Research Article

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Biosynthesized silver nanoparticles using *Ulva lactuca* as a safe synthetic pesticide (*in vitro*)

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Abstract: In this era, we must synthesize safe pesticides from inexpensive sources to avoid the diseases caused by most of the previously used pesticides. Therefore, nanotechnology was used to produce biologically synthesized pesticides from very cheap sources such as seaweed, especially green algae, as it is safe to synthesize a pesticide against various pests such as bacteria and fungi that affect various agricultural crops. *Ulva lactuca* is used for the biosynthesis of silver nanoparticles by a bottom-up bioreduction reaction of silver nitrate to silver nanoparticles (Ag-NPs) observed by the formation of brown color. The biosynthesis reaction has been proven by using UV-VIS, FT-IR, EDAX, SEM, TEM, and DSC-TGA and has been tested against a wide range of bacteria and fungi that affect plants, poultry, fish, rabbits, animals, and humans. Antioxidant activity was also determined. Silver nanoparticles (Ag-NPs) have proven to be good and safe synthetic pesticides. The results of the spectroscopy demonstrated the success of the biological synthesis of the pesticide. Also, the results of the antimicrobial activity demonstrated the success of the pesticide that was biologically synthesized to fight bacteria and fungi that cause different diseases of different agricultural crops and should be used as a safe synthetic pesticide.

Keywords: biosynthesis, green synthesis, antimicrobial, safe synthetic pesticides, spectroscopy, silver nanoparticles, *Ulva lactuca*

1 Introduction

Nanotechnology is a branch of the biological sciences with dimensions of nanoparticles of 1–100 nm. It involves

the nuclear, atomic, or molecular level. Nanotechnology is used in many applications such as agriculture, medicine (suppression of various human diseases), cosmetics, and other fields. The use of minerals in the synthesis of nanoparticles is more appropriate because it is cheap, nontoxic, safe, and ecofriendly as plant extracts are used as a reducing agent called a bottom reaction (Nabi et al. 2014; Rath et al. 2014).

The marine environment is rich in many creatures and hence should be well studied, and their chemical composition should also be analyzed. Algae such as green, red, and brown algae are marine organisms rich in beneficial chemical components. *Ulva lactuca* is a green macro algal called green sea lettuce or green laver. The chemical composition of *U. lactuca* consists of carbohydrates, proteins, fats, vitamins, minerals, phenolic compounds, chlorophylls, carotenoids, flavonoids, alkaloids, terpenes, phytosterols, and phytohormones (Amin 2019). Their biological activities include antioxidants, antimicrobials and antitumor (Amin et al. 2015) anticoagulant, anti-inflammatory, antihyperlipidemic, hypocholesterolemic, hepatoprotective, and insecticidal activities (Yu-Qing et al. 2016), and they are used as animal feed (Abd El-Galil and Amin 2017) and as prebiotic in food industry (Shalaby and Amin 2019).

Microbes are living organisms that cause diseases in humans, plants, animals, poultry, rabbits, and fish. Therefore, we should search for new technologies to eliminate their harmful effects on crops, vegetables, and fruits in agriculture and to avoid side effects of other synthetic pesticides and also resistance gained from microbes against most pesticides.

Free radicals and reactive oxygen species are generated as by-products of biological reactions. Pollutants and radiation cause damage to biomolecules. Antioxidants protect the body from oxidation by scavenging free radicals and facilitating them as a defense against free radicals.

In this article, I hope to highlight new, good, and safe synthetic pesticide that point to the possibility of using nanotechnology in agriculture to produce safe pesticides that do not cause human diseases and any

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harm to the environment and to reduce pollution caused from the use of previous pesticides.

Family: Ulvaceae
e.g.: *Ulva lactuca*

2 Material and methods

2.1 Materials

Silver nitrate (AgNO_3) was purchased from Morgan Specialty Chemicals in Egypt.

2.2 Collection of algae (*Ulva lactuca*)

Algae were collected from the Mediterranean (Abu Qir) in the spring of 2018 and cleaned with sea water to remove sand pebbles, epiphytes, and shells, and then, the algae were transported to the laboratory in plastic bags and washed with a dilute solution of sodium chloride and then distilled water. Algae were shade dried, grounded in an electric mixer, and stored in the refrigerator for further use (Amin 2019).

2.3 Classification of *Ulva lactuca*

The classification of *U. lactuca* is as follows:

Division: Chlorophyta
Subdivision: Chlorophytina
Class: Cladophorophyceae

3 Methods

3.1 Aqueous extract of *Ulva lactuca*

A total of 10 g of seaweed were added to 100 mL of distilled water, stirred for 1 h at 6,000 rpm, and the filter and extracts were kept in dark bottles in the refrigerator (Amin 2019).

