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Synthesis of silver nanoparticles using *Ulva lactuca, Sargassum denticulatum, Spirulina platensis* and *Chlorella vulgaris*

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Abstract:

Nowadays, we need to develop eco-friendly nanoparticles synthesis process that does not use toxic chemicals in the synthesis methods. Biological synthesis of nanomaterials is cheaper, innovative and environmental friendly. From this point, the present study focused on the synthesis of silver nanoparticles from the extracts of *Ulva lactuca*, *Sargassum denticulatum*, *Spirulina platensis* and *Chlorella vulgaris*. Characterizations were performed by UV-Visible Spectroscopy (UV-Vis), X-ray Diffraction (XRD) and particle morphology, and size of silver nanoparticles were observed by transmission electron microscope (TEM) It was found spherical shaped nanoparticles sizes with average diameter of approximately 20,78.8, 31 and 50 nm from the extracts of the four algal species (*Ulva lactuca, Sargassum denticulatum, Chlorella vulgaris* and *Spirulina platensis*), respectively. So, the extracts from algae were screened for phytochemicals analysis followed by FT-IR to know the present chemical functional groups. Therefore, the present study illustrates silver nanoparticles can play an influential role in nanobiotechnology field in future.

Keywords: Characterization, Silver nanoparticles, *Ulva lactuca, Sargassum denticulatum, Chlorella vulgaris* and *Spirulina platensis.*

1. Introduction and Review of Literature:

The development of nanotechnology and synthesis of metal nanoparticles (NPs) is a big challenge. The fundamental building blocks of nanotechnology are the nanoparticles. The biological synthesis process of nanoparticles is evolving into an important branch of nanotechnology. Nanoparticles of free metals are attractive

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research materials because of their chemical activity, unique physical properties and potential applications in catalysis [1,2]. Also, nanoparticles play a vital role in a lot of fields such as biological labeling, drug delivery [3], biosensing, antifungal [4], environmental application [5], antibacterial activity and antiviral activity

The development of nanoscience is a valuable gift for the development of all branches of science, and although much research has been done in recent decades there are still considerable gaps in knowledge about the biotechnology potential of synthetic green nanoparticles. Biosynthesis of NPs is low-cost, eco-friendly and reduces the use of toxic chemicals and solvents. The use of biological nanoparticles in medicine reduces any side effects and increases equipment efficiency [6,7].

Numerous species of microalgae have been exploited through the use of their extracts in the biosynthesis of nanomaterials. For example, the fine powder of *Spirogyra insignis* was used in the biosynthesis of gold NPs and silver NPs. There are a lot of methods for the biological synthesis of Ag-NPs such as the cell-free filtrate of disrupted cells of *Euglena gracilis* and *Euglena intermedia*, the cell-free extract of *Amphora* sp. and the cell-free supernatant of *Anabaena* sp., *Cylindrospermopsis* sp., *Lyngbya* sp., *Limnothrix* sp., *Synechocystis* sp., *Synechococcus* sp., *Microcoleus* sp. and *Spirulina platensis* [8,9].

Biological production of nanoparticles by algae takes relatively shorter time than the other biosynthesizing procedures [10,11]. Therefore, several seaweeds such as *Chaetomorpha linum* [12], *Enteromorpha flexuosa* [13], *Fucus vesiculosus* [14], *Turbinaria conoides* [15], *Sargassum wightii* [16], *Stoechospermum marginatum* [17], *Ulva fasciata* [18] and *Ulva reticulata* [19] have been used for the biological synthesis of AgNPs with different sizes and shapes to use in different scientific purposes.

Chlorella vulgaris and *Spirulina platensis* have been used for the extracellular synthesis of gold and silver NPs because their extracts have the strong ability of binding [20, 21].

2- Materials and Methods:

-Culturing of microalgae

Spirulina platensis and *Chlorella vulgaris* were obtained from the National Institute of Oceanography and Fisheries, in land water and aquaculture branch. *Spirulina platensis* and *Chlorella vulgaris* were cultivated on culture media of the authors [22, 23, 24] Zarrouk and Blods basal media (BBM), respectively.

-Biomass collection

The biomass was collected after filtration for *Spirulina plantensis* and centrifugation for *Chlorella vulgaris*, then air dried and weighed.

-Collection of seaweeds

Seaweeds were collected from the intertidal zone of the red sea. *Ulva lactuca was* collected during spring season from Ras El-adabiya, which located on the western shore of Suez Bay at latitude, longitude (29.681737, 32.508970), respectively. Where, *Sargassum denticulatum* was collected during summer season from Ras Sedr, which located north–east of the Gulf of Suez at latitude, longitude (29.622328, 32.687970), respectively**.**

-Preparation of seaweed for algal extracts:

Seaweeds were carefully washed by seawater, collected in plastic bags and kept in an ice –box at 20°C. The frozen seaweeds were lifted to thaw and washed with distilled water to get rid of salts, then lift to dry in indirect light. After dryness, the samples were ground for the following analysis.

-Preparation and algal aqueous extraction for the biological production of silver nanoparticles

Five gram of each algal species (*Ulva lactuca*, *Sargassum denticulatum*, *Spirullina platensis* and *Chlorella vulgaris*) were ground by using a mortar and pestle, thereafter, extracted by incubation at 37ºc for 24 hours with 50ml water and 60% methanol separately as shown in Figure (1). The extracts were boiled and carried out in dark at room temperature using Ultrasonicator. The mixture was subsequently centrifuged at 5000 rpm for 20 minutes at 4 °C and filter by using sterilized 0.2 µm membrane syringe [25].

Fig.1: Algal extracts for the biological production of silver nanoparticles.

-Phytochemical analysis for the algal extracts

-Total sugars were measured by using a phenol–H_2SO_4 reaction, with d-fractose standard [26].

- Protein content was determined by using Coomassie Brilliant Blue reagent and bovine serum albumin as standard [27].

- Phenolic compounds were determined by the Folin Ciocalteau method [28].

-Determination of flavonoid content [29].

-Determination of total sterols in the algal extracts by using acid hydrolysis and extraction method [30].

- Synthesis of silver nanoparticles

Five ml of each algal extract was dissolved in 95 ml of 1mM $AgNO_3$ solution and were kept in the water bath at 60°C for 15 mins (dark). We monitored the color change from15 mins to 30 mins with constant pH maintenance as shown in Figure (2). Stability was monitored periodically by spectral analysis [31].

Fig. 2: Synthesis of silver nanoparticles and change in color.

-Characterization of silver nanoparticles:

- Double beam UV-Visible Spectrophotometer (Perkin Elmer).
- The particle size, polydispersity index and zeta potential of silver nanoparticles were performed by Dynamic Light Scattering (DLS) using Malvern Zetasizer (Malvern Instruments, UK) with a wave length of 532 nm at 25°C with an angle detection 90º .
- Particle morphology and size of silver nanoparticles were observed by TEM (Philips CM200, Mahwah, NJ, USA).
- XRD diffraction patterns of the samples were recorded on an X-ray diffractometer (Panlytical X'pert Pro, Philips, Netherlands) with wavelength λ = 0.154 nm at a voltage of 45 kV and a current of 30 mA according to [32]. The scanning rate was 3°/min and the scanning scope of 2θ was from 4° to 80° in a continuous scan mode in steps of 0.02 at room temperature.
- Concentration of the samples was determined by Inductively Coupled Plasma (ICP) Atomic Emission Spectrometer as shown in Figure (3).

Fig. 3: Inductively Coupled Plasma (ICP) Atomic Emission Spectrometer.

- FT-IR analysis was done according to [33] to confirm the chemical interaction. Powdered samples by a freeze-drying system were loading on KBr pellets under 1:99 ratio, respectively. The results were recorded by ALPHA II (Bruker Optik GmbH, Germany), in the range of 4000 to 400 cm^{-1}.

3. Results and Discussion

3.1 Results

-Determination of chlorophyll content for *Spirulina plantensis* and *Chlorella vulgaris*

Chlorophyll content for *Spirulina plantensis* was shown in Fig.4. It revealed that its minimum value of 0.191 mg/l was recorded in 2 nd day, while its maximum value of 1.321 mg/l was recorded in 14th day.

