*OnLine Journal of Biological Sciences*

Original Research Paper

# Comparative Effect of Ulvan and Biosynthesized Silver Nanoparticles on Different Cell Lines Cytotoxicity and Gene Expression

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*Article history*
Received: 04-04-2020
Revised: 29-05-2020
Accepted: 29-06-2020

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**Abstract:** Until now, researchers searching for compounds that contribute for treating the most serious disease of this age, cancer. Researchers hope to contribute to this through this research. Activities of both Ulvan extracted from *Ulva lactuca* and silver nanoparticles (Ag-NPs) were examined against seven different cancer cell types called (Caco2 cells, A549 cells, Mcf7 cells, Hep2 cells, HepG2 cells, PC3 cells, HELA cells). Green synthesized Ag-NPs; via *Ulva Lactuca*; was characterized by UV-VIS, SEM and TEM. Cell viability assay was conducted to study cytotoxicity effects of both Ulvan and Ag-NPs treatments. RTPCR; using different sense and antisense primers; was carried out to study the expression of GAPDH, Bcl-2, Bax, P21 and P53 (apoptosis related genes) in treated cell lines. SEM and TEM showed that Ag-NPs shape is spherical with average size of 3.89-55 nm. Also, cell viability study showed that both Ulvan and Ag-NPs have cytotoxicity effects on different cancer cells. However, Ag-NPs are more effective than Ulvan. Gene expression data illustrated that both treatments induced down-regulation of Bcl2 and induced up-regulation of P53 genes. This work showed that both Ulvan and Ag-NPs have activities against the investigated cancer cell types.

**Keywords:** Ulvan, Silver Nanoparticles, Cell Lines, Gene Expression, Cancer, Biosynthesis

## Introduction

Nanotechnology is a modern science used for the synthesis of nanoparticles ranging from 1-100 nm at nuclear atomic or molecular level. Bottom up nanotechnology technique; by a biochemical reduction; using plants or algae is used in the medicine, agriculture and cosmetics fields as it is easily available, safe, inexpensive, ecofriendly and has no side effects (Nabi *et al*., 2014; Rath *et al*., 2014).

Oceans and seas are good sources of many marine organisms such as algae that classified into many families depending on their pigments. The largest families are green, red and brown algae. Green algae such as macro algal *Ulva lactuca* called also sea lettuce or green laver has many chemical constituents and has many biological activities such as anticoagulant, anti-inflammatory, anti-hyperlipidemic, hypocholesterolemic, hepatoprotective, cytotoxic and insecticidal activity (Amin, 2019; Amin *et al*., 2015; Yu-Qing *et al*., 2016). as animal feed (Abd El-Galil and Amin, 2017) and as prebiotic in food industry (Shalaby and Amin, 2019). Ulvan is extracted from the cell walls structure of thallus of *Ulva lactuca* with yield of dry weight from 8 to 29% and is used in enhancing gastrointestinal immunity, dropping blood glucose and lipids, reducing colorectal cancer and cardiovascular risks. Ulvan has also hepatoprotective effects and can be used as antiviral, as antioxidant and as anticancer (Yu-Qing *et al*., 2016) and as prebiotic in food processing (Shalaby and Amin, 2019), as Ulvan is non-toxic (Tabarsa *et al*., 2018).

Cancer is responsible for million deaths yearly in the world. Cancer means uncontrolled production of unhealthy cells. There are many factors that caused cancer as radiation, chemicals, tobacco and viruses cause oxidative attack on DNA, leading to mutations. Traditional treatments of cancer have a lot of disadvantages especially the delivery of the drug to cancer tissues. Nanotechnology is widely used in cancer treatment and different types of metals in Nano forms are used to treat various types of cancer (Nabi *et al*., 2014; Rath *et al*., 2014).

This paper focuses on the effect of Ulvan extracted from *Ulva lactuca* and green synthesized silver nanoparticles using *Ulva lactuca* against many types of cancer cell lines, making comparison of both of them and studying also their effects on gene expression of cancer cells.

## Materials and Methods

### *Materials*

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

*Ulva lactuca* was obtained from the department microbiology and phycology, Faculty of Science, Zagazig University, Egypt.

