The green synthesis, characterization and antimicrobial activities of silver nanoparticles synthesized from green alga Enteromorpha flexuosa (wulfen) J. Agardh

ARTICLE *in* MATERIALS LETTERS · DECEMBER 2014

Impact Factor: 2.49 · DOI: 10.1016/j.matlet.2014.08.110

3 AUTHORS, INCLUDING:

Morteza Yousefzadi
Hormozgan University
66 PUBLICATIONS **505** CITATIONS
SEE PROFILE

Vahid Ghafouri
Shahid Beheshti University
19 PUBLICATIONS **37** CITATIONS
SEE PROFILE

Materials Letters 137 (2014) 1–4

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

The green synthesis, characterization and antimicrobial activities of silver nanoparticles synthesized from green alga *Enteromorpha flexuosa* (wulfen) J. Agardh

Morteza Yousefzadi [a,*], Zohreh Rahimi [b], Vahid Ghafori [c]

[a] Department of Marine Biology, Faculty of Sciences, University of Hormozgan, Bandar Abbas, Iran
[b] Department of Fisheries, Faculty of Agricultural and Natural Resources, University of Hormozgan, Bandar Abbas, Iran
[c] Research Institute of Applied Sciences, Academic Center of Education, Culture and Research (ACECR), Shahid Beheshti University, Tehran, Iran

ARTICLE INFO

Article history:
Received 8 November 2013
Accepted 20 August 2014
Available online 28 August 2014

Keywords:
Green synthesis
Algae
XRD
X-ray techniques
Nanoparticles

ABSTRACT

In the recent decades, development of green nanotechnology is generating interest of researchers toward ecofriendly biosynthesis of nanoparticles, because of its incredible applications in all fields of science. In this study, to explore the novel approaches for the biosynthesis of silver nanoparticles, the seaweed *Enteromorpha flexuosa* (wulfen) J. Agardh extract was mixed with silver nitrate to synthesize silver nanoparticles. The reduced silver nanoparticles were characterized by UV–vis spectrophotometer, energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD) and transmission electron microscopy (TEM). The *in vitro* antimicrobial activity of the synthesized nanoparticles of *E. flexuosa* exhibited high antibacterial activity against Gram-positive bacteria and low activity against the Gram-negative organisms. The algae materials mediated synthesis of silver nanoparticles have comparatively rapid, less expensive and wide applications to antimicrobial therapy in modern medicine.

1. Introduction

The field of nanotechnology is one of the most attractive areas of research in modern material science. Currently, there is a growing need to develop environmentally friendly and sustainable methods for the synthesis of nanomaterials that do not use toxic chemicals in the synthesis protocols, to avoid adverse effects in medical applications [1]. Route of synthesis of nanoparticles by physical and chemical methods may have considerable environmental defect, technically laborious and economically expensive. Many researchers have explored the technological approach for the synthesis. For example, silver ions reduced by chemical, radiation, photochemical methods and biological techniques were reviewed [2,3]. In the recent years, biologically synthesized nanoparticles, especially the development of "green" synthetic approaches for nanoparticles, are of considerable interest in the area of medicinal and technological aspects due to their unique particle size and shape-dependent physical, chemical and biological properties. Synthesis of nanoparticles through biological method is advantageous over chemical and physical methods as it is a cost-effective, environment-friendly and economically alternative method, where it is not necessary to use high pressure, energy, temperature and toxic chemicals [4,5]. The synthesis of silver nanoparticles has attracted much attention because their unique shape-dependent optical, electrical, and chemical properties have potential applications in nanotechnology. Silver nanoparticles are used in photographic reactions, catalysis and chemical analysis [6,7,8]. During the past three decades, marine algae research has been increased considerably for the search of new and effective medicines of natural origin. Several compounds including carbohydrates, alkaloids, steroids, phenols, saponins and flavonoids synthesized by seaweeds are promising source for both industrial and biotechnological applications [5,9,10].

