

Extracellular Synthesis of Eco-friendly Bio Silver Nanoparticles by using Seaweeds from Arabian Gulf, Saudi Arabia and their Potential Applicability for Pharmaceutical Uses

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Currently, there is a growing need to develop environmentally benign nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol. The present study reports an environmentally friendly method for synthesis of silver nanoparticles from two seaweeds named *Cladophora nitellopsis* and *Sargassum latifllium*. Ag-NPs bactericidal impact on *Bacillus subtilis* and *Escherichia coli*. Scanning and Transmission electron microscopy (SEM &TEM) were used to illustrate the size and shape of the obtained Ag-NPs. Energy dispersive X-ray (EDX) spectrometer established the existence of elemental sign of the silver and homogenous allocation of silver nanoparticles. Diffraction by using X ray (XRD) analysis for the formed AgNPs revealed spherical plus cubical shapes structure with different planes ranged between 111 to 311 planes. Further, these Ag-nanoparticles show effective antibacterial activity against human pathogens, *Bacillus subtilis* and *Escherichia coli* due to high surface to volume ratio. Our work showed a rapid, eco-safety and suitable method for the synthesis of AgNPs by using *Cladophora nitellopsis* and *Sargassum latifllium* seaweeds. Therefore, they can be used in pharmaceutical and other biomedical applications.

Key words: Nanoparticles; TEM; EDX; Green biotechnology; *Chladophra*; *Sargassum*.

Synthesis of metal nanoparticles is enormous and expanding area due to their potential applicability in various areas such as electronics, chemistry, energy, and medicine development. Nanoparticles exhibit new or improved properties depending upon their size, morphology and distribution (Song *et al.*, 2009 and Nalwa, 2000).

Nanoparticles are classified primarily into two types, viz organic and inorganic nanoparticles. The nanoparticles of carbon are called the organic nanoparticles. Magnetic nanoparticles, noble metal nanoparticles (platinum, gold and silver) and semiconductor nanoparticles (titanium dioxide, zinc oxide and zinc sulfide) are classified as inorganic nanoparticles (Kathiresan and Asmathunisha, 2013). The biosynthetic method employing marine seaweed extract has received more attention as being simple, eco-friendly and less time consuming compared to usual chemical and physical methods.

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Recently, biosynthetic methods employing both biological microorganisms such as bacteria (Joerger *et al.*, 2000) and fungus or plants extract (Shankar *et al.*, 2003 and Chandran *et al.*, 2006), have developed speedily as a trouble-free and feasible choice to obtain nanomaterials alternative to more complex chemical synthetic procedures. The particular distinctiveness like size, allocation and shape give the nanoparticles different properties from the bulk material (Gardea-Torresdey *et al.*, 2003).

Current nanotechnology developments have led to nanomedicine, a new field which includes many diagnostic and therapeutic applications involving nanomaterials and nanodevices (Kagan *et al.*, 2005).

The green production of AgNPs requires three main process, which have to be checked based on green chemistry phenology, include (1) the assortment of solvent medium, (2) the choice of environmentally kind reducing agent, and (3) the choice of safe substances for the steadiness of AgNPs.

Bioreduction of silver and gold ions to yield metal nanoparticles using plant extract (Gardea-Torresdey *et al.*, 2003 and 2005), *Geranium* leaf broth (Shivshankar *et al.*, 2003), Neem leaf broth (Shivshankar *et al.*, 2004) lemongrass extract (Shivshankar *et al.*, 2005), *Tamarind* leaf extract (Ankamwar *et al.*, 2005) and *Aloe vera* plant extracts (Prathap *et al.*, 2006) have been reported. Shankar *et al.* (2004) reported on the synthesis of clean spiky nanoparticles of silver and gold by the reduction of Ag⁺ and Au³⁺ ions by Neem (*Azadirachta indica*) leaf broth. Most of the reported green synthesis methods using plants took more than 1 hour for the formation of colloidal silver (Shreesh and Dharamvir, 2009 and Mukunthan *et al.*, 2011).

Nanobiotechnology has enhanced the production of minor AgNPs with little toxic effect to human and more effectiveness alongside bacteria (Malabadi *et al.* 2012a, 2012b; Xia *et al.* 2010; Zhang *et al.* 2008). Furthermore, nanoparticles are alternative to antibiotics viewing better action against multidrug opposing bacteria and consequently, plant derived nanoparticles proved better to other methods (Savithramma *et al.* 2011a, 2011b; Song and Kim, 2009). The method of the AgNPs antibacterial action is efficiently explained

in conditions of their interaction with cell membranes of bacteria by troubling its permeability and respiratory role (Vankar and Shukla, 2012; Ghosh *et al.* 2012). In the present study, the extracellular biosynthesis of AgNPs using seaweeds *Cladophora* and *Sargassum* and their antibacterial effects against some human pathogens are testified.