### *Methods*

### *Biosynthesis of Silver Nanoparticles using Ulva lactuca (Ag-NPs)*

10 mL of aqueous extract of *Ulva lactuca* (10 g of seaweed + 100 mL of distilled water stirring for 1h at 6000 rpm) (Amin, 2019) and 1 m M of silver nitrate were mixed together and subjected to stirring for 24 h at 100°C (Amin, 2020b).

Spectroscopic methods were applied for proving the success of the reaction such as (UV-VIS, SEM and TEM). Ultra Violet-Visible (UV-VIS) Spectroscopy Analysis was identified by Schimatzu UV-1800 at the central laboratory of the Faculty of Science, Ain Shams University, Egypt (Fig. 1). Shape and size of Ag-NPs were determined by Scanning Electron Microscope (SEM) at National Research Center (NRC), Egypt (Fig. 2). Success of the reaction was also proved by using Transmission Electron Microscope (TEM) at National Research Center (NRC), Egypt (Fig. 3). SEM was done at an applied potential of 15 kV, but samples of TEM were prepared by placing two drops of Ag-NPs solution or film onto carbon-coated TEM grids. Then the film on the TEM grid was dried prior to measurement.

### *Extraction of Ulvan from Ulva lactuca*

Ulvan polysaccharide was extracted by using methods of cold or hot water-extraction and ethanol-precipitation (Luo *et al*., 2010) and purified according to the Sevag method (Luo and Fan, 2011) as described by (Amin, 2020a).

**Table 1:** Types of cancer cells

| Cancer cell lines | Tissue | Disease |
|---|---|---|
| Caco2 cells | Colon | Colorectal adenocarcinoma |
| A549 cells | Lung | Carcinoma |
| Mcf7 cells | Mammary gland, breast | adenocarcinoma |
| Hep2 cells | Hela contaminant | Carcinoma |
| HepG2 cells | Liver | Hepatocellular carcinoma |
| PC3 cells | Prostate | Grade IV, adenocarcinoma |
| HELA cells | Cervix | adenocarcinoma |

