Stability of silver nanoparticles reduced by *Ulva fasciata*

UV-spectroscopy: The UV-spectroscopy of biosynthesis AgNPs shows intense peak due to the surface plasmon resonance (SPR) of AgNPs at 446, 428 and 426 nm where the intensity was 0.186, 0.317 and 0.341 after 24 h, 1 and 2 weeks, respectively. The results indicated that no significant changes in peak intensity were noticed with 1st and 2nd weeks and also AgNPs synthesis by *U. fasciata* aqueous extract had stability when storage at room temperature for 2 weeks, Fig. 1. These results agree with Govindaraju *et al.*[37], who reported that silver (SPR) band presented at 424 nm and steadily increased in intensity as increase of time reaction without any shift in the peak wavelength. The increase of intensity within 2 weeks indicated the increase of concentrations of nanoparticles[38]. The broadened peak indicating increased concentration of nanoparticles[39] and also the nanoparticles are polydispersed[30]. The UV-spectroscopy of biosynthesis AgNPs by *U. flexuosa* show intense peak at 430 nm[40].The surface plasmon resonance SPR band characteristics of AgNPs were detected around 400-450 nm depending on its size, this results suggested that the spherical shape of nanoparticles and confirmed by TEM[41].

Characterization of silver nanoparticles biosynthesized using *Ulva fasciata* silver ions

Scanning Electron Microscope (SEM): The results in Fig. 2 show that scanning electron microscope of biosynthesis of AgNPs. These results investigated that the nanoparticles were spherical in shape, well distributed on *U. fasciata* aqueous extract and low density dispersion.

Transmission electron microscope (TEM): The result in Fig. 3 shows that the micrograph of silver nanoparticles synthesis by *Ulva fasciata* aqueous extract has spherical shaped, well distribution in solution and the range of particles size is 11-37 nm as shown in Fig. 4. Rahimi *et al.*[40] investigated that the silver nanoparticles synthesis by *U. flexuosa* had size range within 2-32 nm. Abirami and Kowsalya[31] observed that the size of AgNPs synthesis by *U. fasciata* was ranging from 28-41 nm and spherical in shape. The average size of silver nanoparticles synthesized by *U. lactuca* was 20 nm and spherical in shape[30].

Energy dispersive x-ray spectroscopy analysis: Energy dispersive x-ray spectroscopy analysis indicated to verify the presence of silver in aqueous AgNPs. The horizontal axis denotes to energy and vertical axis display the number of counts of X-ray[42]. The presented spectrum could clearly showed five peaks which located between 1-6 KV. The quantitative analysis cleared that higher percentage of silver content (46%) and present of other elements Na, S, CL and Fe which represent 2.79, 25.49, 24.2 and 1.51%, respectively in nanoparticles suspension synthesis by *U. fasciata*, Fig. 4 and Table 1.

Fourier transform infrared spectroscopy: Fourier transform infrared spectroscopy was used to identify the functional thesis by *U. fasciata*. The results in Fig. 5 denote that the groups that coating Ag nanoparticles syn.absorption peaks at 1633 and 1385 cm^{-1} which indicated that the presence of amide band in proteins. These peaks indicated that reducing and stabilizer $AgNO_3$ by alga extract to Ag^0 [38]. The primary amines of proteins in plant extracts play a major role in the reduction of $AgNO_3$ to AgNPs[43].

