

PHYSICAL CHARACTERIZATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING BROWN SEAWEED *SARGASSUM BINDERI*

Yu Ke-Xin^{1,*}, Divashini Navaneetharajah¹, Nurshahida Amat¹, Norliza Zulkifli¹,
Ng Chean Hui², Shahida Mohd Said³ and Shirley Gee Hoon Tang⁴

¹Faculty of Health and Life Sciences, Management and Science University, Seksyen 13,
40100 Shah Alam, Selangor, Malaysia.

²School of Pharmacy, Management and Science University, Seksyen 13, 40100 Shah Alam
Selangor, Malaysia.

³Department of Restorative Dentistry, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur,
Malaysia.

⁴Center for Toxicology and Health Risk Studies, Faculty of Health Sciences, Universiti
Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

*kxyu@msu.edu.my

Abstract. Green synthesized silver nanoparticles are new in nanotechnology, proven to be eco-friendly and cost effective. Nanoparticles derived from seaweeds have high value in biomedical field. This study was aimed to characterize the green-synthesized silver nanoparticles (AgNPs) using brown seaweed *Sargassum binderi*. A total of 10 g dried fine cut seaweed *S. binderi* was boiled in 100 mL distilled water to light yellow colour. The extract was filtered and centrifuged. The pellet of extract was freeze-dried. Synthesis of AgNPs was done by mixing seaweed extract (0.08 mg/ml) with silver nitrate (2.0 mM) at pH 9, room temperature, in 20 minutes. The solution was centrifuged to obtain the pellet, and it was oven-dried for 24 hours to yield dry form of AgNPs. Total of 4.4 mg dark-coloured AgNPs using seaweed *S. binderi* was synthesized. UV-vis analysis (ranged 200-600 nm) of *S. binderi* extract showed peak at 305 nm while AgNPs formation at peak 425 nm. FTIR analysis (ranged 4000–400 cm⁻¹) of *S. binderi* extract showed peaks at 3347, 1642, 672 and 600 cm⁻¹, while AgNPs showed peaks at 3350, 1650, 680 and 600 cm⁻¹. SEM revealed spherical shape of AgNPs with the size of 39.63 ± 9.72 nm in diameter. EDX spectrum illustrated elemental constituents of silver (82.3%), carbon (8.5%), oxygen (7.9%) and nitrogen (0.3%). Quantitative measurements have shown the parameter setting of AgNPs synthesis is valid. Results indicate the potential use of brown seaweed *S. binderi* in the synthesis of AgNPs which has enormous possibilities in biomedical applications.

Keywords: *Sargassum binderi*, silver nanoparticles, green synthesis, physical characterization

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Introduction

Nanomaterials have a length scale of 1-100 nm in size and will exhibit different properties affecting physical, chemical and biological behaviours. The nanomaterial opened a huge opportunity in material science research. Nanomaterials come in different types, according to size and shapes, such as nanoparticles, nano films, nano rods, etc. [1]. The synthesis of uniform sized nanoparticle synthesis has increased worldwide. The most studies done are on silver nanoparticles (AgNPs) due to the diverse benefits serving as catalysts in chemical reactions, solar energy absorption coats, biolabeling and good electrical conductivity.

Seaweed is referred to as macroalgae consisting of aquatic biomass. These seaweeds consist of red, brown and green seaweeds and are simple and non-flowering. *Sargassum binderi* is a brown seaweed species commonly found in Malaysia [2]. Pheophytine, sargaquinoic acids, polyphenols, sterols, sulfated polysaccharides and terpenoids are all biologically active compounds derived from *Sargassum* species. These compounds were reported to have valuable biomedical values, which include anti-inflammatory, anti-viral, anti-coagulant, anti-tumor, anti-microbial and analgesic bioactivities [3].

Green synthesized silver nanoparticles using seaweed are able to neutralize the free radical mechanism such as cancer or cardiovascular diseases and are applied in the biomedical field [4]. Green synthesized nanoparticles also tend to possess more biological activities when compared to chemically synthesized particles [5]. *Sargassum tenerrimum*, *Sargassum siliculosom*, *Sargassum muticum*, *Sargassum wightii*, *Sargassum pollyphyllum* and *Sargassum angustifolium* are reported to be used as reducing agents for silver nanoparticles synthesis. The nanoparticles synthesized ranged from 5-480 nm. Most of the particles are shaped spherically. These nanoparticles have been reported effective in antibacterial and anti-toxicity activities [6-8]. The findings of this study will be an important addition to the record of green-synthesized AgNPs using *Sargassum* seaweeds in Malaysia.