3.2 Biosynthesis of silver nanoparticles using *Ulva lactuca* (Ag-NPs)

A total of 10 mL of aqueous extract of *U. lactuca* were added to 1 mM of silver nitrate, mixed together, and subjected to stirring for 24 h at 100°C. The reaction color is converted from transparent to brown (Figure 1), which indicates the success of the reaction (Bhimba and Kumari 2014).

3.3 Ultra violet-visible (UV-VIS) spectroscopy

UV-VIS was used to prove the success of the bio-reduction of silver nitrate by aqueous extract of *U. lactuca* into silver nanoparticles (Figures 2a and b).

UV-VIS was identified by Shimatzu UV-1800 at the Central Laboratory of Faculty of Science, Ain Shams University, Egypt.

Figure 1: (A) Extract of *Ulva lactuca* and (B) biosynthesized silver nanoparticles using *U. lactuca*.

Figure 2: (a) UV-VIS of Ag-NPs (first experiment) and (b) UV-VIS of Ag-NPs (second experiment).

3.4 FT-IR (Fourier-transform infrared)

The chemical composition of active groups of silver nanoparticles was determined by NICOLET-6700-FT/IR-Thermo Scientific, and the spectra were recorded in the wavelength interval of $4,000\text{--}400\text{ cm}^{-1}$ (Figure 3a and b and Table 1) in the Central Laboratory of the Faculty of Science, Ain Shams University, Egypt and the National Research Center (NRC), Egypt.

3.5 Scanning electron microscope (SEM) and transmission electron microscope (TEM)

The shape and the size of the silver nanoparticles were determined at the National Research Center (NRC), Egypt. SEM was done at an applied potential of 15 kV, but samples of TEM were prepared by placing two drops of Ag-NPs solutions onto carbon-coated TEM grids. Then, the film on the TEM grid was dried before measurement (Figures 4 and 5).

3.6 Energy-dispersive X-ray spectroscopy (EDX)

The elemental composition of the sample was determined at the National Research Center (NRC), Egypt, by the SEM operated at 15 kV (Figure 6).

3.7 Thermal analysis

DSC (differential scanning calorimeter) analysis and TGA (thermo gravimetric analysis) of the same sample (DSC-TGA).

Thermal decomposition analysis of silver nanoparticles was studied using SDTQ600 TG-DSC instrument (TA Company, USA) at National Research Center (NRC), Egypt (Figure 7).

3.8 Antimicrobial activity of silver nanoparticles by Kirby–Bauer method

Antimicrobial activity of the tested samples was determined using a modified Kirby–Bauer disc diffusion

Figure 3: (a) FT-IR spectroscopy of *Ulva lactuca* and (b) FT-IR of Ag-NPs.

method (Bauer et al. 1966). Briefly, 100 μ L of the test bacteria was grown in fresh media (Pfaller et al. 1988). A total of 100 μ L of bacterial suspension was spread onto agar plates. NCCLS (1997) recommended Mueller–Hinton agar. Disc diffusion method was carried out for yeasts as recommended by NCCLS (2003). Plates inoculated with the Gram-negative bacteria *Desulfomonas pigra* ATCC 29098T were incubated for 24–48 h, and the diameters of the inhibition zones were measured in

millimeters (Bauer et al. 1966). Standard discs of Ampicillin (Antibacterial agent) are used as positive controls, but DMSO was used as a negative control. The zone diameters were measured as recommended in NCCLS (1993).

Agar-based methods such as E-test and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods (Liebowitz et al. 2001; Matar et al. 2003; Table 2).

Table 1: FT-IR of Ag-NPs

Frequency (cm^{-1})	Bond/stretching	Functional groups
3447.13	O–H and N–H stretch	Alcohols–phenols and amides
3227.29	C–H stretch	Alcohols and amides
1,639	C=O stretch	Carbonyl compounds containing N–O bond (amides) and aldehydes, ketones and carboxylic acid.
1,156	C–O and C–N stretch

Figure 4: SEM of Ag-NPs.