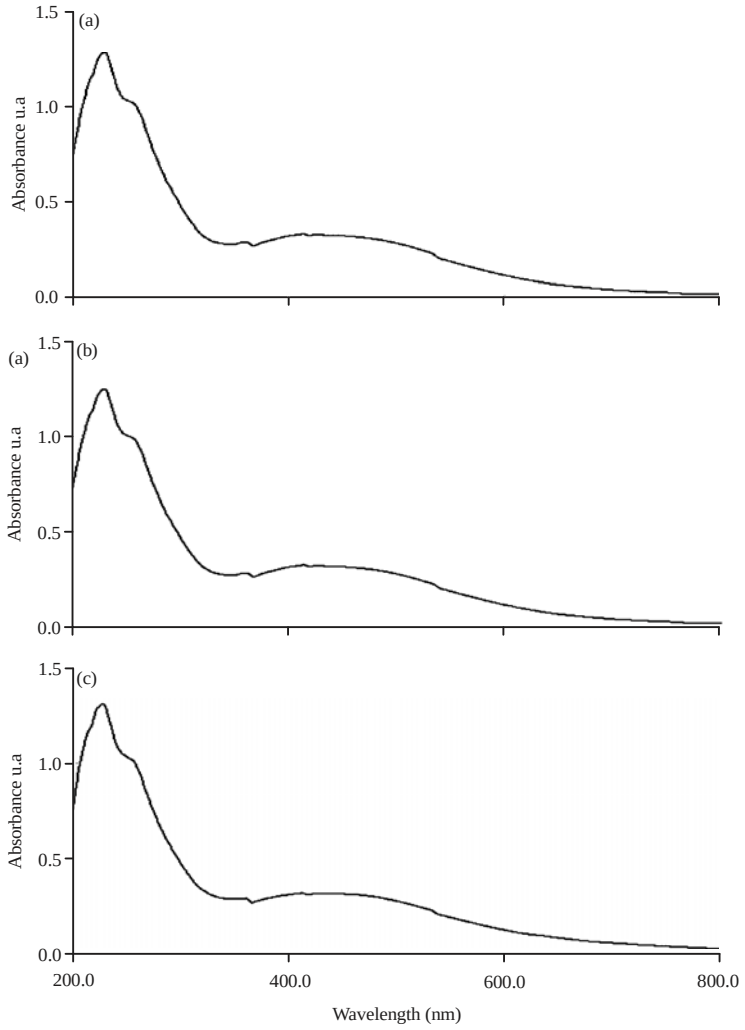

Fig.1(a-c): Ultra-violet visible spectrums (UV) of Ag nanoparticle suspension reduced with *Ulva fasciata* aqueous extract and stabilized by SDS (a) after 24 h (b) after 1 week and (c) after 2 weeks

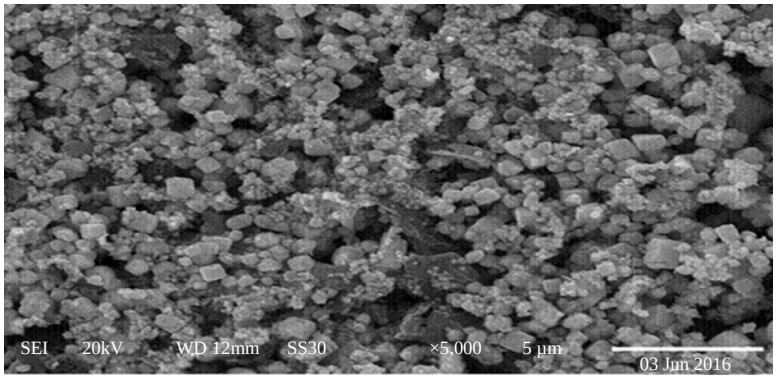

Fig. 2: Scanning electron microscope (SEM) of Ag nanoparticles

Zeta potential: The zeta potential is refer to surface charge of silver nanoparticles (AgNPs) and it is useful parameters to analyze the long term stability of nanoparticles. Zeta potential is important parameter to predict the stability of AgNPs. Zeta potential less than -20 mV and more than +20 mV predicts good physical stability. The zeta potential value of

Table 1: Energy dispersive X-ray spectroscopy analysis of AgNPs suspension reduced with *Ulva fasciata* aqueous extractand stabilized by SDS

Elements	Weight (%)	Atomic (%)
Na	0.95	2.70
S	12.04	25.49
CL	12.64	24.20
Ag	73.12	46.00
Fe	1.24	1.51
Total	100.00	100.00

biosynthesis of silver nanoparticles by *Ulva fasciata* was 30.7 mV. The results in Fig. 6 indicated that AgNPs synthesis by *Ulva fasciata* is a good stability because the repulsive forces prevent aggregation with aging.

Particle size: The results of Fig. 7 indicated that a distribution range between 30 and 500 nm with an average nanoparticles size around 53.50 nm. These results refer to AgNPs synthesis by *U. fasciata* has some aggregation.