MATERIALS AND METHODS

Materials

Silver nitrate (AgNO₃) was purchased from Merck. The glass wares used in this experimental work were acid washed. Ultrapure water was used for all dilution and sample preparation.

Sample collection

The green seaweeds *Cladophora nitellopsis* and *Sargassum latiflillum* collected from Al-Khober costal region (70°8'E, 10°16'N) in Arabian Gulf, the eastern coast of Saudi Arabia. Immediately after the collection, the samples were transferred to the laboratory in new plastic bags containing natural sea water to prevent desiccation.

Extraction of seaweeds

The seaweed was washed thoroughly with distilled water and was shade dried for 10 days. Fine powder of the seaweed were used to make the extract. 25 g of seaweeds were systematically washed with distilled water followed by double distilled water to take away the dust particles and other pollutants. Then the plant substance was grinded into fine powder and taken in a clean 250 ml Erlenmeyer conical flask and 100 ml of germ-free double distilled water was added and incubated on a sand bath at 60 °C for 30 mins to facilitate the formation of aqueous leaf extract. The extract was then filtered using Whitman No. 1 filter paper. The algal extract was used for the synthesis of AgNPs and the extract can be stored at 4°C for further use.

Preparation of 1mM Silver nitrate solution

For the preparation of 1mM Silver nitrate (AgNO₃) 0.02 gm of AgNO₃ was added to 100 ml of double distilled water. The solution was mixed thoroughly and stored in an amber colored bottle in order to prevent auto oxidation of silver.

Synthesis of Silver nanoparticles

For the production of 5% plant mediated

Ag-NPs; 5mL of seaweed extract was added to 95 ml of 1mM silver nitrate solution and incubated on a sand bath at 60°C for 30 minutes after that the color change was observed. This indicates the preliminary confirmation for the formation of AgNPs. The brown color formation indicates that the AgNPs were synthesized from the seaweed extracts and they were centrifuged at 5000 rpm (Hettich EBA20S Portable Centrifuge) for 10 minutes in order to obtain the pellet which is used for further study.

Transmission electron microscope (TEM)

TEM measurements and photographs were carried out on a JEOL-TEM 1200 EX II Transmission Electron Microscope in the Faculty of science at the Alexandria University (Alexandria, Egypt). The sample was dried in order to take the photograph.

Energy-dispersive X-ray spectrometer (EDX) analysis

Ag-NPs were cut off by centrifuging 20 ml of solution in water including Ag-NPs for 10 min at 15,000 rpm (Hettich EBA20S Portable Centrifuge). The pellets were obtained and dehydrated in oven at 50 °C to get rid of water. The Ag-NPs obtained in the powder and was used for EDX investigation. To perform EDX investigation, the leaf extract Ag-NPs were dehydrated and fall covered on to carbon layer. EDX investigation was then done using electron microscope (SEM) set

with EDX. EDX can be used to confirm the composition and distribution of the nanoparticles through spectrum and elemental mapping by using an EDX spectrometer incorporated into a scanning electron microscopy (SEM) system.

UV-Visual Observation

UV-Vis absorption spectra were measured using LKB-spectrophotometer.

Microorganisms

The evaluation of antibacterial action was done using various strains. The subsequent microorganisms were used: *Bacillus subtilis* (Thermo Fisher Scientific, AS Polyvalent 2 R691360, Waltham; USA) and *Escherichia coli* (Thermo Fisher Scientific, Set R761372, Waltham; USA). The microbial cultures were maintained by the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

Antibacterial activity study

Antiseptic action of the synthesized AgNPs was performed using the agar well diffusion examine process (Perez *et al.*, 1990). In this technique, disinfected Mueller- Hinton Agar plates were arranged. Pathogenic bacteria used in the current research were widening above the agar plates by sterile cotton wipe down. The plates were allowed to dry and a sterile well-cutter of diameter 5.0 mm was used to bore wells in the agar plates. Subsequently, a 50µl of the synthesized