3.9 Antioxidant activity of silver nanoparticles (1.5 mg/mL)

Adding 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 μL of silver nanoparticles (1.5 mg/mL) were put in tubes and raised each solution in each tube to 1 ml by

Figure 5: TEM of Ag-NPs.

adding ethanol and 4 mL of DPPH (0.1 mM of 2,2'-biphenyl picryl hydrazyl (DPPH) were also added to each tube

(Amin H. H. 2019)). After 30 min of the incubation period at room temperature in the dark, the absorbance was read against the blank at 517 nm. Inhibition of free radical DPPH was calculated according to the following equation:

$$\% \text{ Scavenging activity} = (A_{\text{control}} - (A_{\text{sample}}/A_{\text{control}})) \times 100$$

3.10 Statistical analysis

The statistical package SPSS (version 20) was used for the statistical analysis. The Propit analysis was performed to calculate the medium effective concentration (EC_{50}) for determining bactericidal and fungicidal activities and the medium inhibition concentration (IC_{50}) to determine the antioxidant activity calculated by the linear regression analysis (Francis et al. 2006).

4 Results

4.1 Biosynthesis of silver nanoparticles (Ag-NPs)

The reaction color is converted from transparent to brown (Figure 1), which indicates the success of the reaction.

4.2 Ultraviolet-visible (UV-VIS) spectroscopy analysis

UV-VIS was used to prove the success of the bioreduction of silver nitrate by the aqueous extract of *U. lactuca* into silver nanoparticles (Figure 2a and b). The absorption spectrum of UV-VIS was shown at 406 nm (Figure 2a) in the first experiment and was 446 nm (Figure 2b) in the second spectrum (in another experiment).

4.3 FT-IR (Fourier-transform infrared)

The chemical composition of active groups of silver nanoparticles was determined, and the spectra were recorded (Figures 3a, b and Table 1).

4.4 SEM of Ag-NPs

Ag-NPs were spherical with an average size of 3.89:55 nm (Figure 4).

4.5 TEM graph

TEM of Ag-NPs showed the success of the reaction (Figure 5).

4.6 EDAX (energy-dispersive X-ray spectroscopy) analysis

EDAX analysis showed peaks located between 0 and 4 kV. Silver nanoparticles showed the absorption peak with 51.72% (Figure 6).

4.7 Thermal analysis

DSC-TGA of silver nanoparticles was carried out to observe weight loss with temperature. DSC-TGA analysis of silver nanoparticles is shown in (Figure 7).

4.8 Antioxidant activity

Antioxidant activity of green synthesized silver nanoparticles of *U. lactuca* was at 1.5 mg/mL with $IC_{50} = 0.3$ mg/mL.

Figure 6: EDAX of Ag-NPs.

Figure 7: DSC-TGA of Ag-NPs.

4.9 Antimicrobial activity

Antibacterial and antifungal activities of green synthesized silver nanoparticles of *U. lactuca* are presented in Table 2.

5 Discussion

Green synthesis of silver nanoparticles using extracts of plants or algae is the most preferred technique as it is safe, inexpensive, and eco-friendly. In this study, silver nanoparticles were synthesized by bio-reduction of silver ions (silver nitrate 1 mM) using a green macro algal *U. lactuca* through biological synthesis where it is eco-friendly and safe. The color of the product after biosynthesis was brown due to the excitation of surface plasmon vibration of silver nanoparticles (Figure 1; Bhimba and Kumari 2014).

UV-VIS, FT-IR, SEM, TEM, EDAX and DSC-TGA were applied to prove the success of green synthesis.

The absorption spectrum of UV-VIS is shown at 406 nm (Figure 2a) in the first experiment and is 446 nm (Figure 2b) in another experiment. This was due to the surface plasmon of various silver nanoparticles, which also proved the success of the reaction, and also was due to the increased reaction period that was overnight for the first experiment and 1 day for the second experiment (Bhimba and Kumari 2014; Khalifa et al. 2016).

FT-IR of both *U. lactuca* (Figure 3a) as determined in the study by Amin (2019) and green synthesized silver nanoparticles of *U. lactuca* (Figure 3b and Table 1) indicated that many biologically active compounds

(primary and secondary metabolites) of *U. lactuca* shown in Figure 3a disappeared in Figure 3b, which indicated that many bioactive compounds involve bioreduction of silver nitrate into silver nanoparticles and suggested that bioactive compounds in the seaweed extract are responsible for the formation of silver nanoparticles, which was also proved by Khalifa et al. (2016). On the other hand, there is another opinion that protein molecules are responsible for the bioreduction (Bhimba and Kumari 2014), but I suggest that biologically active compounds in the presence of proteinated enzymes of *U. lactuca* are responsible for bioreduction of silver nitrate into silver nanoparticles.

SEM of Ag-NPs was spherical with an average size of 3.89:55 nm (Figure 4).

The TEM graph showed the success of the reaction between silver nitrate and phytochemical components of *U. lactuca*, and the silver nanoparticles were of spherical shape with 3.89:55 nm (Figure 5).