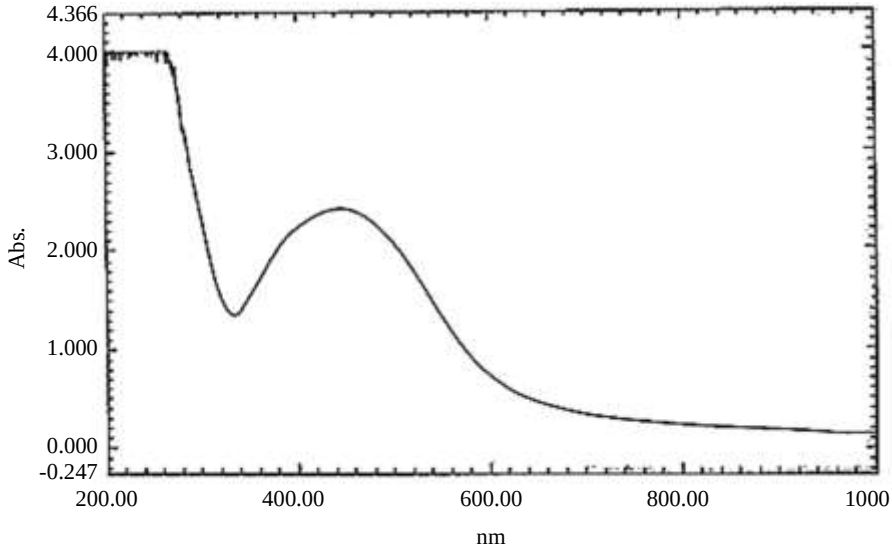


**Fig. 1:** UV-VIS of Ag-NPs

**DOI: 10.3844/ojbsci.2020.125.133**

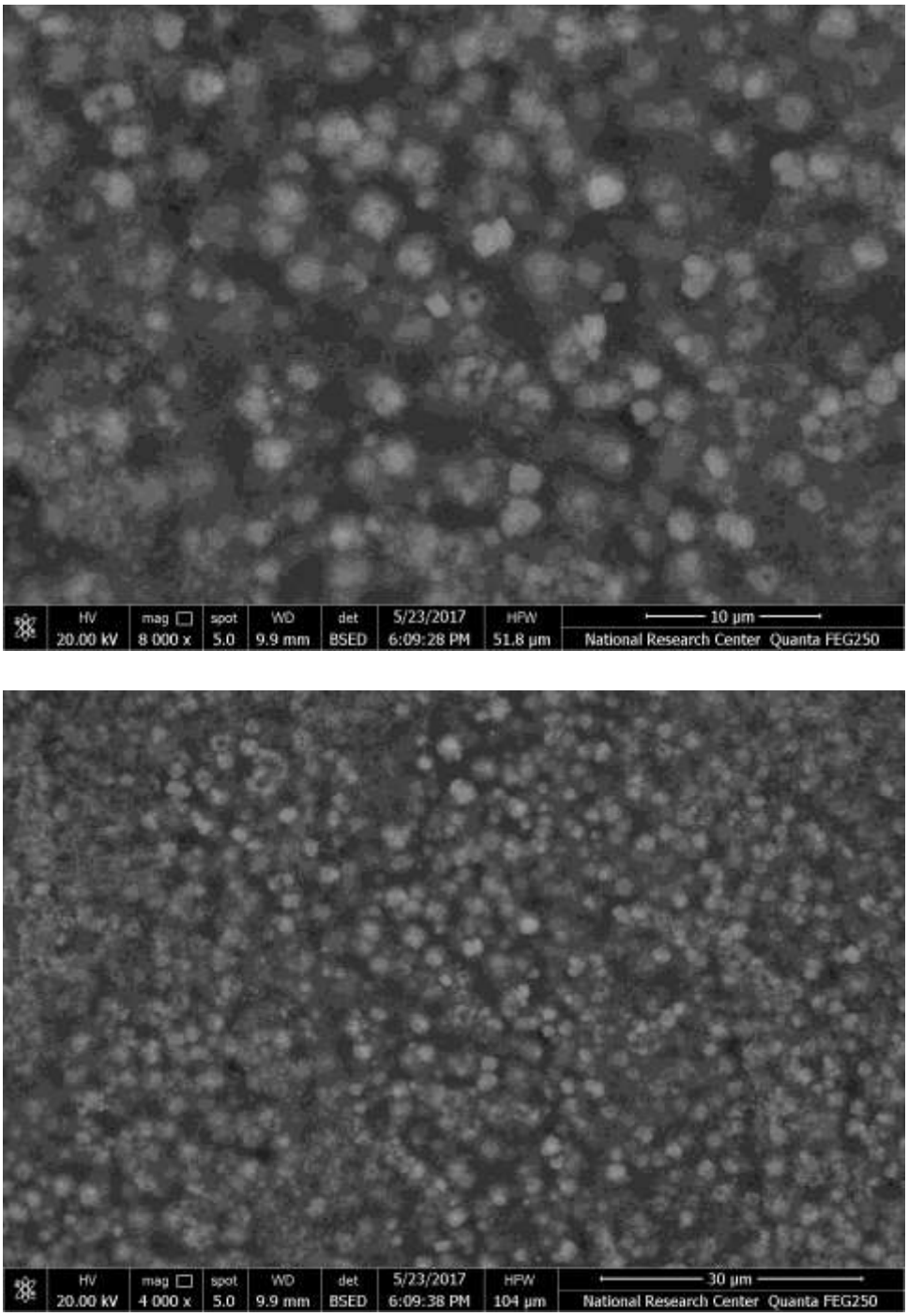


**Fig. 2:** SEM of Ag-NPs

*Cell Viability Assay*

*Determination of Sample Cytotoxicity on Cells (MTT Protocol)*

0.1 mL of each dilution was tested in wells (3 wells as control) receiving only maintenance medium. Plates were incubated at 37°C. 20 uL MTT solutions were added to each well, shaken (150 rpm for 5 min) and incubated for 1-5 h. Dump off the media. Suspend (MTT metabolic product) in 200 uL DMSO and shaken. Optical density at 560 nm was red (Fig. 4), (Table 1) (Abdel-Reheem *et al*., 2014).