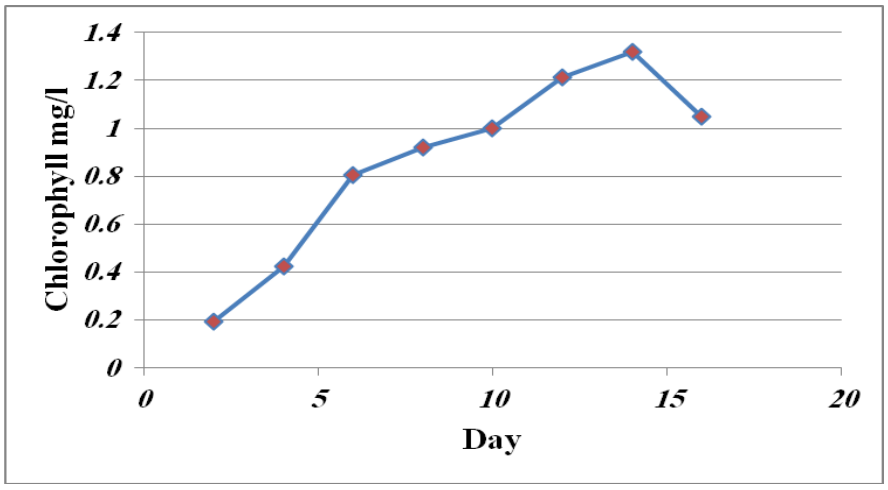

Fig.4: Chlorophyll content curve for *Spirulina plantensis.*

Chlorophyll content for *Chlorella vulgaris* was shown in Fig.5. It revealed that its minimum value of 0.232 mg/l was recorded in 2 nd day, while its maximum value of 1.23 mg/l was recorded in 14th day.

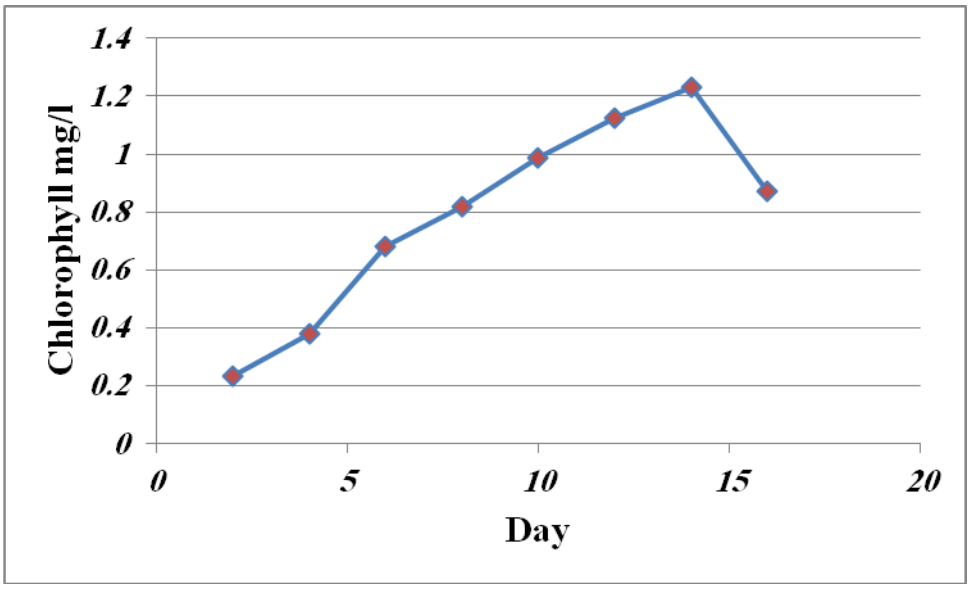

Fig.5: Chlorophyll content curve for *Chlorella vulgaris.*

-Phytochemical analysis for the algal extracts

Results of total sugars (%) for the algal extracts are shown in Fig.6. It revealed that its minimum value of 44.59 % was attained by *Sargassum denticulatum*, while its maximum value was 51.35 % was attained by *Chlorella vulgaris.*

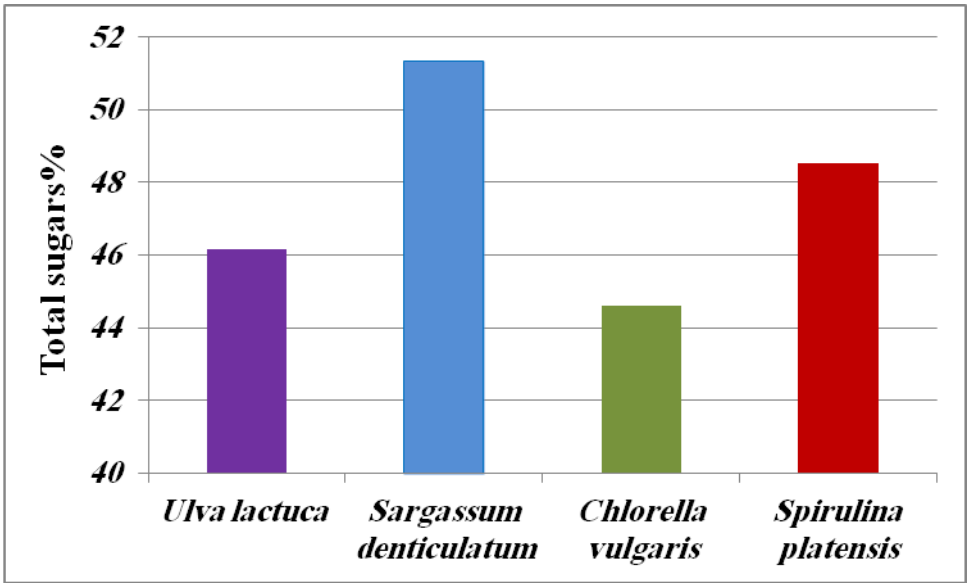

Fig.6: Total sugars (%) (Means values) for the algal extracts.

Results of total protein content (%) for the algal extracts are shown in Fig.7. It revealed that the minimum value of 8.24 % was recorded by *Sargassum denticulatum*, while its maximum value was 11.56 % was recorded by *Spirulina platensis*.

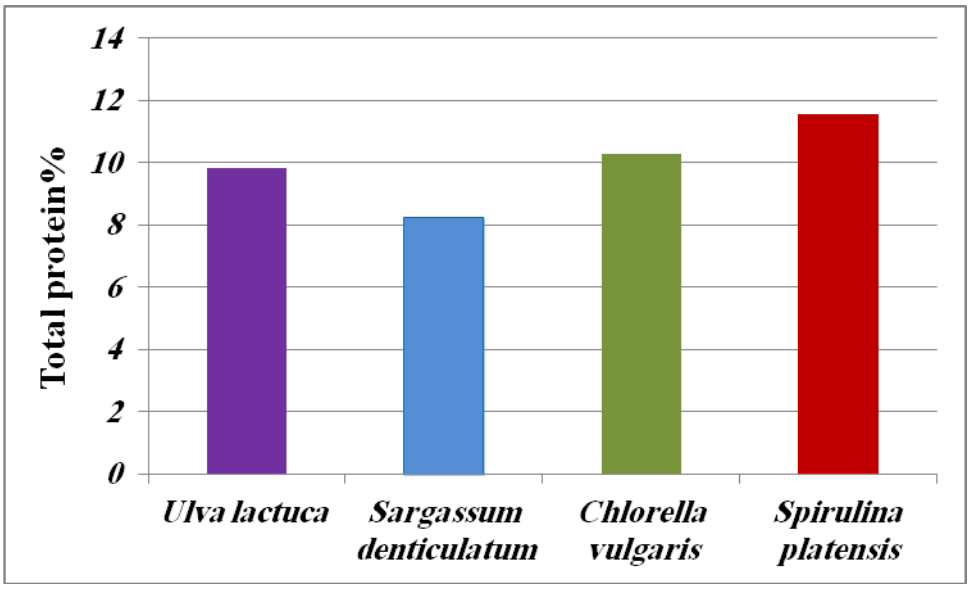

Fig.7: Total protein content (%) (Means values) for the algal extracts.

Results of phenolic compounds (%) for the algal extracts are shown in Fig.8. It revealed that its minimum value of 0.421% was recorded by *Sargassum denticulatum*, while its maximum value of 0.82 % was recorded by *Spirulina platensis*.

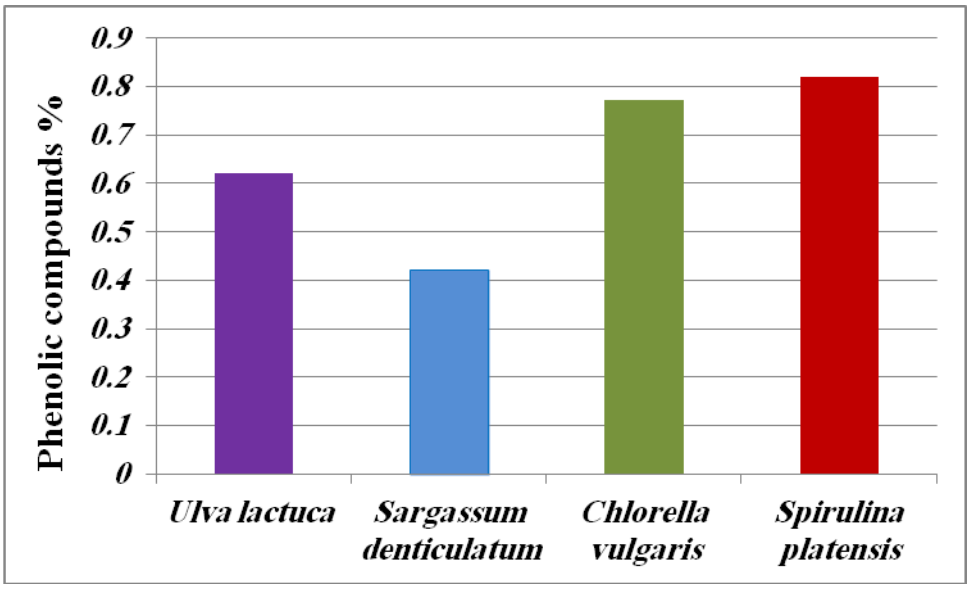

Fig.8: Phenolic compounds (%) (Means values) for the algal extracts.