EDAX (energy dispersive X-ray spectroscopy) analysis was used to confirm the presence of silver nanoparticles (Figure 6) and showed peaks located between 0 and 4 keV. Silver nanoparticles showed the absorption peak with 51.72%, which indicated high silver contents in the colloidal solution of silver nanoparticles. Other elements such as O, Si, Cl, Na, and Ca of 19.21, 1.01, 23.85, 3.1, and 1.1%, respectively, may be found due to some salt or protein residue in the marine environment.

Thermal analysis (DSC-TGA) of silver nanoparticles was carried out to observe weight loss with temperature (Figure 7). The green synthesized silver nanoparticles

Table 2: Antimicrobial activity of Ag-NPs

Microorganism	Inhibition zone diameter (mm/mg)	Standard
<i>Bacillus subtilis</i>	G+ 30	32
<i>Escherichia coli</i>	G– 26	30
<i>Klebsiella</i> sp.	G– 17	18
<i>Pseudomonas aeruginosa</i>	G– 27	28
<i>Staphylococcus aureus</i>	G+ 25	26
<i>Streptococcus faecalis</i>	G+ 13	30
<i>Neisseria gonorrhoeae</i>	G– 11	25
<i>Alternaria alternate</i>	F 0.5	—
<i>Aspergillus fumigatus</i>	F 1	14
<i>Candida albicans</i>	Y 0.5	16
<i>Fusarium oxysporum</i>	F 1	—
<i>Penicillium</i> sp.	F 1	—

F = fungus G+ = Gram-positive bacterium.

G– = Gram-negative bacterium Y = yeast – = not determined.

were subjected to heating at room temperature up to 250°C. Thermal decomposition of silver nanoparticles took place at around 113.65°C and that proved the formation of silver nanoparticles where thermal decomposition of Ag atom molecules occurs at high temperature (over 200°C).

Antioxidant activity of green synthesized silver nanoparticles was at 1.5 mg/mL with $IC_{50} = 0.3$ mg/mL, and the scavenging activity increased with the increasing concentration of silver nanoparticles, and therefore, Ag-NPs may contribute to crop protection from harmful light of sunlight or ozone oxidation.

5.1 Antimicrobial activity

5.1.1 Antibacterial and Antifungal activities of green synthesized silver nanoparticles

Silver nanoparticles showed high toxic effects against bacteria, which are human, plant, poultry, fish, and animal pathogens, such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* and a moderate effect against bacteria such as *Klebsiella* sp., *Streptococcus faecalis* and *Neisseria gonorrhoeae*, but low effect against all fungi (Table 2). There are many theories that inquire the mode of action of silver nanoparticles, but I think the most accepted theory is that silver nanoparticles are characterized by their small size that managed them to reach the cell wall and penetrate it and disturb the permeability of cell wall by interacting with phosphorus and sulfur of DNA, cofactor of enzymes, and protein and damage the cell wall of bacteria, especially at larger surface to volume ratios of their reaction. Also, silver nanoparticles have higher antibacterial activity against Gram-negative bacteria than Gram-positive bacteria, which may be due to the thinner peptidoglycan layer and the presence of beta barrel proteins called porins (Geoprincy et al. 2013).

Antifungal activity of green synthesized silver nanoparticles is due to pits formation on the surface of the fungal cell membrane that causes partial reduction of the cell shape (Devi and Bhimba 2014).

The researcher aims to produce a pesticide that can be used against insects, bacteria, fungi, viruses, nematodes, mice, snakes, and harmful animals and that can be used when growing economic crops. But this needs further research. To make a pesticide for this combination of a group of compounds against various pests

using nanotechnology, which should be safe and harmless to humans, the researcher prefers to use biological synthesis as it is safe, harmless to humans, low cost and not expensive.

6 Conclusion

The biosynthesis of silver nanoparticles was achieved in this article by spectroscopic methods and also showed good effects on different microbes causing diseases in agricultural crops *in vitro*, and we should test this green synthesis *in vivo* in near future as a safe synthetic pesticide in fields and in animals, poultry, rabbits, and fish farms. I hope that I succeeded in persuading the use of nanotechnology to produce agricultural pesticides biologically using organisms available in our environment such as algae, as they are safe, cheap, do not cause harm to humans like previous pesticides, and are effective against different agricultural microbes. Many researches should be done in this field to produce an agricultural pesticide that has effects on other organisms such as insects, bacteria, fungi, viruses, nematodes, mice, snakes, and harmful animals. Biosynthesized silver nanoparticles should also be used in food packaging and food preservation.

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