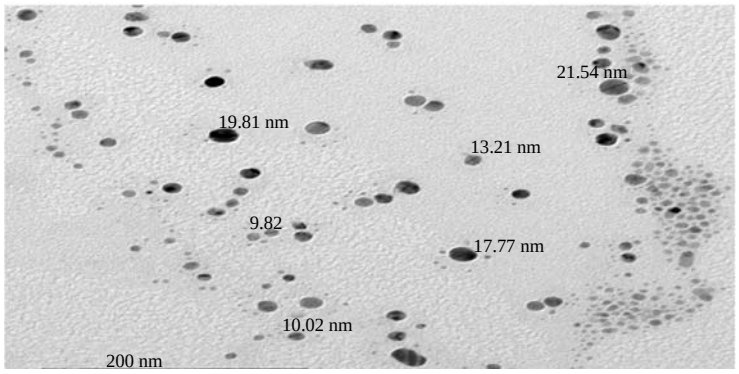

Fig. 3: Transmission electron microscopic image of silver nanoparticles biosynthesized by *Ulva fasciata* aqueous extract and stabilized by SDS

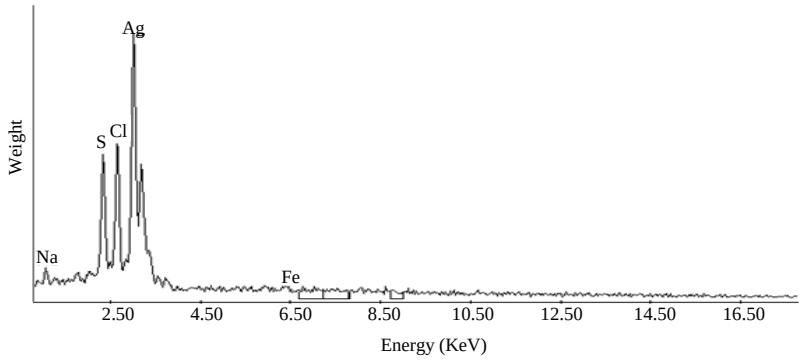

Fig. 4: Energy dispersive X-ray spectroscopy analysis of Ag nanoparticle suspension reduced with *Ulva fasciata* aqueous extract

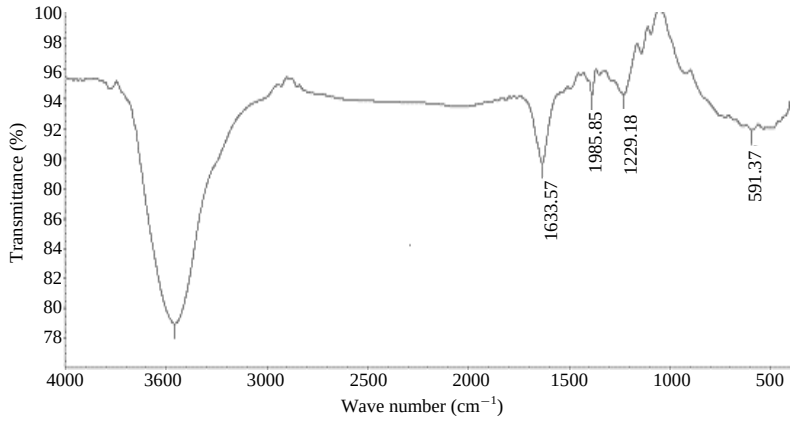

Fig. 5: FTIR of Ag nanoparticle suspension reduced with *Ulva fasciata* aqueous extract and stabilized by SDS

Antibacterial assay: The results obtained from Fig. 8 show different concentrations of biosynthesis of AgNPs had antibacterial activity against two Gram positive bacteria Micrococcus *leutus* and *Kocuria varians* and Gram negative bacteria *Escherichia coli* ATTCC 8739 and *Proteus mirabilis* ATTCC 9240. The best concentrations effects on the tested bacteria had 100 μg mL^{-1} followed by 75, 50 and 25 μg mL^{-1}, respectively. *Proteus mirabilis* ATTCC 9240 was most sensitive strain with AgNPs among other tested bacteria. When added sodium dodecyl sulfate (SDS) as stabilizer against to AgNPs reduced by *Ulva fasciata* aqueous extract, the zone of inhibition around the disk was increased, indicated that SDS effect on the stability and polydispersity of silver nanoparticles and biosynthesis of AgNPs with SDS as stabilizer was highest activity against pathogenic bacteria than biosynthesis AgNPs without stabilizer. The highest antibacterial activity was observed against Gram positive bacteria *Micrococcus leutus*, followed by *Escherichia coli* ATTCC 8739. The most concentrations that effect on the AgNPs stabilizers were 6 mM (Fig. 9, 10).