Results of flavonoids (%) for **t**he algal extracts are shown in Fig.9. It revealed that its minimum value of 0.191% was recorded by *Sargassum denticulatum*, while its maximum value 0.46% was recorded by *Spirulina platensis.*

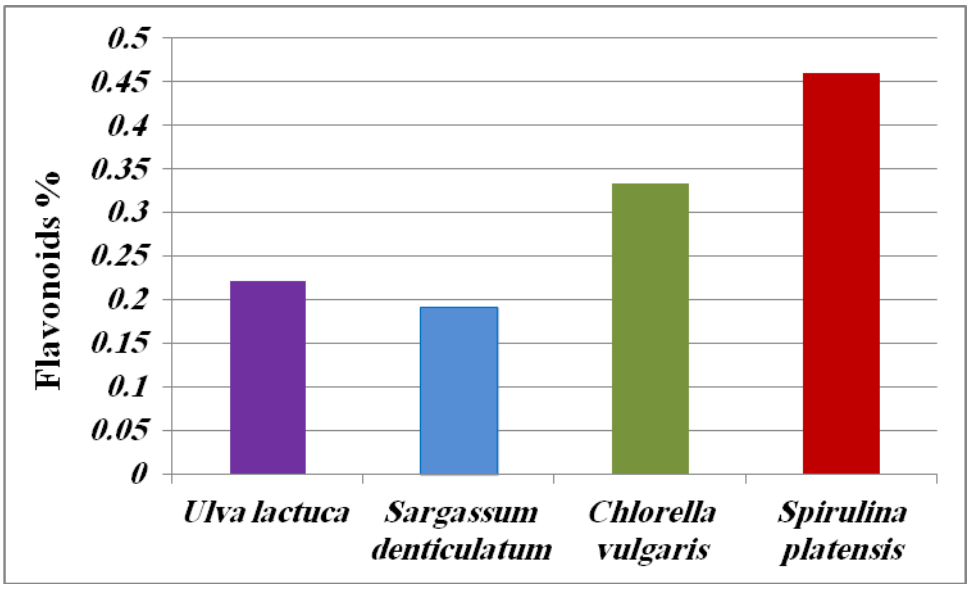

Fig.9: Flavonoids (%) (Means values) for the algal extracts.

Results of sterols (%) for the algal extracts are shown in Fig.10. It revealed that its minimum value of 1% was recorded by *Spirulina platensis*, while its maximum value of 4.07 % was recorded by *Chlorella vulgaris.*

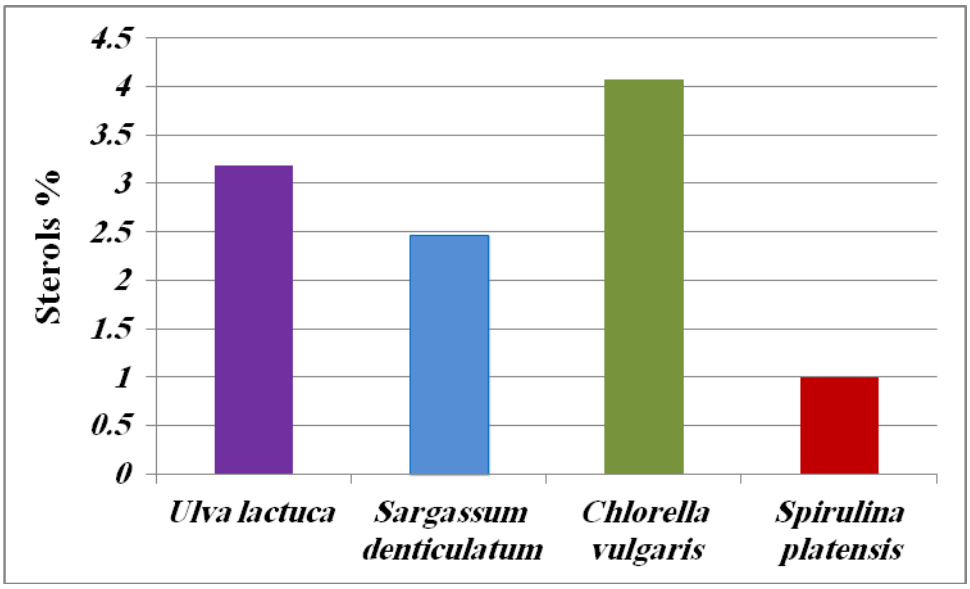

Fig.10: Sterols (%) (Means values) for the algal extracts.

- Characterization of silver nanoparticles:

-- UV–visible absorption

Figs. 11, 12, 13 and 14 show the UV absorption spectra of the synthesized silver nanoparticles using the four algal extracts recorded as the function of reaction time. The spectrum illustrates that the peak at 416 nm illustrated the synthesis of silver nanoparticles by *Chlorella vulgaris* extract, 420 nm by *Sargassum denticulatum* extract, 430 nm by *Ulva lactuca* extract and 379 nm by *Spirulina platensis* extract.

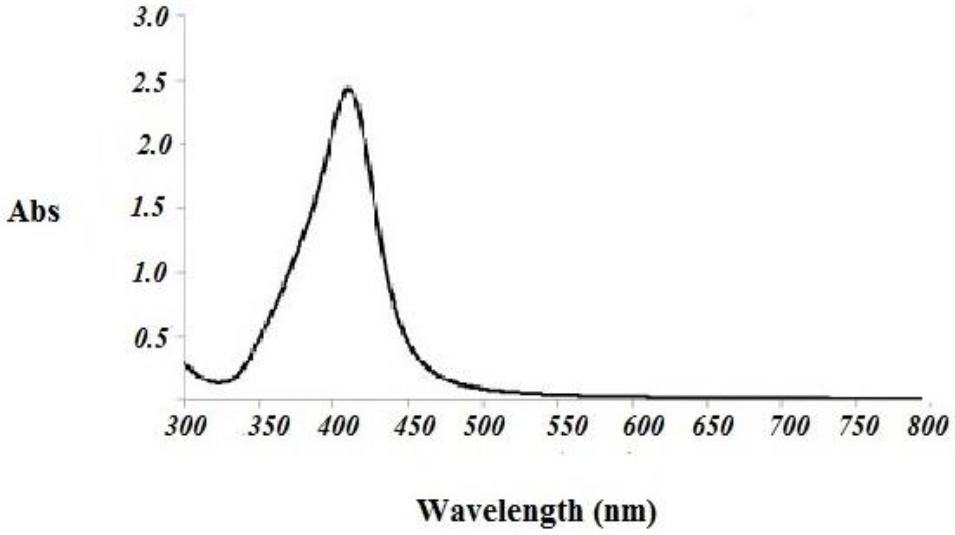

Fig.11: UV-Visible absorption spectra for Ag-NPs synthesized by *Ulva lactuca* extract.

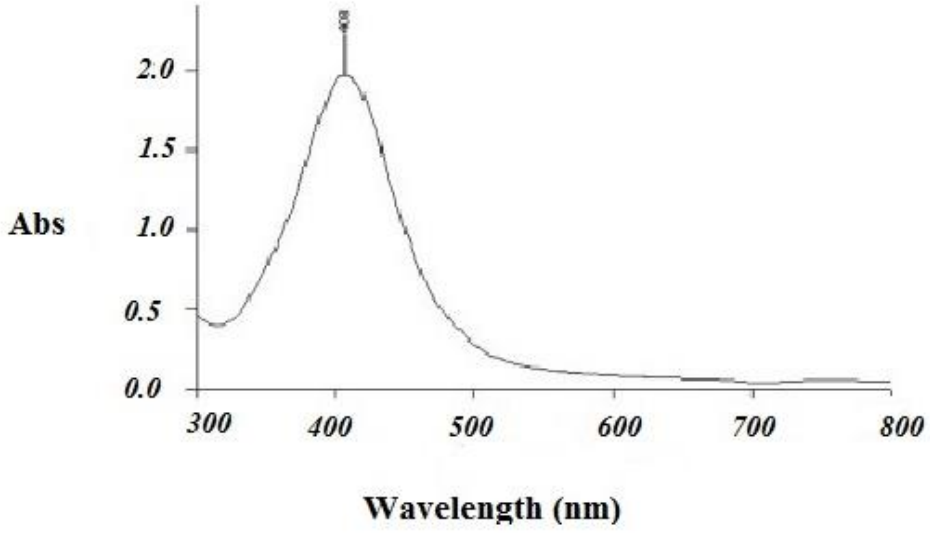

Fig.12: UV-Visible absorption spectra for Ag-NPs synthesized by *Sargassum denticulatum* extract.