The characteristic features of nanomaterials, such as size, shape, size distribution, surface area, solubility, etc. need to be evaluated before assessing their bioactive properties. To characterize the synthesized nanomaterials, a variety of instruments are used which include ultraviolet visible spectroscopy (UV-vis spectroscopy), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), electron microscopy (SEM), atomic force microscopy (AFM) and dynamic light scattering (DLS). It is very important to characterize the silver nanoparticles before application [9].

This study aimed to green synthesize silver nanoparticles by using brown seaweed *S. binderi* and physically characterize green synthesized silver nanoparticles using UV-vis spectrophotometer, fourier transform infrared spectroscopy (FTIR) and scanning electron microscope.

Materials and Methods

Preparation of Seaweed Extract

Washed dried fine cut dried seaweed (10 g) was placed in a glass beaker along with 100 mL distilled water. The mixture was boiled until color changed from watery to light

yellow was observed for 15 minutes. The extract was then filtered by using a sieve with filter paper (Whatman No.1) and centrifuged at 6000 rpm for 30 minutes. The filtrate was then collected, kept and stored at 4 °C. After that, the filtrate was sent for freeze-dry for 48 hours at 0.05 mbar and -45 °C. Lastly, the freeze-dried extract was stored at -20 °C [10].

Synthesis of Silver Nanoparticles

Seaweed extract of 20 mg was weighed and mixed in 250 ml of distilled water in beaker A (to yield for 0.08 mg/ml). The seaweed was dissolved by rotating on a hot plate without heat for one hour. In beaker B, 84.9 mg of silver nitrate (AgNO_3) (molecular weight of 167.87 g/mol) was measured and mixed into 250 ml of distilled water, to yield a final concentration of 2 mM. The contents of beaker B were added into beaker A and mixed. The pH was checked and kept at 9 which was the optimum pH for synthesis. Colour changes were observed. Synthesis was done by centrifugation of mixed solution at a speed of 5000 rpm for 20 minutes. The pellet was obtained after two times centrifugation, then it was air-dried in the oven for 24 hours to obtain the dried silver nanoparticles (in powder form) [10].

Ultraviolet-visible (UV-vis) Spectrophotometry

Physical characterization using UV-vis was done at Instrumentation Laboratory, Management and Science University (MSU). The reduction of silver nitrate solution and seaweed extract was monitored using the UV-vis spectrophotometer (DR 6000, Hach, USA) between 200-600 nm at a resolution of 0.5 nm. The peaks were observed and recorded in graph and absorbance values [10].

Fourier- Transform Infrared Spectroscopy (FTIR)

Physical characterization using Fourier- Transform Infrared Spectroscopy (FTIR) (IRSpirit, Shimadzu, Japan) was done at Instrumentation Laboratory, MSU. The FTIR analysis was observed at region 4000–400 cm^{-1} with a resolution of 4 cm^{-1} [11]. The measurement of the FTIR sample was done by obtaining the graph with transmittance peaks.

Scanning Electron Microscope with Energy Dispersive X-ray Spectroscopy (SEM-EDX)

Physical topography characterization of green synthesized nanoparticles was done using an electron microscope (Schottky Field Emission SEM SU5000, Hitachi, Japan) with EDX detector (EDAX Octane Elite Super) and EDX Software (APEX software) at Hi-Tech Instrumental Sdn Bhd. SEM stub was prepared and conductive double-sided carbon tape was stucked on the stub. Toothpick was used and picked up the nanoparticles powder and sprinkled onto the carbon tape. The stub was knocked and blow using a blower to ensure the powder which was not firmly stucked on the carbon tape was blown off and left the powder which was stucked well on the tape. The stub was then screwed to SEM holder and loaded to VP-FESEM. The parameters used for VP-FESEM: accelerating voltage: 15 kV, vacuum mode: high vacuum, signal: secondary electron (SE). SEM Micrograph was obtained under 100 000 and 150 000x of scanning.