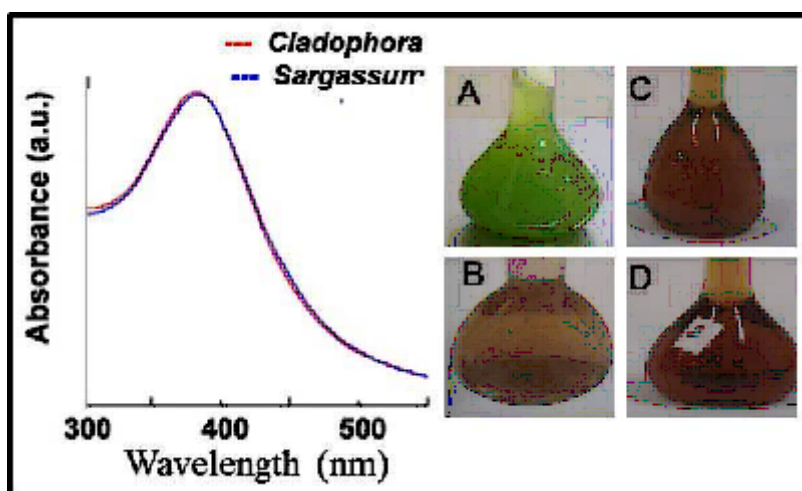


Fig.1. UV-Vis spectra viewing absorption presented as a purpose of 1mM AgNO₃ with aqueous extract of *Cladophora nitellopsis* and *Sargassum latiflillum*. Color development with time for *Cladophora nitellopsis* (A and C) and (B and D) for *Sargassum latiflillum*.

nanoparticle suspension (mass concentration=0.02 $\mu\text{g}/\mu\text{l}$) was introduced into wells of the inoculated Mueller – Hinton Agar plates. Another two concentrations 10 and 25 μl of the synthesized Ag-nanoparticles were used and introduced into wells of the Agar. The plates containing the bacterial and AgNPs were stand for 1h to allow diffusion to take place and then incubated at 37 $^{\circ}\text{C}$ for 24 h, and then observed for indication of zones of inhibition, which show as a clear region around the wells (Cheesbrough, 2000). The length of such inhibition zone was calculated using a meter ruler, and the significant value for each type of bacteria was documented and spoken in millimeters.

RESULTS

When the seaweed extracts were uniformed in aqueous solution of 1mM AgNO_3 , the reduction of Ag was measured by UV–Vis spectrum of the process. The reduction of AgNO_3 into AgNPs during contact to extracts is followed by a slow raise in color progress from clear yellow to brown (Fig.1). The absorption spectra of AgNPs formed, shows the creation of AgNPs with almost

100% algal reduction of Ag ions as supported by qualitative testing of supernatant after the decontamination of silver nanoparticles by heat (Fig.1).

SEM examination was approved out to recognize the topology, surface morphology and the dimension of the AgNPs, which viewed the production of higher density poly dispersed round Ag-NPs of different sizes that varied between 4.83-13.7nm for *Cladophora* and from 2.44-15.12 nm for *Sargassum*. The majority of the AgNPs collected and only some of them were spread, as examined under SEM (Fig.2 A and B). From the images, the nanoparticles are appearing to be aggregated and the surface of the aggregates is rough. Scanning electron micrograph (SEM) was equipped with energy dispersive spectroscopy (EDX). The presence of silver was confirmed from the Ag peak obtained from the EDX spectrum as shown in Fig. 2C and D.

Examination of AgNPs by Energy dispersive X-ray (EDX) spectrometer established the existence of elemental indication of the Ag and homogenous distribution of AgNPs (Fig.2C and D). The pointed sign peak of Ag powerfully established the reduction of AgNO_3 to AgNPs. The