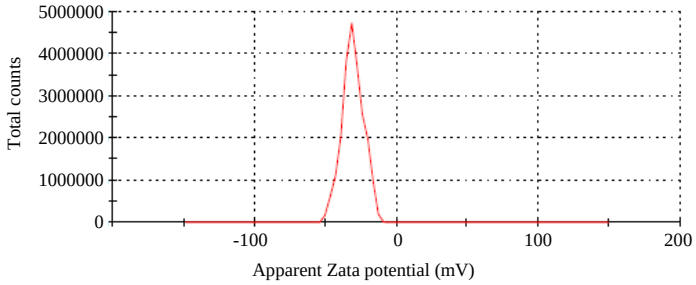

Fig. 6: Zeta-potential analysis

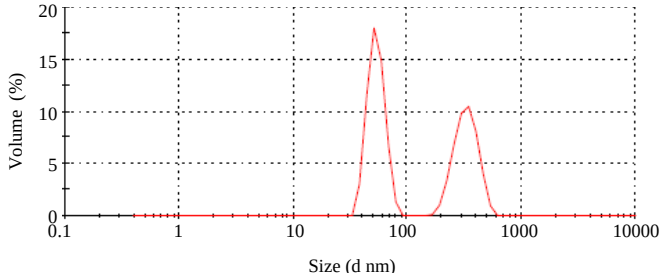

Fig. 7: Particle size distribution of silver nanoparticles reduced with *Ulva fasciata* aqueous extract and stabilized by SDS

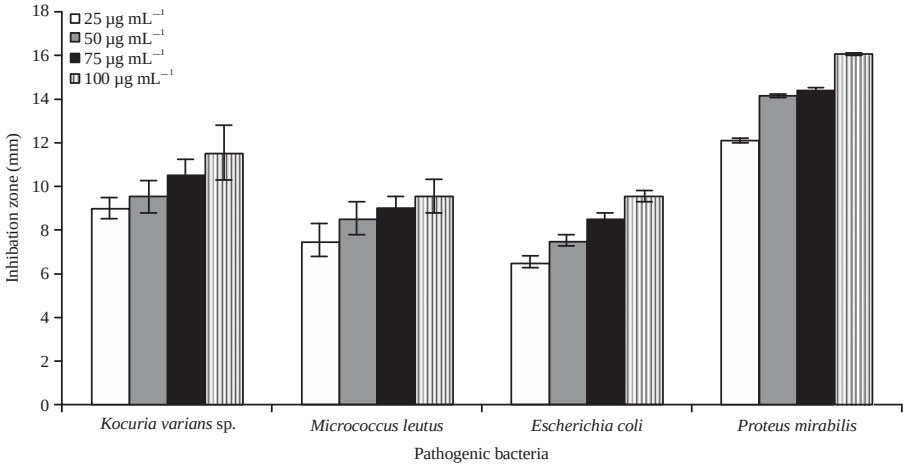

Fig. 8: Effect of different concentrations of silver nanoparticles reduced with *Ulva fasciata* aqueous extract against pathogenic bacteria

Inhibition zone mm+the zone of paper 3 mm, Bars represent are standard error of means