**DOI: 10.3844/ojbsci.2020.125.133**

### *DNA Fragmentation*

The $IC_{50}$ of tested sample was added and incubated for 24 h with incubated mixture of cultured cells ($10^5$ cells/mL) and 5 mL of medium. The cell layer was rinsed twice with 5 mL of PBS, lysed, incubated for 5 min and the cell lysates were collected. Proteins were digested by incubation and centrifuged. The supernatant was separated, incubated and centrifuged. Then DNA in the supernatant was precipitated with ethanol. The mixture was centrifuged and the supernatant removed. The pellet was rinsed with 70% ethanol, dried and suspended in 200 µL of TE 20-1 for DNA quantification by UV spectrophotometry at 254 nm (Fig. 5), (Abid-Essefi *et al*., 2014).

### *Gene Expression*

Using Reverse Transcriptase-PCR (RT-PCR), 105 cells seeded in 3 mL in 6-well multi-dishes were incubated with $IC_{50}$ of tested samples for 48h at 37°C. GAPDH genes were used as control and centrifuged for 5 min, the pellet was used for RT-PCR studies, total RNA was isolated and 10 µL of CDNA product (generated by Reverse Transcription System) was used as templates. PCR was carried out using specific primers. Finally the products were separated and stained (Fig. 6), (Yusup *et al*., 2011).