Results and Discussion

Green synthesis of silver nanoparticles is a cost-effective, and environment-friendly alternative compared to chemical synthesis. The fabrication of green-synthesized nanoparticles using seaweed extract is cost-effective and eco-friendly with simple procedure [11]. In this study, the synthesis of silver nanoparticles was done at pH 9 mixing 250 ml of 20 mg seaweed extract (0.08 mg/ml) and 250 ml of 84.9 mg silver nitrate (0.34 mg/ml) at room temperature. The colour changes from light brown to dark brown was observed 20 minutes after mixing (Figure 1). Changes in the colour indicate the complete synthesis of silver nanoparticles. The synthesis to obtain silver nanoparticles was repeated six times. The total of silver nanoparticles (in powder form) yielded was 4.4 mg (Figure 2). The average yield of silver nanoparticles for each synthesis obtained was 0.733 ± 0.165 mg.

The concentration of silver nitrate concentration has effects on the outcome of the morphology and quantity of the silver nanoparticles [12]. In the study by Sobczak et al. [13], it was shown that when the concentration of silver nitrate was increased, the formation of more silver nanoparticles was observed. The nanoparticle's size was also increased causing larger silver aggregates to be formed. However, with lower silver nitrate concentration, lesser formation of silver nanoparticles along with smaller size particle was observed.

In the synthesis of silver nanoparticles in the present study, the change of colour turning from light brown to dark brown was observed after mixing. This indicates ion excitation forming the synthesized silver nanoparticles. Compared to the study done by Azizi et al. [14] using *Sargassum muticum*, change in colour to dark brown was also observed. The changes in colour indicate the completion of the silver nanoparticle formation. It was explained to be due to the excitation of surface plasma resonance and reduction of silver nitrate. The time taken for changes of colour in the synthesis of nanoparticles was observed to be similar to the present study which was 20 minutes. The speed of the centrifuge to obtain pellet of nanoparticle for extraction was different in several reports. In the present study, the speed for the centrifuge used was 5000 rpm for 20 minutes, however in the study of Deepak et al. [11], it was 10 000 rpm for 10 minutes while in the study of Azizi et al. [14], it was 6000 rpm for 10 minutes.

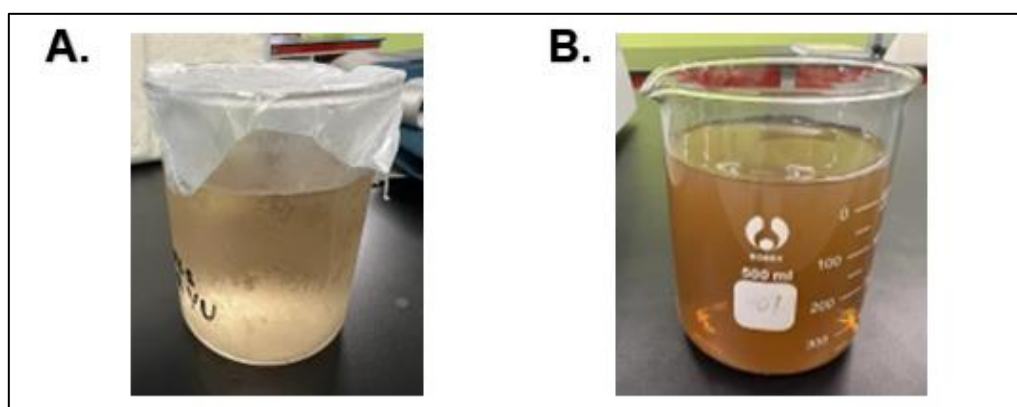


Figure 1: Synthesis of silver nanoparticles using seaweed *Sargassum binderi*. (A) The colour of mixture before reaction (light brown) and (B) after 20 minutes of reaction (it has turned into dark brown)