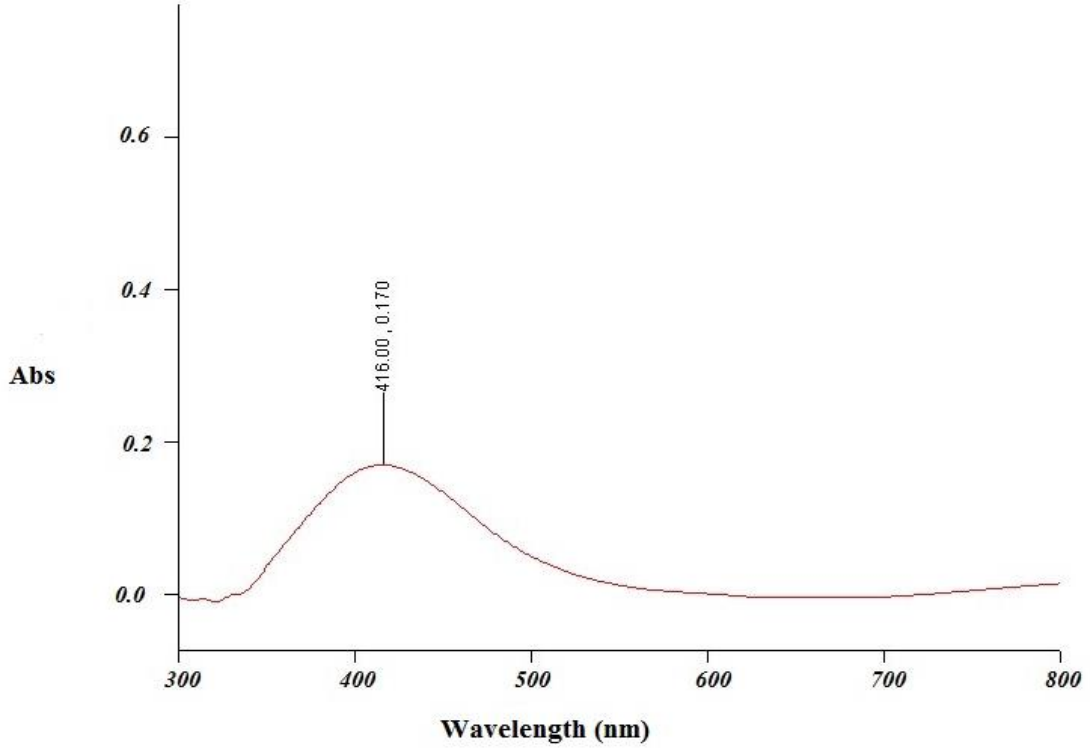

Fig.13: UV-Visible absorption spectra for Ag-NPs synthesized by *Chlorella vulgaris* extract.

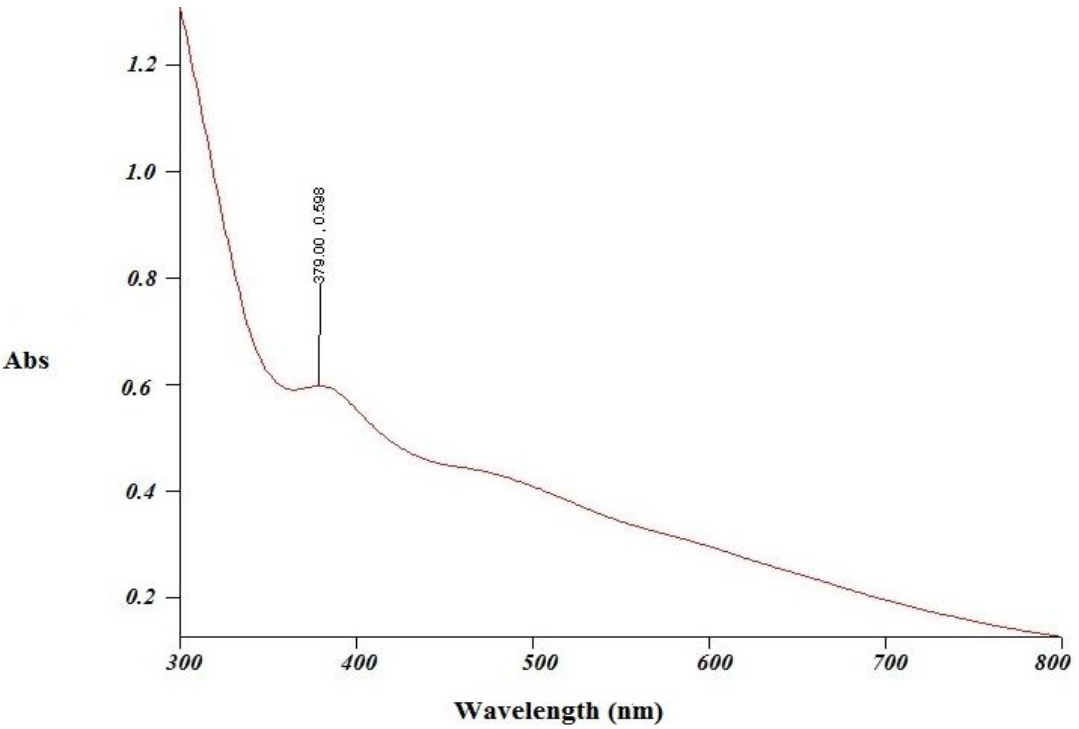

Fig.14: UV-Visible absorption spectra for Ag-NPs synthesized by *Spirulina platensis* extract.

-The x-ray diffraction patterns:

The XRD pattern (Fig.15) indicates the synthesized silver nanoparticles are highly purified, no other substances appear. Also, no peaks of the XRD pattern of Ag_2O. The nanoparticles had a spherical structure.

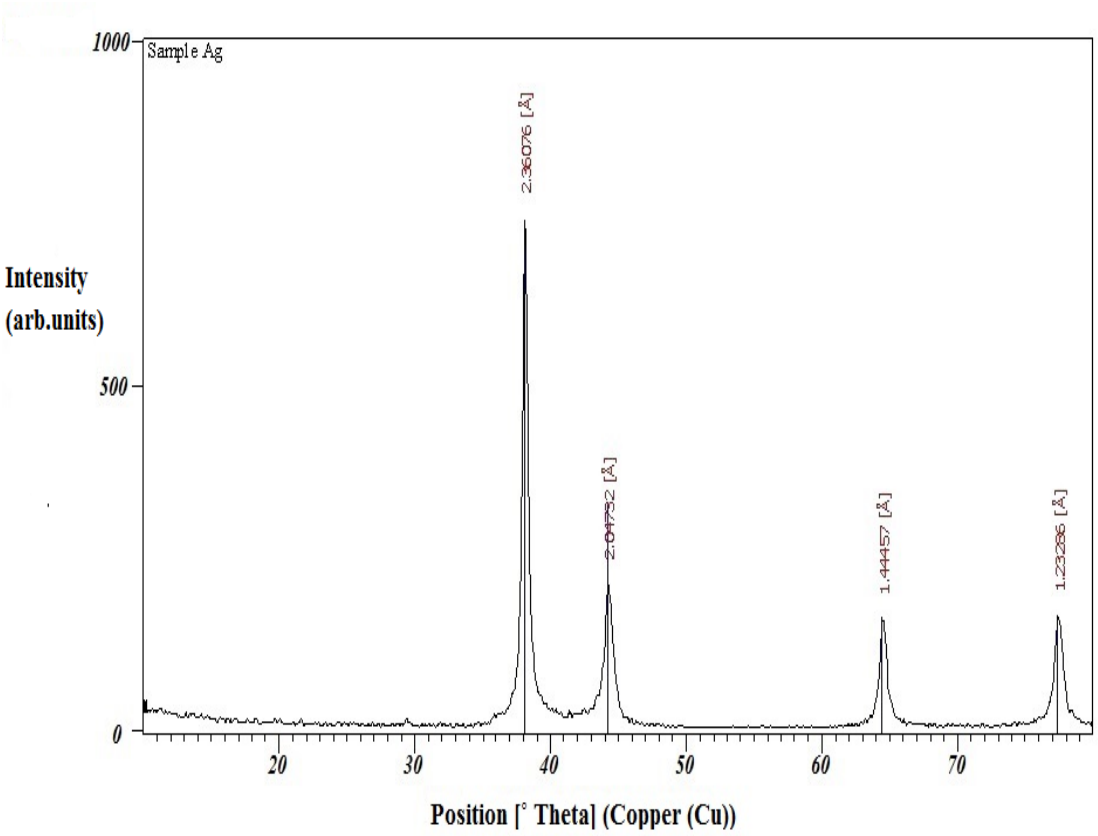

Fig.15: XRD patterns of Ag-NPs nanoparticles**.**

-Particle size and zeta potential

Dynamic light scattering (DLS) cleared that diameters of Ag-NPs in suspension were approximately 25.23, 84.46 , 33.44 and 51.56 nm from the four algal extracts (*Ulva lactuca, Sargassum denticulatum, Chlorella vulgaris* and *Spirulina platensis*) respectively as shown in Figs. 16, 17, 18 and 19.