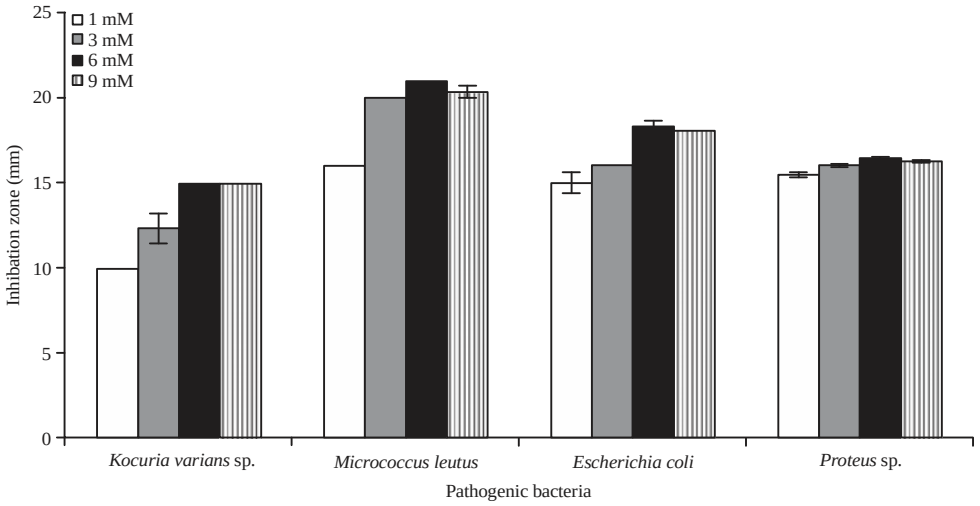

Fig. 9: Antibacterial activity of AgNPs reduced with *U. fasciata* and with different concentrations of sodium dodecyl sulfate (1, 3, 6 and 9 mM) as stabilizer against pathogenic bacteria

Inhibition zone mm+the zone of paper 3 mm. Bars represent are standard error three replicates of means