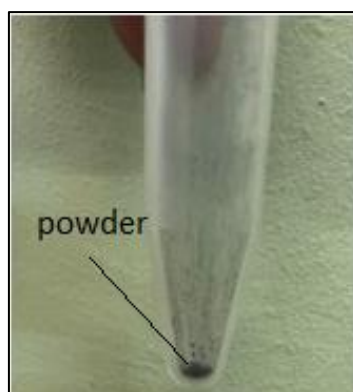


Figure 2: Silver nanoparticles obtained were black colour in powder form

UV-vis spectroscopy is used to observe and keep track of the synthesis and stability of the silver nanoparticles (AgNPs) by analyzing the UV-vis graph. In the present study, the UV-vis scan was optimized to 200-600 nm and managed to get the accurate peak and absorbance values for the seaweed extract of *S. binderi* and silver nanoparticles. UV-vis analysis of seaweed extract showed highest peak at 305 nm and silver nanoparticles showed highest peak at 425 nm (Figure 3). In the study of Deepak et al. [8] which uses *Sargassum wightii*, showed a peak at 300 nm which appears to be very close to the absorbance peak in the present study. On the other hand, a study using seaweed *S. muticum* for the synthesis of nanoparticles, showed a peak at 320 nm in the UV-vis spectrum [14].

In this study, UV-vis absorbance of green synthesized silver nanoparticles using *S. binderi* showed a peak at 425 nm. The highest absorbance value shows the highest yield of nanoparticles at the wavelength of 425 nm. In this case, wavelength of more than 500 nm was not productive for the synthesis of nanoparticles. Synthesis of silver nanoparticles using *S. muticum* showed the highest peak at 420 nm, which indicates the formation of AgNPs [14]. Synthesis of silver nanoparticles using *S. wightii* also showed similar peak of 420 nm as highest peak of absorbance [11]. Therefore, it is observable that the wide absorption peak is due to wide size of distribution of the silver particles.

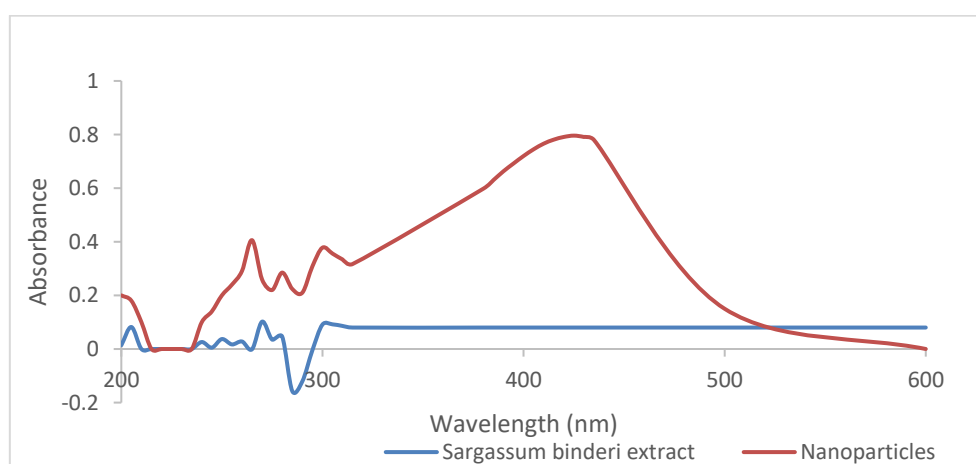


Figure 3: UV-vis analysis of *Sargassum binderi* extract shows a peak at 305 nm while silver nanoparticles show a peak at 425 nm

Fourier-transform infrared spectroscopy (FTIR) has also been used in studying nanomaterials to identify functional molecules. It is a valuable, cost-effective and non-invasive method. In the present study, FTIR analysis of silver nitrate revealed peak vibrations at 3380.5, 1642, 660 and 600 cm^{-1} . The peak reading for seaweed extract of *S. binderi* was 3347, 1642, 672 and 600 cm^{-1} . FTIR analysis for silver nanoparticles showed peaks at 3350, 1650, 680 and 600 cm^{-1} (Figure 4). The results of FTIR analysis have been compared to other reports and tabulated in Table 1.

FTIR analysis of *S. binderi* with peak at 3347 cm^{-1} indicates the presence of OH functional group. Peak of 1642 cm^{-1} in the FTIR analysis of present study was compared to the report of Anand & Suresh [15] with peak 1654 cm^{-1} , which indicates the C–O stretching and N–O asymmetric stretching of the ester group. The peak obtained in this study of 672 cm^{-1} is comparable to the report with a peak of 669 cm^{-1} , which indicates the presence of carbohydrates [15].