Zeta potential of Ag-NPs were approximately 29.8, -26, , -29 and -28.8 mV from the four algal extracts (*Ulva lactuca, Sargassum denticulatum,Chlorella vulgaris,* and *Spirulina platensis*), respectively as shown in Figs. 16, 17, 18 and 19.

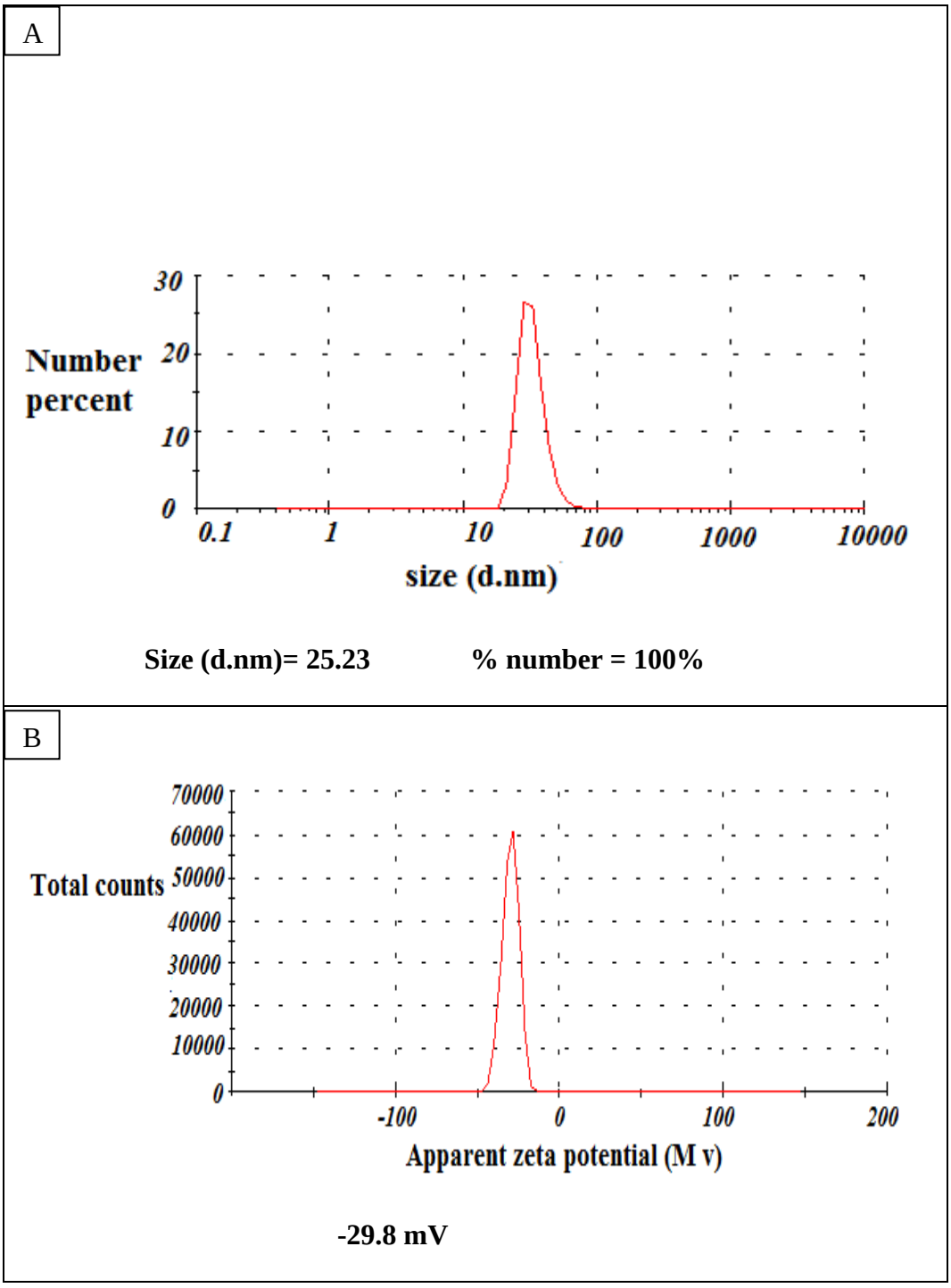

Fig.16: The size distribution by number (A) and zeta potential distribution (B) of Ag-NPs nanoparticles synthesized from *Ulva lactuca* extract.

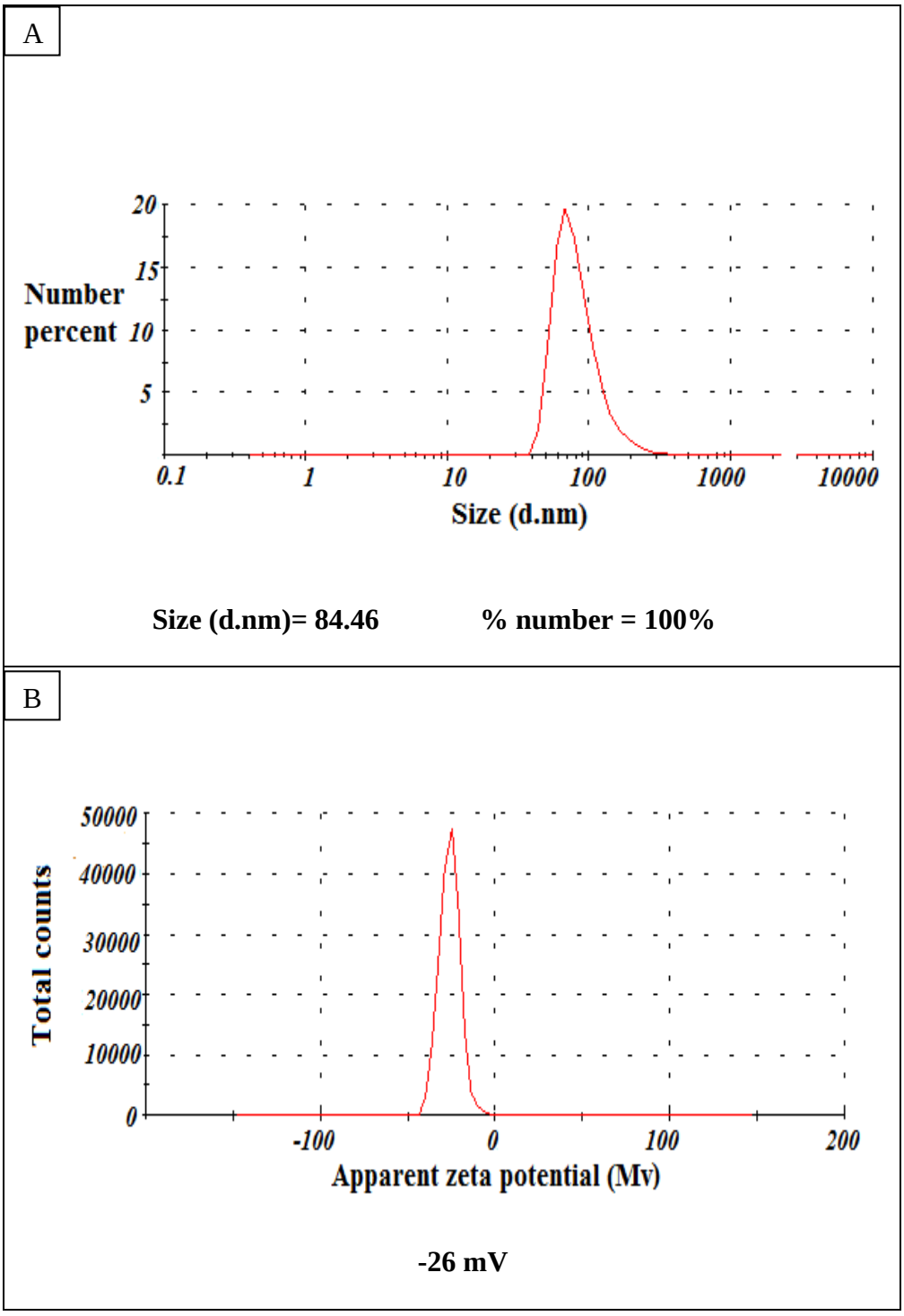

Fig.17: The size distribution by number (A) and zeta potential distribution (B) of Ag-NPs nanoparticles synthesized from *Sargassum denticulatum* extract.