To date, there is no report on the synthesis of silver nanoparticles by utilizing the aqueous extracts of *Enteromorpha flexuosa* (wulfen) J. Agardh. Hence, the present study was designed to synthesize and characterize silver nanoparticles and to investigate the antimicrobial activities of the synthesized nanoparticles.

2. Materials and methods

The seaweed *E. flexuosa* was harvested manually from Qeshm island, Persian Gulf, Iran, and thoroughly washed in seawater to remove epiphytes and detritus and then brought to the laboratory in an ice box. The cleaned fronds were then washed in distilled

* Corresponding author. Mobile: +989121886139.
E-mail address: morteza110110@gmail.com (M. Yousefzadi).

http://dx.doi.org/10.1016/j.matlet.2014.08.110
0167-577X/

water and shade dried for five days. Five grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for ten minutes. After cooling, the extract was filtered with Whatman no.1 filter paper. The filtrate was stored at 4 °C for further use.

Ten milliliter of the aqueous extract of algae was mixed with 90 ml of 1 mM aqueous AgNO3 solution to make up final volume to 100 ml for reduction of Ag^+ ions. The reaction mixture was kept in dark room condition until the color change was observed. The reaction solution color changes were observed for the characterization of silver nanoparticles. The reduction of pure silver ions was monitored by measuring the UV–vis spectra of the solution at regular intervals after diluting 2 ml aliquot of the sample. Energy-dispersive X-ray spectroscopy (EDS) was performed using the HITACHI model: S-4160 machine. X-ray diffractometer (XRD) [CuK_α radiation ($\lambda = 1.54$ nm) with the scanning 2θ angle ranging from 20 to 80 degree by Step Size 0.0390, Generator Settings: 40 mA, 40 kV, Model: X'Pert PRO] was used for characterization of nanoparticles crystalline. Transmission Electron Microscopic (TEM) analysis was done by using a transmission electron microscopy (Philips, CM-30) for characterizing size and shape of biosynthesized silver nanoparticles. Antibacterial activity of the biosynthesized silver nanoparticles was determine using the disc diffusion method as well as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against pathogenic Gram-negative and Gram-positive bacteria, as well as fungi [11].

3. Results and discussion

3.1. Synthesis of silver nanoparticles

In this study, silver nanoparticles were formed by the reduction of Ag^+ into Ag° with the addition of seaweed extraction to the solution of 1 mM $AgNO_3$. The colorless reaction mixture turned into dark brown color solution after 60 minutes of incubation in light room condition, indicating the biotransformation of ionic silver to reduced silver, as a result of the surface plasmon resonance phenomenon. The control $AgNO_3$ solution (without seaweed extract) showed no color change (Fig. 1). It is well known that Ag-NPs exhibit reddish-brown in water [12]. The color change occurred because the active molecules present in the extract reduced the silver metal ions into silver nanoparticles. The intensity of the color change was increased which was directly proportional to the incubation period of nanoparticle synthesis. It may be due to the excitation of surface plasmon resonance (SPR) and reduction of $AgNO_3$ [3,13]. The formation of silver nanoparticles was monitored by UV–vis absorption spectra at 200 to 600 nm

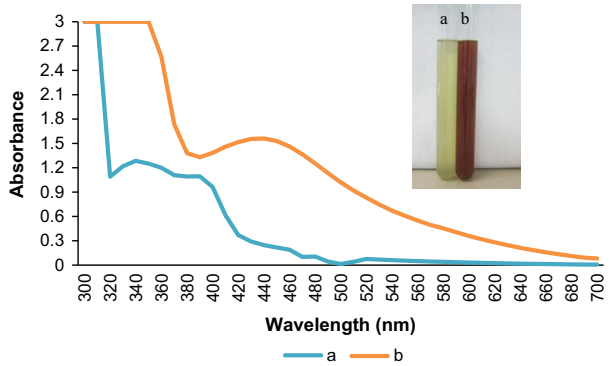

Fig. 1. UV–vis spectra of aqueous extract alone showed SPR peak at 320–340 nm (a), aqueous extract reduced silver nanoparticles SPR peak at 430 nm (b). The figure inset shows extract alone (a) and synthesized silver nanoparticles (b).