FTIR analysis of silver nanoparticles revealed peak vibrations at 3350, 1650, 680 and 600 cm^{-1} . From the study of Deepak et al. [11], peak vibrations of silver nanoparticles present at 3448, 1635 and 1045 cm^{-1} . The 3448 cm^{-1} peak was linked to the NH functional group of amines that are involved in the reduction of silver ions. The peak at 1635 cm^{-1} revealed the C=O functional group of amides. The peak at 1045 cm^{-1} showed functional group O–H of alcohols [11]. Based on the findings of Roy & Ananthraman [16], the peak obtained at 669 cm^{-1} belonged to the alkyl halide group (also known as haloalkanes).

SEM-EDX was done on *S. binderi* green-synthesized silver nanoparticles revealed the morphology and size measurement of the silver nanoparticles. The morphology of the silver nanoparticles observed was spherical in shape. The size of silver nanoparticles observed under 150k was ranging from 25.1 to 54.2 nm (Figure 5). Based on the measurements obtained, the average size of the silver nanoparticle is 39.63 ± 9.72 nm in diameter.

The shape of nanoparticles in the report of Thangaraju et al. [19] using *Sargassum polycystum* was also mostly spherical shape, ranging in size from 25.1 to 54.2 nm., which is larger, as compared to the nanoparticles of this study (39.63 ± 9.72 nm).

The results of EDX spectra revealed strong signals from the silver atoms present in nanoparticles and confirmed the presence of elemental silver as a major constituent. The EDX analysis confirmed the elemental constituents of silver (82.3%), carbon (8.5%), oxygen (7.9%) and nitrogen (0.3%) (Table 2). Weaker signals were obtained such as carbon, oxygen and nitrogen which must have been proteins or sugars that were present in the brown seaweed (Table 2). The energy of silver (at peak 3 keV) as a strong characteristic determined by EDX spectra (Figure 6) was similar to another study which explains 3 keV as the absorption of metallic silver nano-crystallites [20].