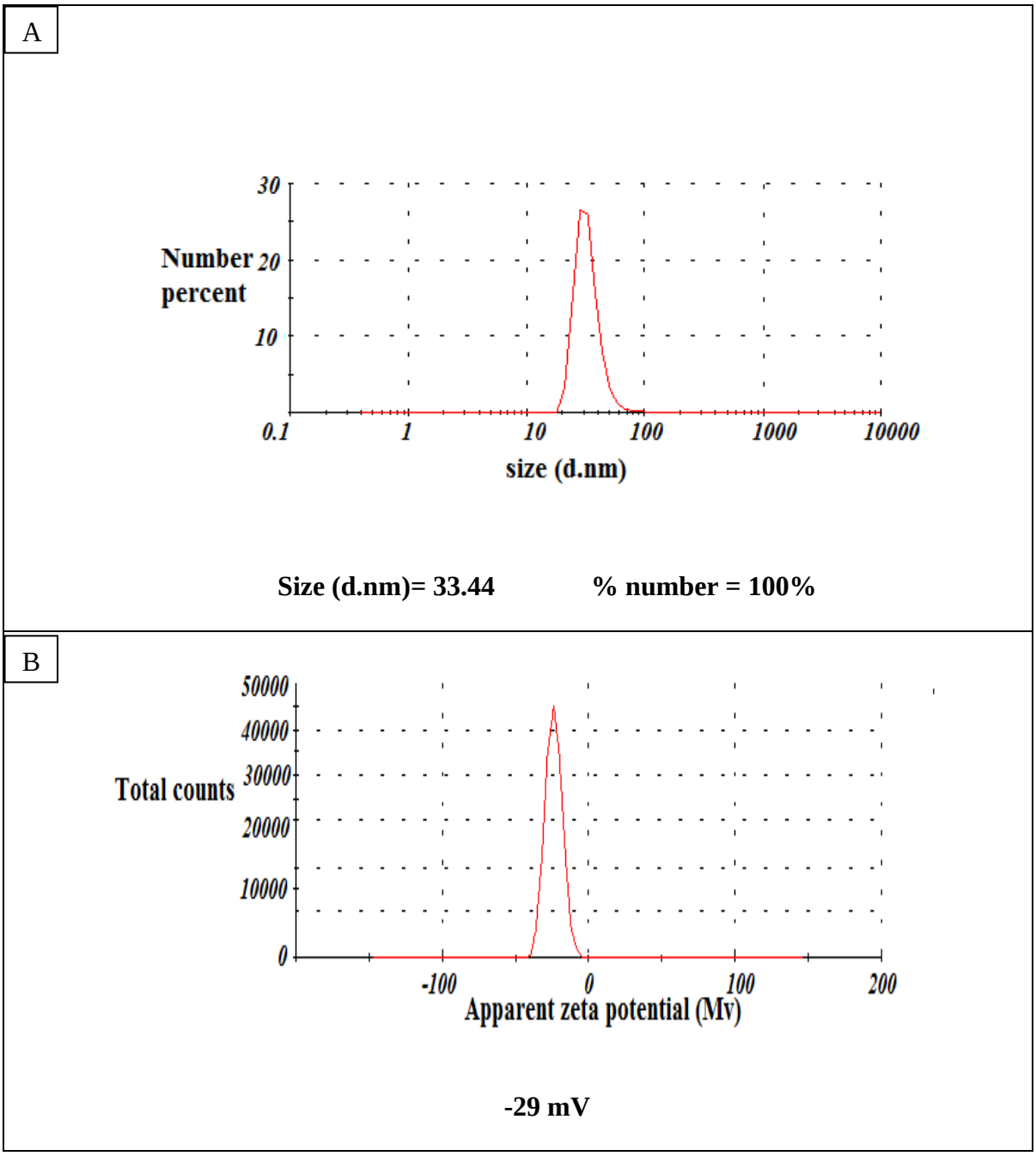

Fig.18: The size distribution by number (A) and zeta potential distribution (B) of Ag-NPs nanoparticles synthesized from *Chlorella vulgaris* extract.

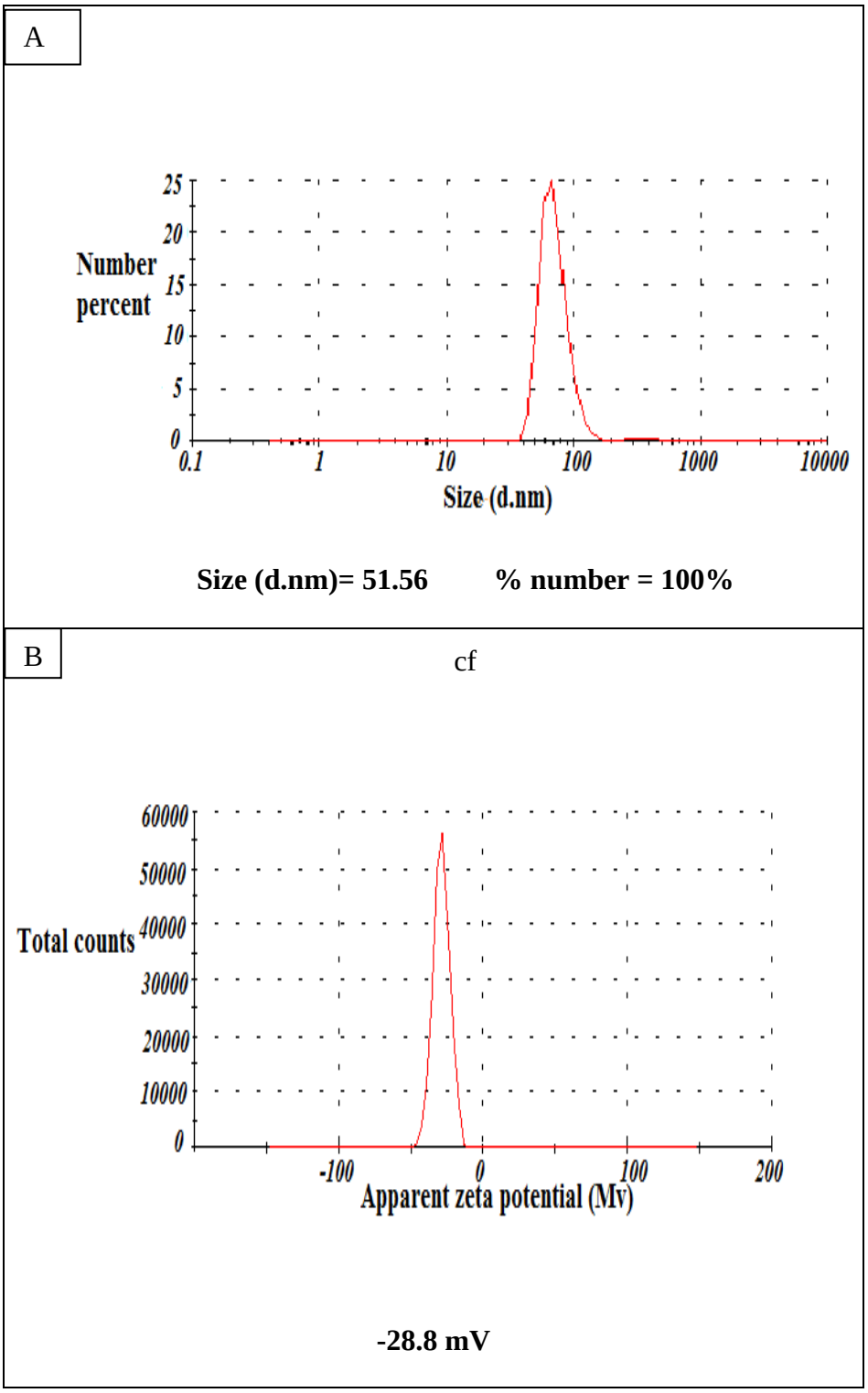

Fig.19: The size distribution by number (A) and zeta potential distribution (B) of Ag-NPs nanoparticles synthesized from *Spirulina platensis* extract.

-Particle morphology and size:

Transmission Electron Microscopy (TEM) showed a homogenous size distribution and a spherical shape with average diameter of approximately 20, 78.8, 31 and 50

nm from the four algal extracts (*Ulva lactuca*, *Sargassum denticulatum*, *Chlorella vulgaris* and *Spirulina platensis*), respectively as shown in Photos 1, 2, 3 and 4.