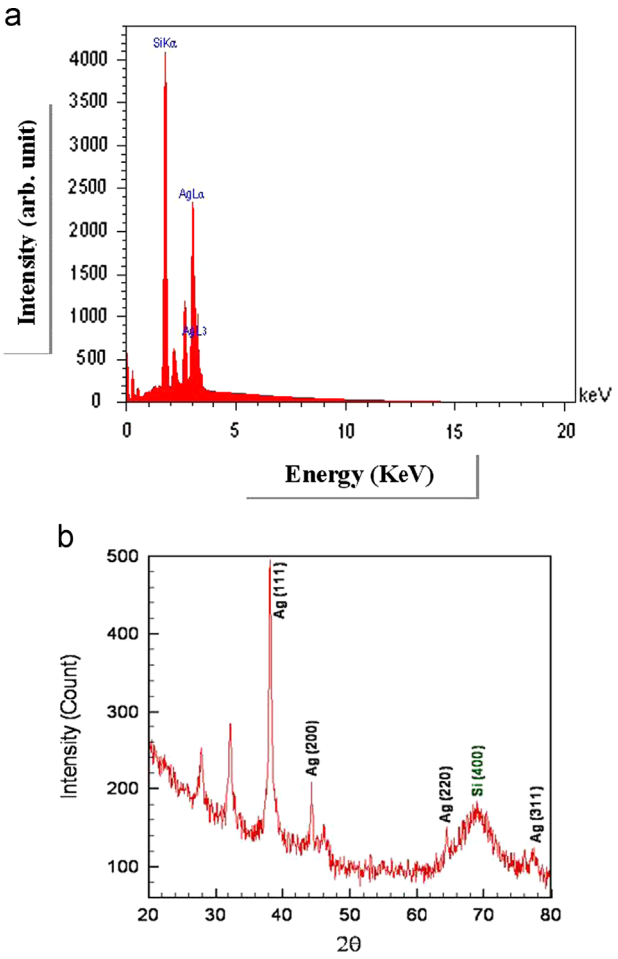

Fig. 2. (a) EDS spectrum of sample with Ag nanoparticle. As it can be seen the EDS indicates the existence of Ag peaks that conformed there are some Ag nanoparticles in the sample. Si peak is related to substrate. (b) XRD pattern of Ag nanoparticles deposited on Si substrate.

where an intense band was clearly detected at 430 nm, which confirmed the formation of silver nanoparticles (Fig. 1). The observed band in this range has been associated with Ag nanoparticles confirming the synthesis of spherical Ag nanoparticles with narrow size distribution [9].

The EDS spectrum of sample is plotted in Fig. 2a. The elements that exist on the table are Carbon, Oxygen, Silicon, Chlorine and Silver. The Si peak is attributed to the Silicon substrate. Due to our procedure of synthesis of nanoparticles, and using bio-materials and green plants, the existence of carbon and oxygen elements are inevitable. However, Ag element is seen in EDS analysis with the greatest percent, which implies that the major part of the product is Ag nanoparticles. XRD is a widely used technique to estimate the size of nanoparticles in the range between 1 to 100 nm, because of the commonly used X-ray wavelength [5]. XRD analysis of the nanoparticles showed intense peaks, corresponding to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) Bragg reflection, based on the face-centered cubic (fcc) structure of Ag nanoparticles, with a lattice constant of $a = 4.086$ Å (Fig. 2b). The mean particle diameter of Ag nanoparticles was calculated from the XRD pattern, according to the line width of the maximum intensity reflection peak. The size of the nanoparticles was calculated through the Scherer equation:

$$D = (0.9\lambda)/(\beta_c \cos\theta), \text{ and } \beta_c = (\beta_s^2 - \beta_r^2)^{1/2},$$

where D is the average crystal size, λ is the X-ray wavelength ($\lambda = 1.5406$ Å), $^\circ 2\theta$ is Bragg's angle, β_c is the corrected full width at half maximum (FWHM) in radians, and β_s and β_r are the FWHM of