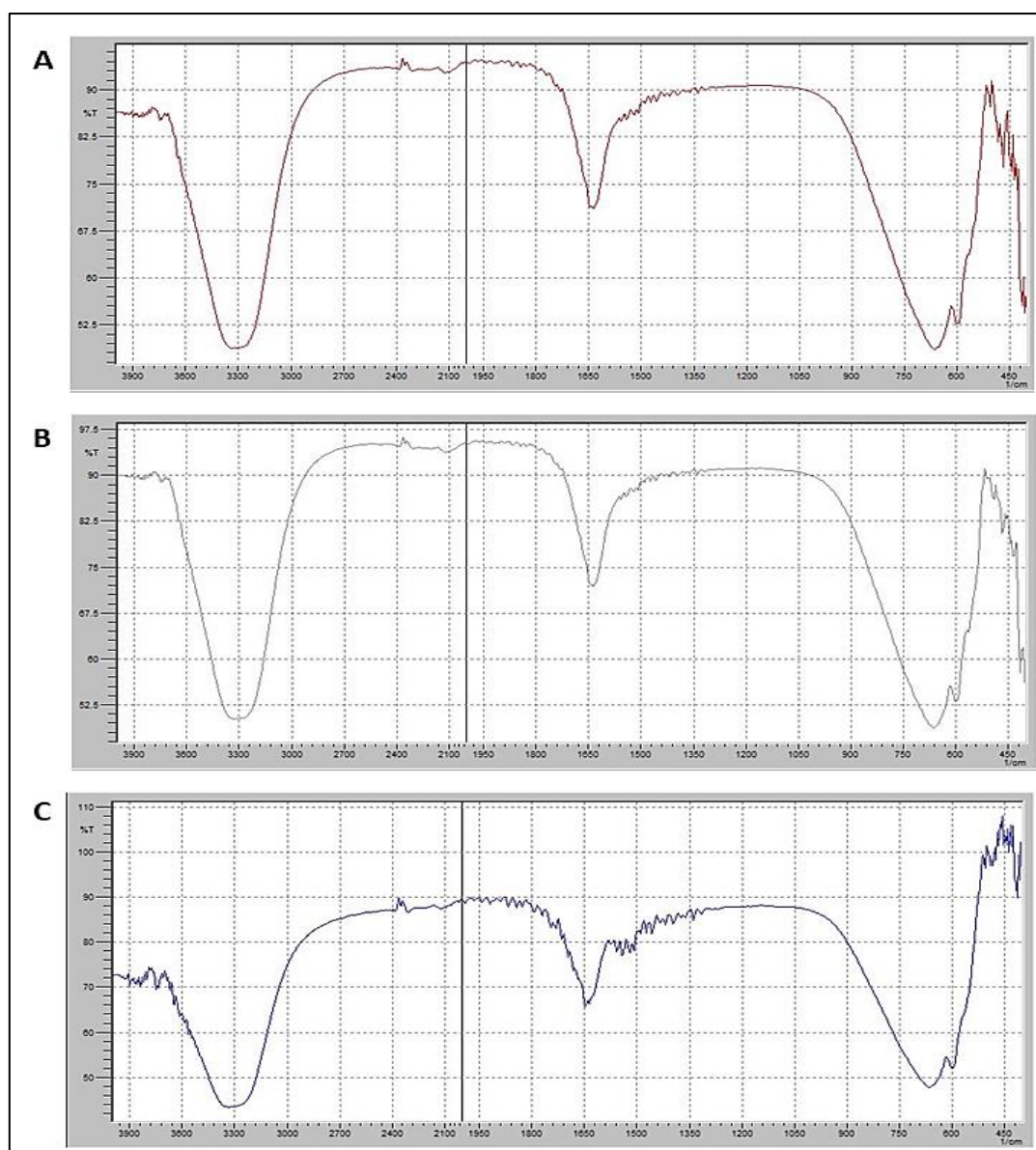


Figure 4: FTIR analysis of different samples: (A) silver nitrate with peaks at 3380.5, 1642, 660 and 600 cm^{-1} , (B) *S. binderi* extract with peak at 3347, 1642, 672 and 600 cm^{-1} and (C) silver nanoparticles with peak at 3350, 1650, 680 and 600 cm^{-1}

Table 1: Comparison of FTIR analysis of green synthesized nanoparticles

Sample	Peak value (transmittance, cm^{-1})	References	Remarks
Silver Nitrate	3380.5, 1642, 665, 600	3328: O-H 2129: C-H 1634: C=C [17]	3328: O-H peak intensity high and broad presence of alcohol. 2129: aromatic C-H stretching. 1634: C=C stretching peak intensity variables with the presence of alkene.
<i>Sargassum binderi</i>	3347, 1642, 672, 600	1610: NH(C=O) [14]	1610: Stretching vibration of (NH) C=O group. Proteins shifted and become shorter.
		1654: C-O, N-O 669: C-H bending [15]	669: Confirms the presence of carbohydrates. 1654: Due to the C-O stretching and N-O asymmetric stretching of the ester group.
		3338: OH 663: O-H [18]	
AgNPs	3350, 1650, 680, 600	3448: NH 1635: C=O 1045: O-H [11]	3448: NH functional group of Amines. 1635: C=O functional group of Amides 1045: O-H functional group of alcohols.
		669: C-Cl, C-Br stretch 1619: C=C bending 3424: N-H stretch [17]	669: Alkyl halide 1619: Aromatic 3424: 2° Amines, Amides