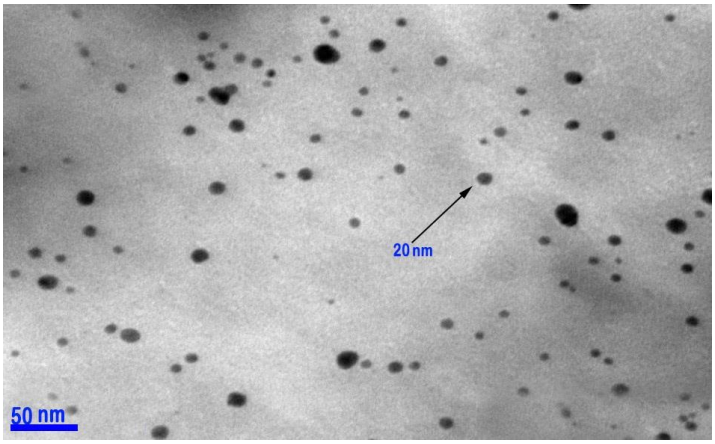

Photo. 1: Transmission Electron Microscopy image for Ag-NPs nanoparticles synthesized from *Ulva lactuca* extract.

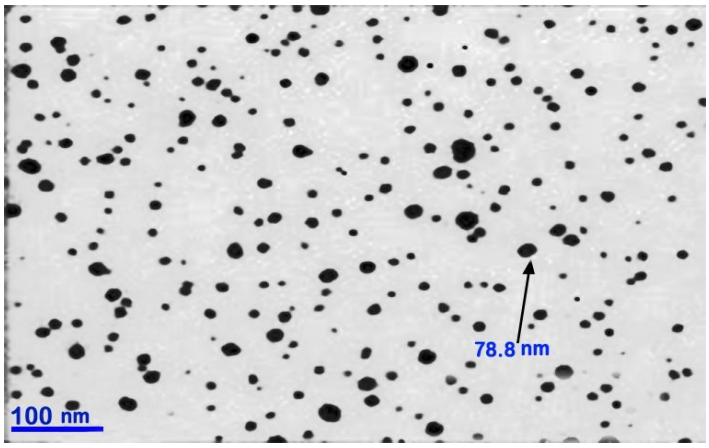

Photo. 2: Transmission Electron Microscopy image for Ag-NPs nanoparticles synthesized from *Sargassum denticulatum* extract.

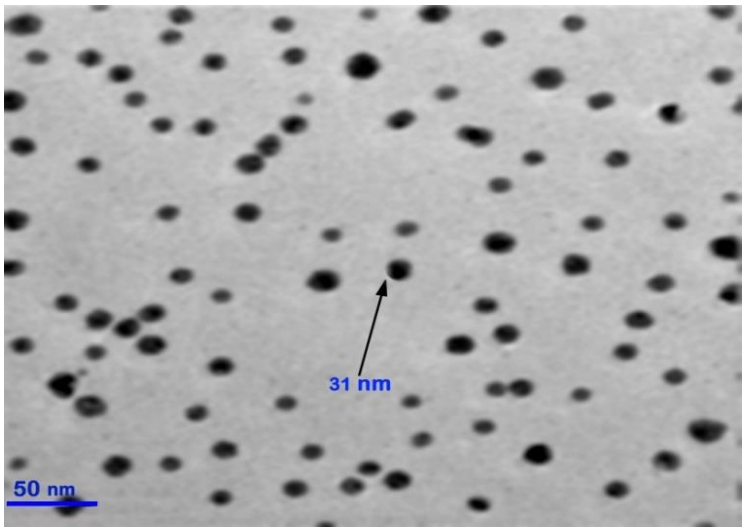

Photo 3: Transmission Electron Microscopy image for Ag-NPs nanoparticles synthesized from *Chlorella vulgaris* extract.

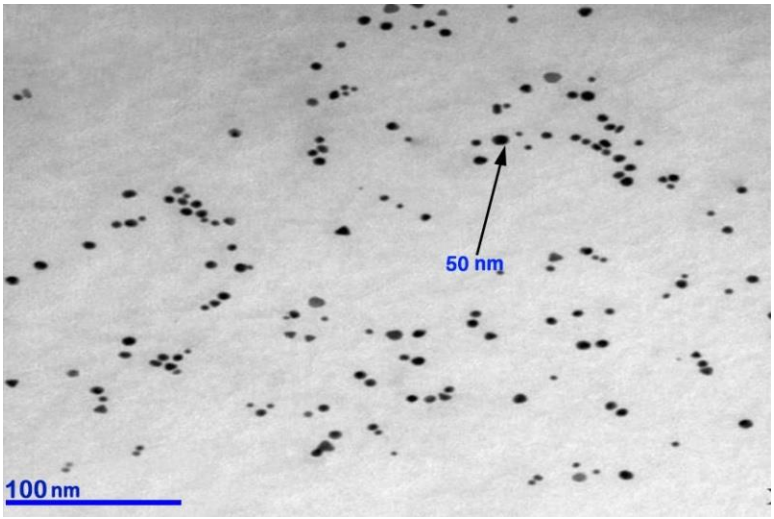

Photo. 4: Transmission Electron Microscopy image for Ag-NPs nanoparticles synthesized from *Spirulina platensis* extract.

-FT-IR analysis:

Fourier Transforms Infrared Spectroscopy analysis was made to know the functional groups on *Ulva Lactuca* extract and prophesy their role in the biosynthesis of AgNPs.

Data in Fig. 20 illustrated the band intensities in different regions of the spectrum. It showed the presence of O-H stretching which was observed by the peaks

around 3320–3427 cm^{-1}, the C-H stretching symmetric and antisymmetric aliphatic and aromatic modes respectively at 2972 and 2879 cm^{-1}.

Also, Fig.20 showed that the amides II and III appeared at 1539 and 1235 cm^{-1} respectively. Also, the secondary structure of the protein between 1735 and 1238 cm^{-1}.

In addition, significant peaks were detected at 2851, 1383 and 1466 cm^{-1} and showed the function of aromatic groups in the reduction of silver ions.

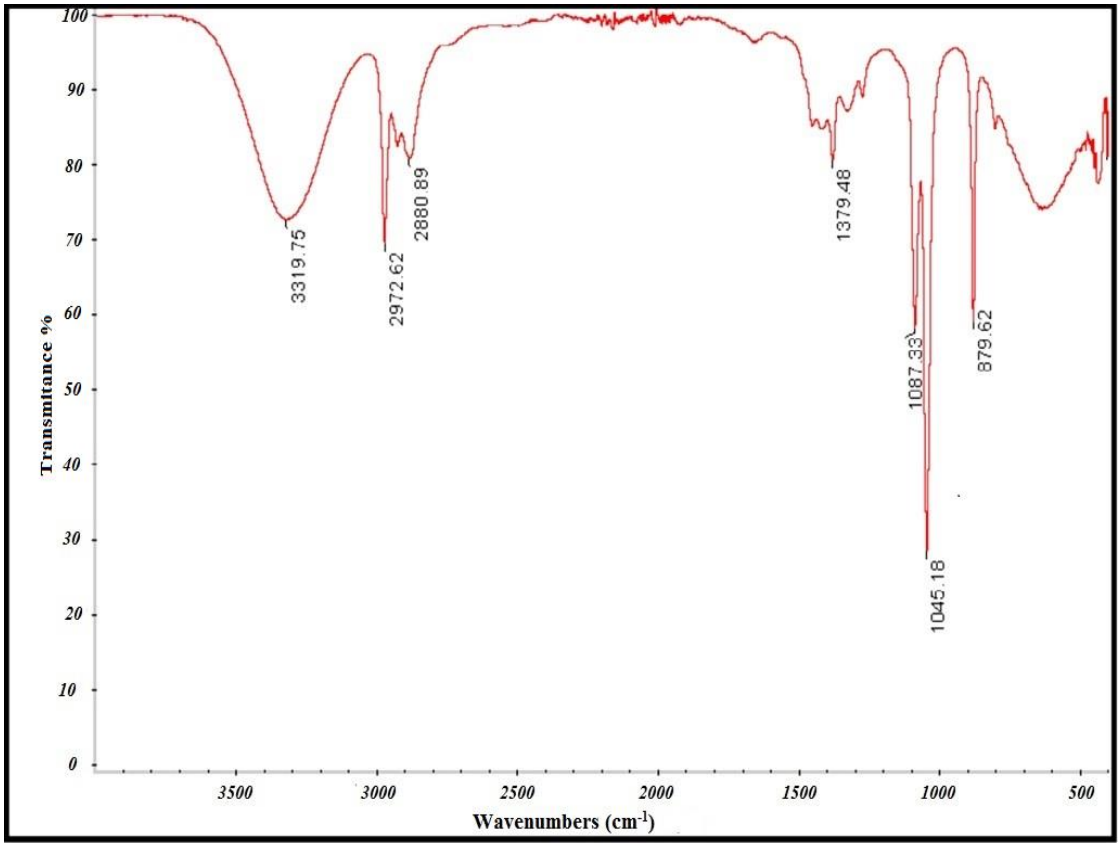

Fig.20: FT-IR spectrum of *Ulva lactuca* extract.