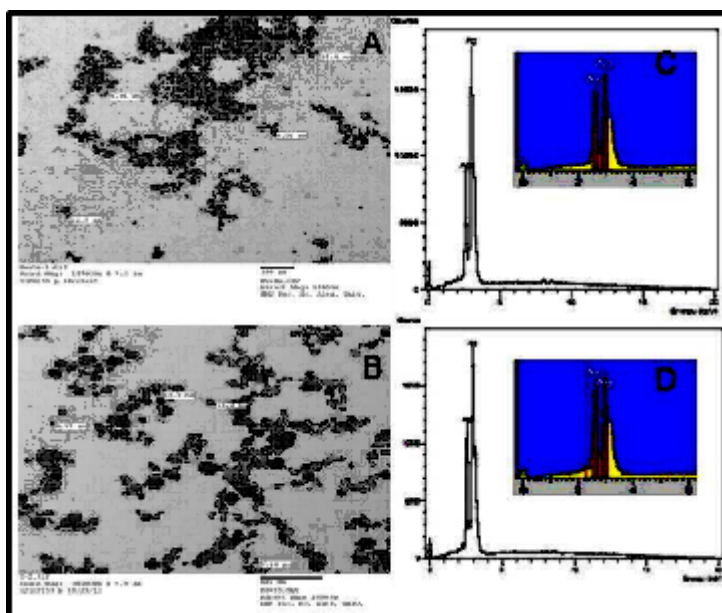


Fig.2. SEM images of AgNPs by leaf extract of (A) *Cladophora nitellopsis* and (B) *Sargassum latiflillum*. Analysis of Energy dispersive X-ray (EDX) spectrometer of the particles formed by leaves extract of (C) *Cladophora nitellopsis* and (D) *Sargassum latiflillum*

upright axis expresses the number of X-ray counts while the parallel axis shows energy in KeV. Detection lines for the main release energy for Ag were clarified and these communicate with peaks in the spectrum, thus giving affirmation that Ag has been properly recognized and present in the solution. The appearance of some other minute signals may be due to the thin film made on the glass slide taken for the EDX.

The X-ray diffraction patterns (XRD) of the formed AgNPs formed by extract of both seaweeds were additionally established by the distinguished peaks examined in the XRD image

(Fig. 3). The XRD pattern showed four intense peaks (26.24°, 30.86°, 45.16° and 54.33) in the full spectrum of 2θ value in between from 19 to 72.

Transmission electron micrographs give the closed view of spherical Ag nanoparticle and indicating that they are also spherical (Fig. 4). AgNPs were not well estranged from each other in the nanotriangles in the extent range 43–58 nm are covered with minor particles due to the occurrence of small crystal and hexagonal particles of about 11–28 nm in width on the triangular face.

The antibacterial activity for silver nanoparticles was done with gram positive bacterial

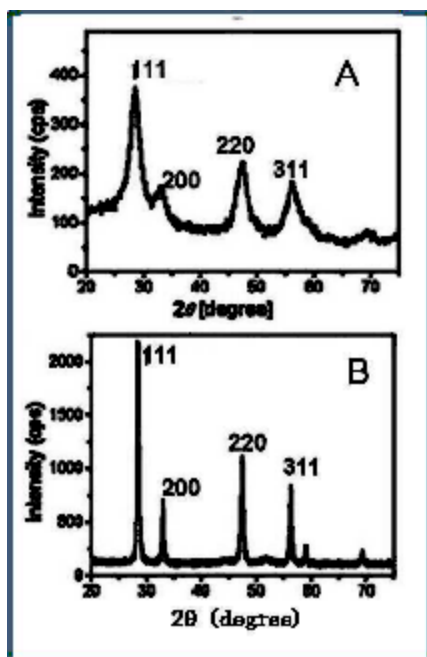


Fig. 3. The XRD pattern of biosynthesized AgNPs in aqueous solution of (A) *Cladophora nitellopsis* and (B) *Sargassum latiflillum*

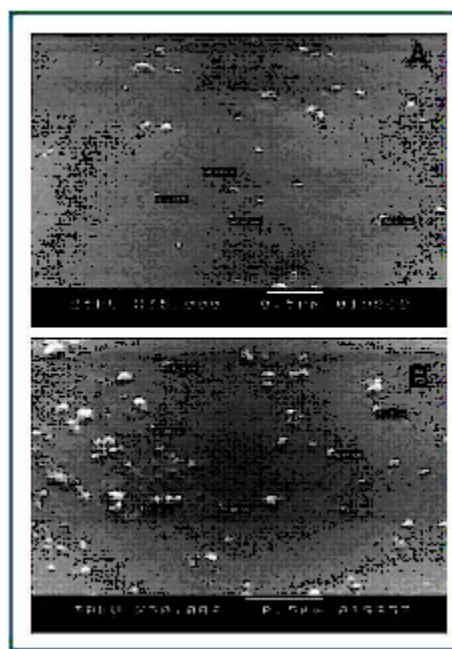


Fig. 4. TEM images and diameter vales of synthesized AgNPs by leaf extract of (A) *Cladophora nitellopsis* and (B) *Sargassum latiflillum*