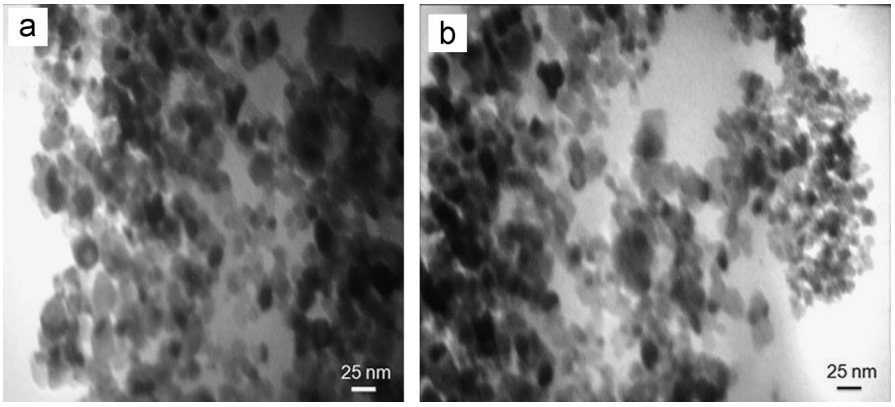

Fig. 3. TEM images of Ag nanoparticles synthesized using *E. flexuosa* extract at 25 nm scale.

Table 1
In vitro antimicrobial activity potential of biosynthesized silver nanoparticles using *E. flexuosa* extract.

Microorganisms	Algae extract			Synthesized silver nanoparticle			Ampicillin[c]	Nystatine[c]	Ag NP[d]
	IZ[a]	MIC[b]	MBC[b]	IZ	MIC	MBC	IZ	IZ	IZ
Bacillus subtilis	15 ± 0.9	25	50	18 ± 0.8	12.5	50	14 ± 0.4	Nt	16 ± 0.6
Bacillus pumulis	16 ± 1.0	25	50	19 ± 1.2	6.25	25	15 ± 0.3	Nt	17 ± 0.9
Enterococcus faecalis	11 ± 0.7	50	100	12 ± 0.9	50	100	11 ± 0.3	Nt	10 ± 0.6
Staphylococcus aureus	12 ± 0.6	50	100	14 ± 0.7	25	50	13 ± 0.3	Nt	11 ± 0.9
Staphylococcus epidermidis	18 ± 1.2	25	50	20 ± 1.5	6.25	12.5	19 ± 0.5	Nt	17 ± 0.8
Escherichia coli	11 ± 0.9	50	100	13 ± 0.9	50	100	12 ± 0.2	Nt	12 ± 0.6
Klebsiella pneumoniae	10 ± 0.5	100	200	10 ± 0.4	50	100	0	Nt	9 ± 0.5
Pseudomonas aeruginosa	0	Nt	Nt	0	Nt	Nt	10 ± 0.3	Nt	0
Aspergillus niger	0	Nt	Nt	0	Nt	Nt	–	16 ± 0.4	0
Candida albicans	12 ± 0.8	50	100	14 ± 0.8	25	100	–	18 ± 0.5	13 ± 0.5
Saccharomyces cerevisiae	13 ± 0.9	25	100	16 ± 0.6	25	50	–	18 ± 0.2	15 ± 0.7

Results (shown as mean ± SD) were obtained from three independent experiments, each performed in duplicate.
Inactive (–). Not tested (Nt).

[a] Inhibition Zone (IZ) includes diameter of disc (6 mm).
[b] Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values as μg/ml.
[c] Tested at 10 μg ampicillin/disc, and 30 μg nystatine/disc.
[d] Ag NP: chemically synthesized silver nanoparticles.

the reference and sample peaks, respectively. In our experiment, the FWHM of reference is equal to 0.0899.