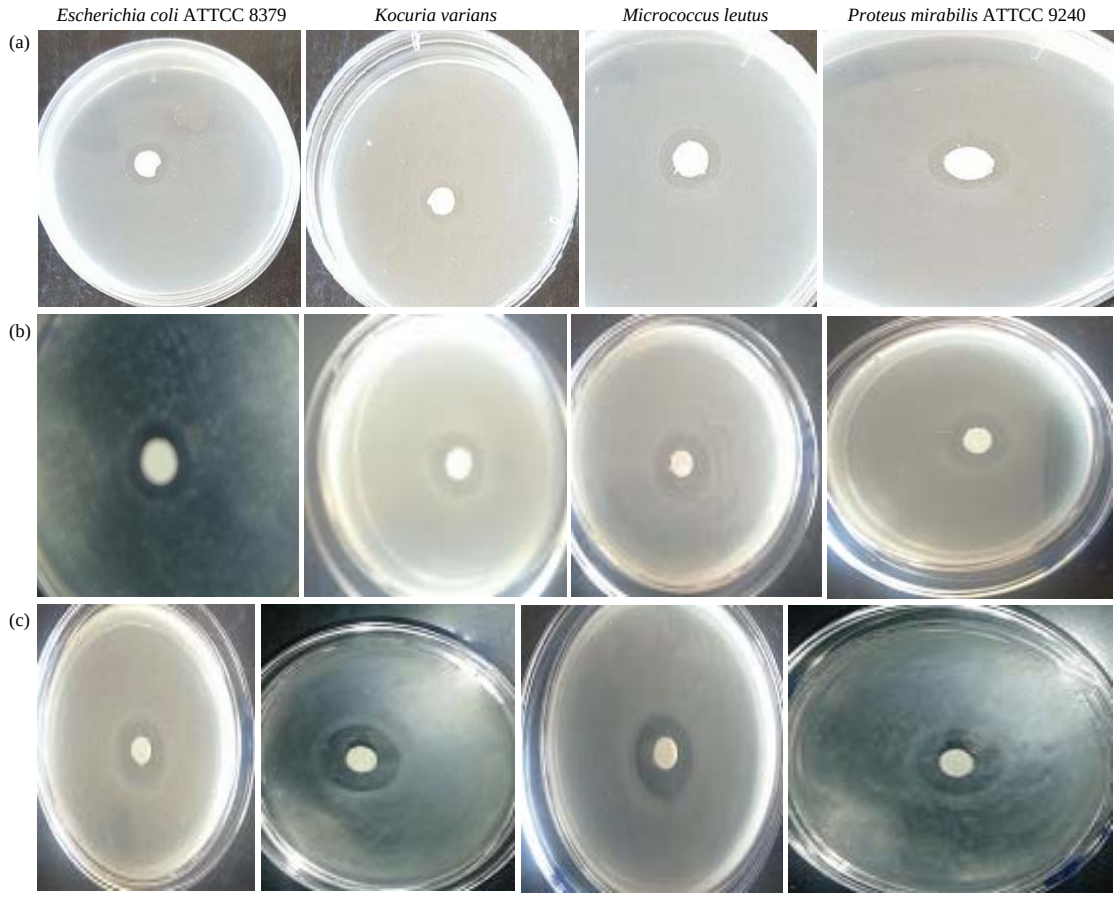

Fig. 10(a-c): Antibacterial activity of silver nanoparticles reduced with *Ulva fasciata* aqueous extract and using 6mm SDS as stabilizer against Gram negative and Gram positive bacteria Treatments: (a) 0.1 mM $AgNO_3$+6 mM SDS, (b) 0.1 mM AgNPs and (c) 0.1 mM AgNPs+6 mM SDS

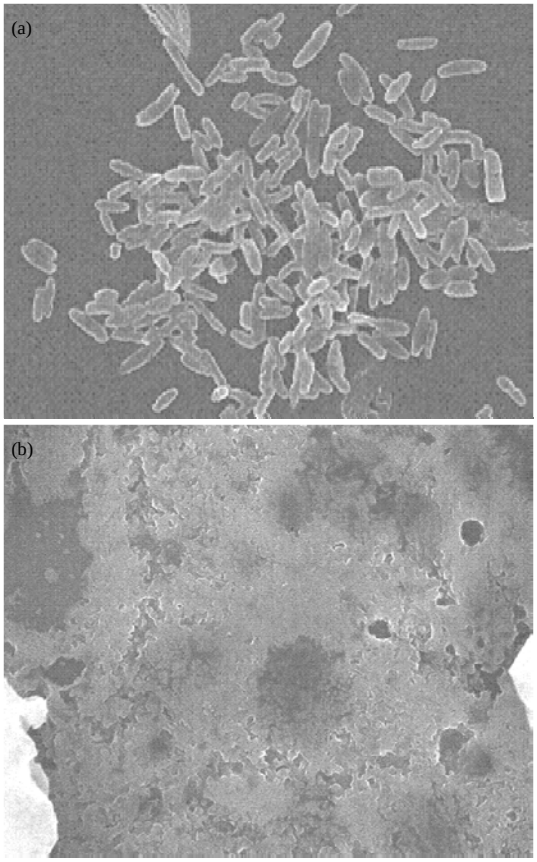

Fig.11(a-b): Scanning electron micrograph showing untreated *E. coli* ATTCC 8739 (a) Untreated *E. coli* ATTCC 8739 and (b) *E. coli* ATTCC 8739 treated with AgNPs, cells disrepute

The results regarding scanning electron micrograph of *Escherichia coli* ATTCC 8739 before and after treatments with AgNPs is shown in Fig. 11. Obtained results indicated that *E. coli* ATTCC 8739 has rod shaped. Silver nanoparticles inhabited cell division, penetrating through cell membrane, damaging of bacterial cells[44]. AgNPs also caused leakage of cell content and destruction of bacterial cells[45]. The inhibition activity of AgNPs was related to free radicals that were caused destruction of the cell membrane. Free radicals (ROS) can exist in extracellular and also intracellular[46].

CONCLUSION

The biosynthesis of silver nanoparticles easily synthesis by using *Ulva fasciata* aqueous extracts as reducing agents and SDS as stabilizing agents. Nanoparticles were produced had stability until 2 weeks, spherical, well distributed and size range between 11-37 nm according to TEM and SEM. Biosynthesis of AgNPs had negative charge and present some aggregation according to zeta potential and zeta sizer. Biosynthesis of AgNPs had powerful antibacterial agent against both Gram negative bacteria and Gram-positive. The treatments *E. coli* ATTCC 8739 by AgNPs caused bacterial cells explosion and damage.

SIGNIFICANCE STATEMENTS

This study is the first one that produces silver nanoparticles by using aqueous extracts of marine alga *Ulva fasciata* as reducing agents and sodium dodecyl sulfate (SDS) as stabilizing agents. Silver nanoparticles possessed antibacterial activities against pathogenic bacteria (*Escherichia coli* ATTCC 8739, *Proteus mirabilis* ATTCC 9240, *Micrococcus leutus* and *Kocuria varians*).

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