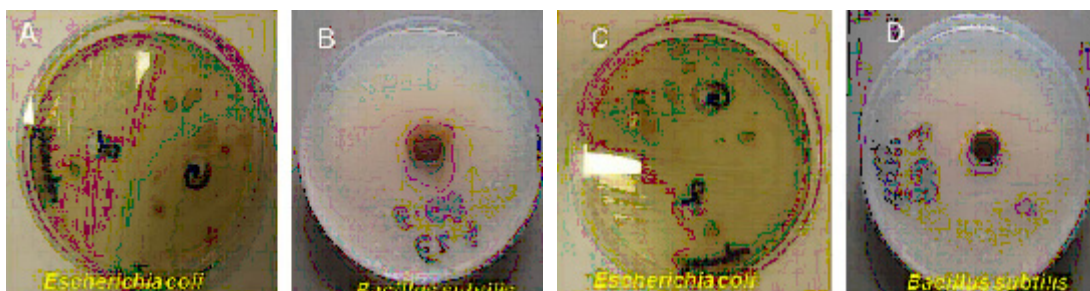


Fig. 5: Effect of AgNPs formed by leaves extracts of (A and B) *Cladophora nitellopsis* and (C and D) *Sargassum latiflillum* seaweeds on the bacterial growth of *Escherichia coli* and *Bacillus subtilis*.

strains like *Bacillus subtilis* and gram negative bacterial strains such as *Escherichia coli*. The inhibition zones caused by 50 µl of synthesized silver nanoparticles extract from *Cladophora* were reached to about 2.3 cm for gram positive strains *Bacillus subtilis* and 2.6 cm for *Escherichia coli* and the values was 3.0 cm for *Sargassum*. On the other side a less effect of Ag NPS on negative strains *Escherichia coli* was observed Fig 5. Lower antibacterial activity was observed for both 10 and 25 µl for gram positive and gram negative bacteria for both algae.

DISCUSSION

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution (Song and Kim, 2009). The out looking of the organized samples shows that considerable alter of the color of *Cladophora* and *Sargassum* algal extracts. This examination was extra established by with UV-Vis spectrophotometer as shown in Fig. 1. According to our results, it could be confirmed that *Cladophora* and *Sargassum* extracts were originate to display the reducing potential in terms of production rate and change to silver nanoparticles.

A little absorption band at 435 nm starts showing in the absorption spectra of the produced sample (0.1 ml sample). This band grew and blue changed from 435 nm to 495 nm with increasing time. This band related to the absorption by AgNPs in the visible area (380–450 nm) due to the occurrence of exterior Plasmon sensations (Njagi *et al.*, 2011). The raise of the peaks strength shows that the absorption of AgNPs was promoted (Zhang *et al.*, 2008). The symmetric and thin absorption peak indicates the fine size allocation of the AgNPs. The peak change of greatest absorption wavelength clarifies that the dimension of AgNPs decreases with increasing time. Our consideration here that, with prolonged time, the rate of nuclei impulsive rises and a high number of nuclei are created throughout the nucleation rupture. Thus, the amount of ending particles rises, and the variance of particle dimension therefore lowers. This examination obviously indicates the occurrence of reduction of AgNPs using *Cladophora* and *Sargassum* seaweed extracts.

The characterization of AgNPs by EDX

outline evidenced physically powerful signals for Ag atoms as shown in Fig.2. The EDX pattern clearly shows that the Ag nanoparticles are crystalline in nature, which is caused by the reduction of silver ions using *Cladophora* and *Sargassum* algal extracts.

The X-ray diffraction (XRD) has confirmed to be an important study means to show the creation of AgNPs, forming the crystal formation of the as-prepared AgNPs and to estimate the crystalline particle dimension.

The antibacterial activity of biologically synthesized silver nanoparticles from seaweed extracts of both *Cladophora* and *Sargassum* were evaluated against *Bacillus subtilis* and *Escherichia coli* showing more effective bactericidal activity in opposition to Gram-negative bacteria than gram-positive one. It could be suggested that AgNPs showed effective antibacterial properties owing to their exceptionally big exterior region, which provides superior contact with microorganisms and its interactions with bacteria are and localized on the membrane of the organism. Our results are consistent with Shrivastava *et al.*, 2007 who reported that the silver nanoparticles have an antimicrobial effect on *S. aureus* and *E. coli*. Similarly, Kim *et al.* (2007) proved bactericidal activities of AgNPs in opposition to *E. coli* and *S. aureus*. They suggested that the cause was quantity needy and was more prominent in opposition to Gram-negative organisms than Gram-positive ones.

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