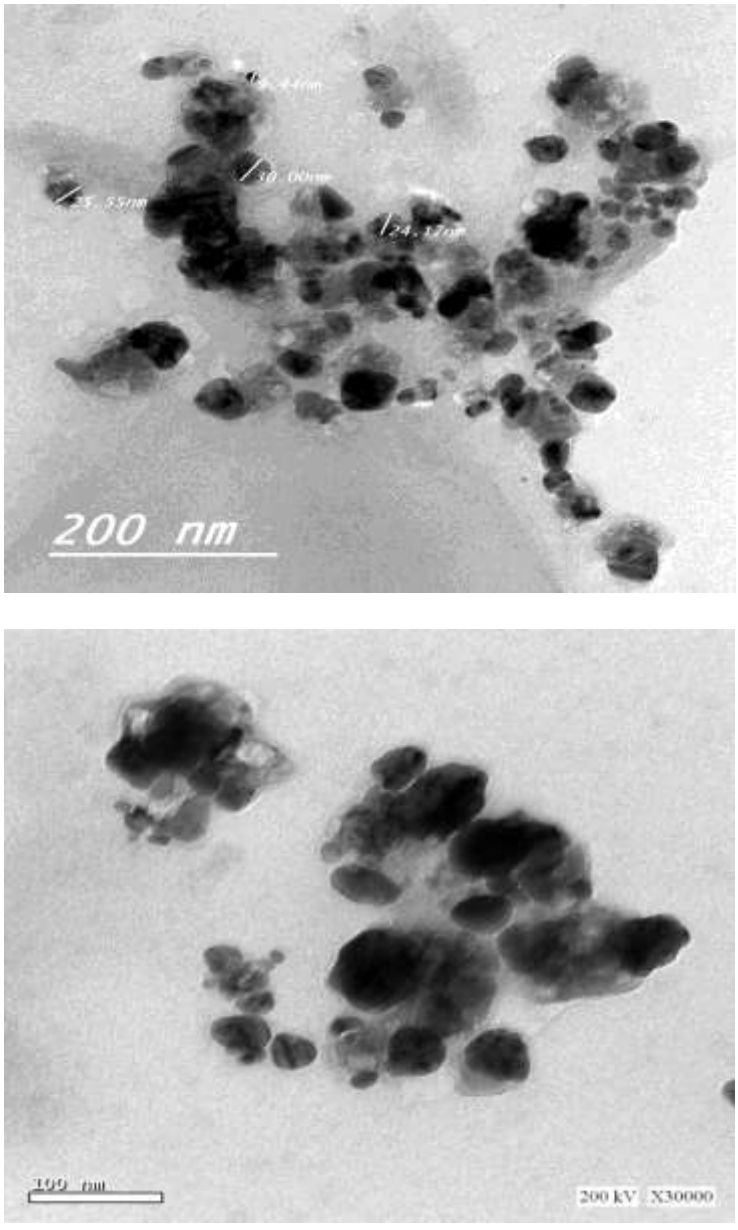


**Fig. 3:** TEM of Ag-NPs

**DOI: 10.3844/ojbsci.2020.125.133**

*Cytotoxicity Effects of Ulvan and Ag-NPs on of Different Cell Lines*

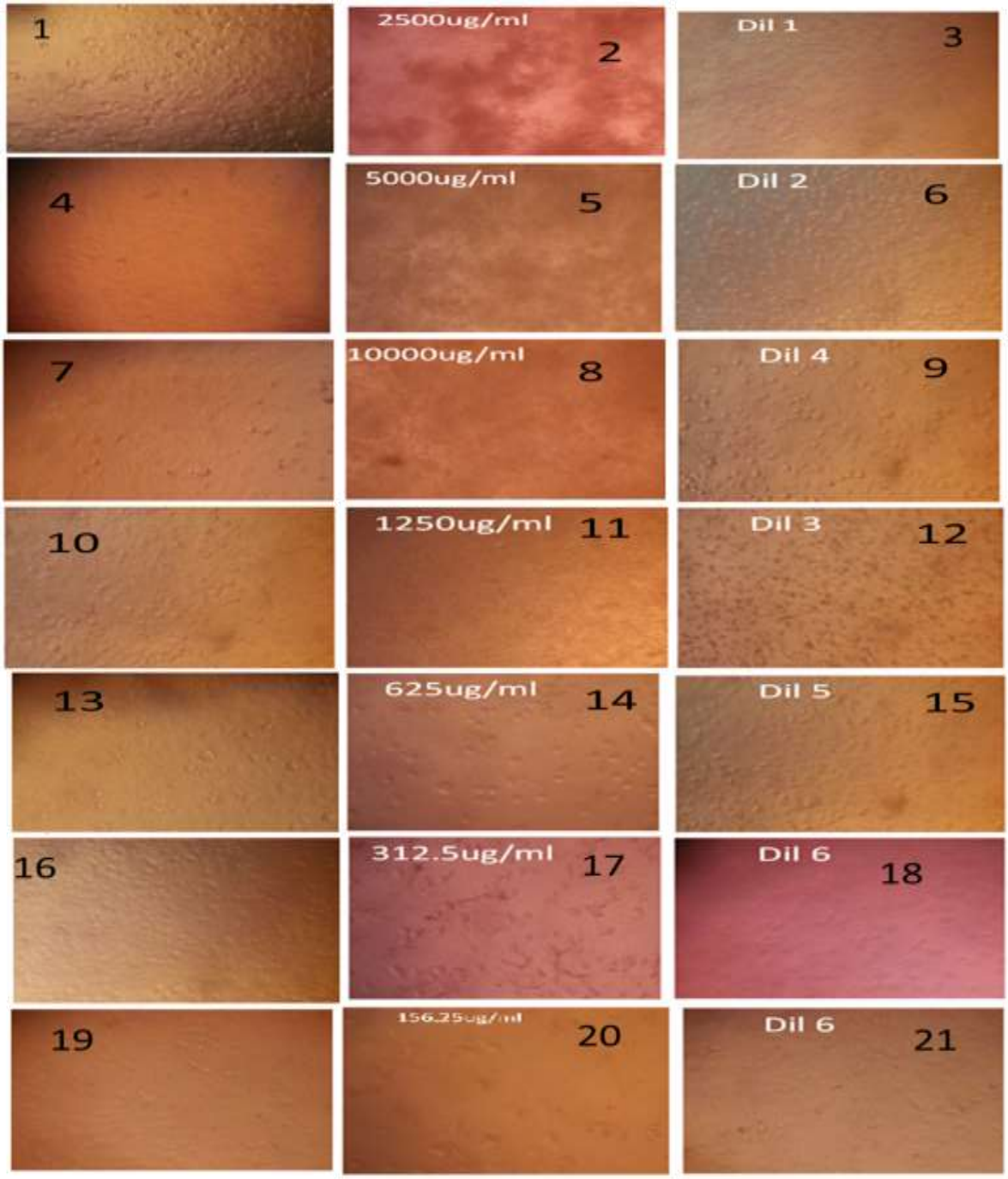


**Fig. 4:** Cytotoxicity effects of Ulvan and Ag-NPs on different cell lines; Where: 1 is Control of Caco2 cells, 2 is Caco2 cells treated with Ulvan (2500 µg/ml), 3 is Caco2 cells treated with Ag-NPs (150 µg/ml), 4 is Control of A549 cells, 5 is A549 cells treated with Ulvan (5000 µg/ml), 6 is A549 cells treated with Ag-NPs (75 µg/ml), 7 is Control of Mcf7 cells, 8 is Mcf7 cells treated with Ulvan (10000 µg/ml), 9 is Mcf7 cells treated with Ag-NPs (18.75 µg/ml), 10 is Control of Hep2 cells, 11 is Hep2 cells treated with Ulvan (1250 µg/ml),12 is Hep2 cells treated with Ag-NPs (37.5 µg/ml), 13 is Control of HepG2 cells, 14 is HepG2 cells treated with Ulvan (625 µg/ml), 15 is HepG2 cells treated