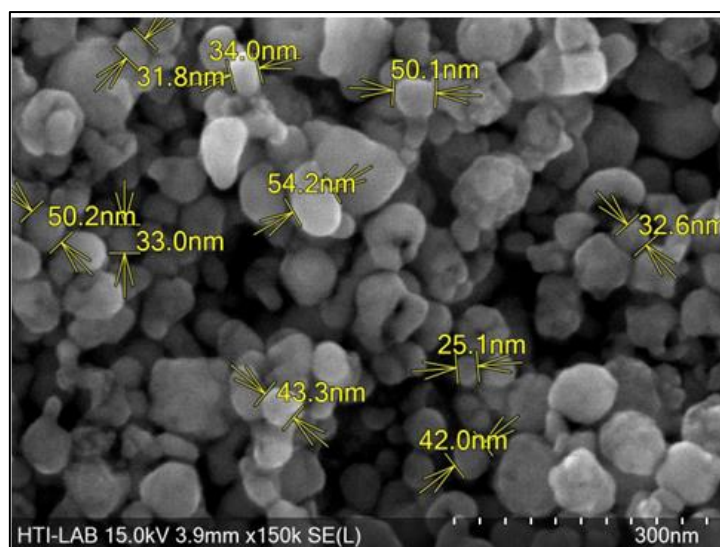


Figure 5: The morphology of the silver nanoparticles observed were spherical in shape

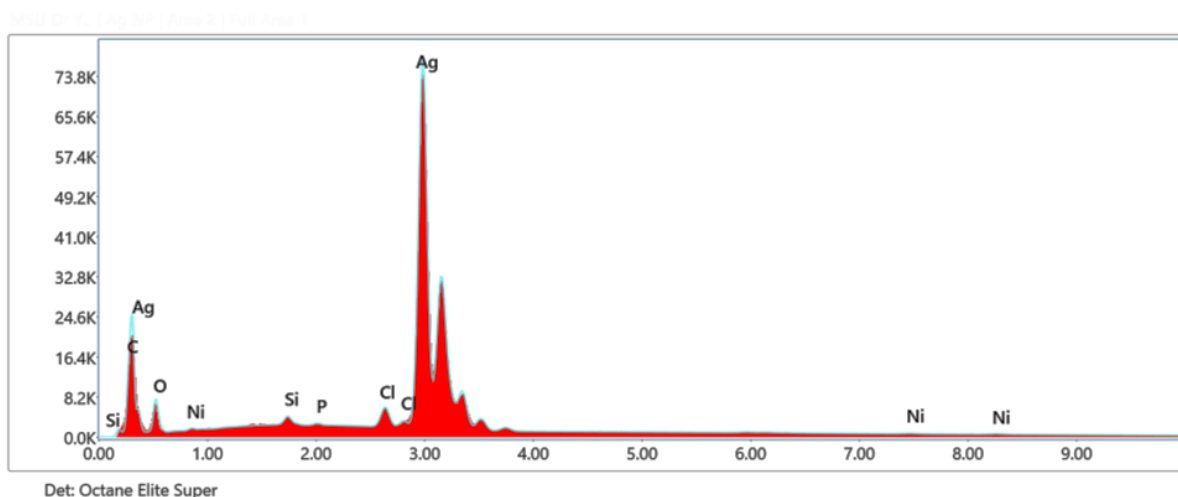


Figure 6: EDX pattern of AgNPs.

Table 2: EDX table showing elemental composition.

Element	Weight %	MDL	Atomic %	Net Int.	Error %	R	A	F
C K	8.5	0.14	35.5	794.6	9.3	0.7398	0.2708	1.0000
O K	7.9	0.12	24.7	562.4	11.2	0.7554	0.0822	1.0000
Si K	0.4	0.04	0.7	205.5	10.2	0.7964	0.5939	1.0187
P K	0.1	0.04	0.2	50.6	19.6	0.8030	0.6877	1.0301
Cl K	0.5	0.06	0.6	205.9	9.6	0.8160	0.8217	1.0705
Ni L	0.3	0.12	0.3	26.4	24.8	0.7690	0.1725	1.0000
Ag L	82.3	0.13	38.0	12485.5	3.0	0.8265	0.8659	1.0040

Conclusions

AgNPs using brown seaweed *Sargassum binderi* have been synthesized. The results of the physical characterization of silver nanoparticles indicate the potential use of brown seaweed *S. binderi*. The findings provide an important reference for the synthesis of AgNPs using Malaysian seaweeds. The limitation of the study was the lack of analysis of certain laboratory equipment such as the zeta potential analyzer. Future work that can be done would be to investigate the bioactivity of the nanoparticles by using biochemical assay and bioactivity testing.

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Author contributions

All authors contributed towards data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of conflict of interest

The authors have no disclosures to declare.

Compliance with ethical standards

The work is compliant with ethical standards

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