The average crystal size of the silver crystallites is calculated from the FWHMs of the diffraction peaks, using the Scherer equation. The size of crystallite in different planes of silver was determined as 13.8, 40.0 and 59.2 nm with the mean value of all three peaks as 37.6 nm. TEM is powerful method to determine the size of nanoparticles. The TEM images of the prepared silver nanoparticles at 25 nm scales are shown in the Fig. 3. It was observed that Ag nanoparticles were circular in shape with maximum particles in size range within 2–32 nm with mean diameter of 15 ± 1.5 nm. It was also observed that silver nanoparticles were evenly distributed in the sample.

3.2. Antimicrobial activities

Results of the evaluation of the antimicrobial properties of the *E. flexuosa* extract and biosynthesized silver nanoparticles using a disk diffusion method, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) are shown in Table 1. Inhibition zones (IZ), MIC and MBC values of the biosynthesized silver nanoparticles showed a variability of inhibition among the bacteria and fungi tested. The results indicated that the biosynthesized silver nanoparticles have strong antimicrobial activity and moderate antifungal activity, except for *Pseudomonas aeruginosa* and *Aspergillus niger*. The mechanism of the bactericidal and fungicidal effect of silver nanoparticles against bacteria and fungi is not very well-known. Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration [5,14].

4. Conclusion

In this study, we have successfully synthesized environment-friendly silver nanoparticles using green algae *E. flexuosa* extract. The amines, peptides and secondary metabolites present in the *E. flexuosa* extract were involved in the bioreduction and stabilization of silver nanoparticles. The morphology of silver nanoparticles was characterized by transmission electron microscope. The XRD results confirm that the particles have a face-centered cubic crystalline structure. The antimicrobial activity of silver nanoparticle was well demonstrated by the clear zone of inhibition against Gram-negative and Gram-positive of bacteria as well as fungi. This method of silver nanoparticles synthesis does not use any toxic reagents and thus has the potential for use in biomedical and agricultural applications.

Acknowledgment

The authors wish to thank the Ministry of Science, Research and Technology of the Iranian government for providing financial support for the research (No. 3/221617).

References

[1] Rajathi FAA, Parthiban C, Ganesh Kumar V, Anantharaman P. Spectrochim Acta Mol Biomol Spectros 2012;99:166–73.
[2] Dipankar C, Murugan S. Colloid Surf B 2012;98:112–9.
[3] Gopinath V, Mubarak A, Priyadarshini S, Meera Priyadharsshini N, Thajuddin N, Velusamy P. Colloid Surf B 2012;96:69–74.
[4] Nabikhan A, Kandasamy K, Raj A, Alikunhi N. Colloid Surf B 2010;79:488–93.
[5] Suriya J, Bharathi Raja S, Sekar V, Rajasekaran R. Afr J Biotechnol 2012;11: 12192–8.
[6] Lengke MF, Fleet ME, Southam G. Langmuir 2007;23:2694–9.
[7] Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Colloid Surf B 2010;76:50–6.
[8] Raja sekharreddy P, Usha Rani P, Sreedhar B. J Nanopart Res 2010;12:1711–21.
[9] Kumar P, Senthamilselvi S, Lakshmiprabha A, Premkumar K, Ganeshkumar RS, Govindaraju M. Nano Biomed Eng 2012;4:12–6.
[10] Thangaraju N, Venkatalakshmi RP, Chinnasamy A, Kannaiyan P. Nano Biomed Eng 2012;4:89–94.
[11] Gnanadesigan M, Anand M, Ravikumar S, Maruthupandy M, Syed A, Vijayakumar V, et al. Appl Nanosci 2012;2:143–8.
[12] Jegadeeswaran P, Shivaraj R, Venckatesh R. Dig J Nanomater Bios 2012;7: 991–8.
[13] Shanker SS, Rai A, Ahmad A, Sastry M. J Colloid Interf Sci 2004;275:496–502.
[14] McDonnell G, Russell AD. Clin Microbiol Rev 1999;12:179–85.