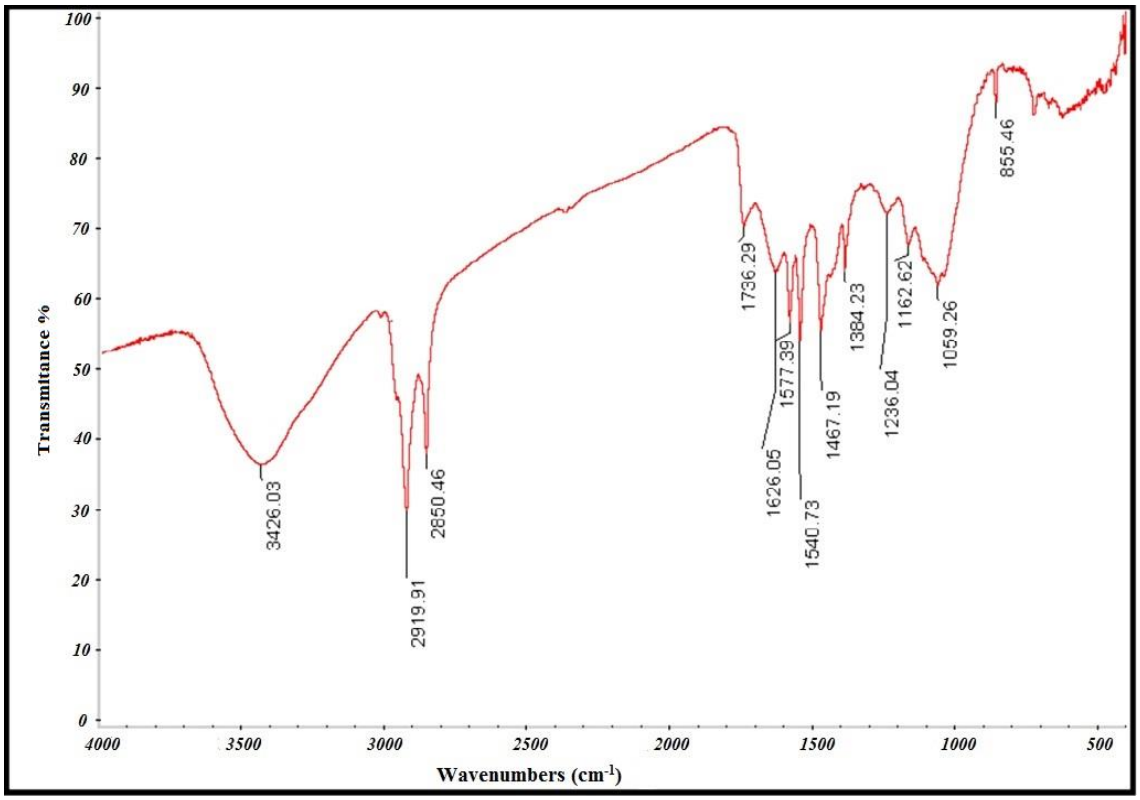

Fig.21: FT-IR spectrum of silverNPs synthesized by *Ulva lactuca* extract.

3.2 Discussion:

The present investigation of total sugars (%) for the algal extracts in Fig.6 agrees with that of [34]. Also, [35] mentioned that the total sugar content in both cold and hot water extracts of some Indian marine green algae were >20 %. As well as, [36] mentioned that sugar content in extracts of the brown algae *Turbinaria ornata* and *Padina tetrastromatica* were 56.42% and 50%, respectively. The current results may be because the carbohydrate content, which relatively influenced by heating.

The present results of total protein contents (%) for the algal extracts (Fig.7) were in agree with that of [37]. Also, [35] mentioned that species of *Ulva* and *Caulerpa* contained 7.0-10 % protein in cold and hot extracts. These results were higher than that found by [38], who reported that the protein contents isolated from marine green algae *Monostroma nitidum* were 0.66–0.99%. This may be due to use of different methods of extraction.

The present results of flavonoids (%) for the algal extracts (Fig.8) were in agreement with a lot of studies, that have illustrated that algae are capable of forming *p*-coumaric acid, the fundamental block of the flavonoid synthesis as the enzyme PAL

has been revealed in the microalgae *Chlorella pyrenoidosa* [39], as well as in the cyanobacteria *Anabaena variabilis* and *Nostoc punctiforme*, suggesting that this enzyme was already present in the ancestors of the chloroplasts [40]. The current results of sterols (%) for the algal extracts (Fig.9) agree with [41].

The current results of the UV absorption spectra of the synthesized silver nanoparticles using the four algal extracts recorded as the function of reaction time Figs. 11, 12, 13 and 14 agreeing with that of [15] who made biosynthesis of silver nanoparticles by the extract of the marine brown alga *Turbinaria conoides.* Also, [42] supported these observations.

The current results of XRD pattern Fig.15 showed that silver ions had been reduced to Ag^0 by using the four algal extracts under reaction conditions. Also, the synthesized silver nanoparticles are highly purified. These results agree with that of [25] who synthesized silver nanoparticles using green and brown seaweeds. Also, [43] supported these observations.

The present results of particle size and zeta potential (Figs. 16, 17, 18 and 19) in agree with that of [44], who made biosynthesis of silver nanoparticles (19 nm) from *Spirulina* microalgae. Also, [45] who made a biological synthesis of silver nanoparticles using the green alga *Pithophora oedogonia* (34.03 nm) supported these observations. In addition, [25] made a synthesis of silver nanoparticles using green seaweeds (*Ulva reticulata* and *Enteromorpha compressa*) with sizes ranging between 40 and 50 nm approximately.

Furthermore, [46] made silver nanoparticles by *Spirogyra* sp., which were spherical in shape and in the range of 40-80 nm and studied the stability of silver nano-particles in aqueous and organic medium.

The current results of morphology and particle size for the synthesized AgNPs by Transmission Electron Microscopy (Photos 1, 2, 3 and 4) in agree with that of [47], who made a green synthesis of AgNPs by using the marine brown alga *Padina pavonia* (49.58–86.37 nm). Also, these results corroborate the findings by [48], who made the screening of cyanobacteria and microalgae for their potential to synthesize AgNPs that ranged between (13 and 31nm).

The present results of Fourier Transforms Infrared Spectroscopy in Figs. 20 and 21 are supported by [14], who mentioned that hydroxyl groups (OH) which are

plentiful in polysaccharides responsible for the reduction process. Also, these results were supported by the findings of [49].

4. Conclusion:

In this study, it has been demonstrated that the algal extracts for *Ulva lactuca, Sargassum denticulatum, Spirulina platensis* and *Chlorella vulgaris* as a reducing agent can effectively produce spherical shaped silver nanoparticles by the biological method. The biosynthesized silver nanoparticles were characterized by UV–vis absorbance, FTIR, particle size, zeta potential and TEM. It was detected by the phytochemical analysis for the algal extracts that the functional groups present in the extracts have reduced the silver ions into Ag^0. Finally, it has been concluded that algae have the potential in the future to produce other valuable nanostructures in the emerging field of nanobiotechnology.

5. References:

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الملخص العربى

تصنيع جسيمات الفضة النانونية باستخدام ***Spirulina ,Sargassum denticulatum , Ulva lactuca*** ***Chlorella vulgaris و platensis***

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فى الوقت الحالى ، هناك حاجة متزايدة لتطوير عملية تخليق جسيمات نانوية صديقة للبيئة لا تستخدم مواد كيميائية سامة فى بروتوكولات التصنيع. تصنيع المواد النانوية بالنهج البيولوجى بالاضافة انه صديق للبيئة يتطلب عمالة أقل. فى هذا الصدد ، ركزت الدراسة الحالية على تخليق جزيئات الفضة النانوية من مستخلصات *Chlorella vulgaris* و *Spirulina platensis* ,*Sargassum denticulatum* , *Ulva lactuca*. تم إجراء التوصيفات بواسطة التحليل الطيفى للأشعة فوق البنفسجية المرئية (UV-Vis) ، وانحراف الأشعة السينية (XRD) وتحديد الشكل المورفولجى وحجم جسيمات الفضة النانونية بواسطة جهاز ال TEM .وجد أن أحجام الجسيمات النانوية الكروية الشكل بمتوسط قطر يبلغ حوالى 20, 78.8, 31و50نانومتر من مستخلصات الطحالب الأربعة (*Ulva lactuca*, *Sargassum denticulatum* ,*Chlorella vulgaris* و *Spirulina platensis*) على التوالي. إجمالاً ، تم فحص المستخلصات من الطحالب بحثًا عن المواد الكيميائية النباتية متبوعة بتنبؤ FT-IR للكشف عن المجموعات الوظيفية الكيميائية الموجودة. لذلك ، توضح الدراسة الحالية أن جسيمات الفضة النانوية يمكن أن تلعب دورًا حيويًا فى مجال التكنولوجيا الحيوية النانوية فى المستقبل.