with Ag-NPs (9.375 µg/ml), 16 is Control of PC3 cells, 17 is PC3 cells treated with Ulvan (312 µg/ml), 18 is PC3 cells treated with Ag-NPs (4.687 µg/ml), 19 is Control of HELA cells, 20 is HELA cells treated with Ulvan (156.25 µg/ml) and 21 is HELA cells treated with Ag-NPs (4.687 µg/ml); Dil is referred to used dilutions of Ag-NPs that mentioned under the figure

*Effect of Ulvan and Ag-NPs on DNA Fragmentation*

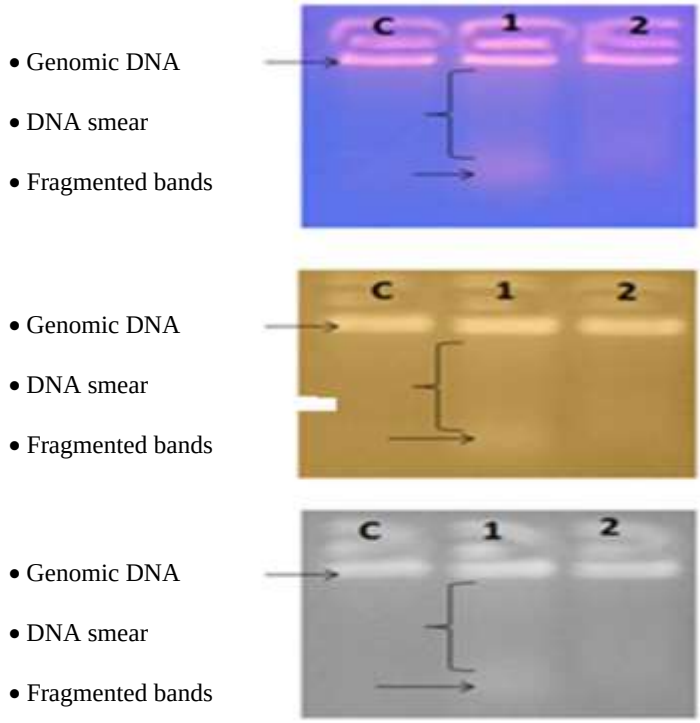


**Fig. 5:** Effect of different concentrations of Ulvan and Ag-NPs on DNA fragmentation; Where: C is control of Caco2 cells (one band, no smear or fragmented bands), 1 is Caco2 cells treated with 205.32 µg/ml of Ulvan (demonstrated DNA smear and fragmented band) and 2 is Caco2 cells treated with 26.445 µg/ml of Ag-NPs (illustrated faint smear of DNA).

*Effect of Ulvan and Ag-NPs on Expression of Some Apoptosis Related Genes in Different Cancer Cell Lines*

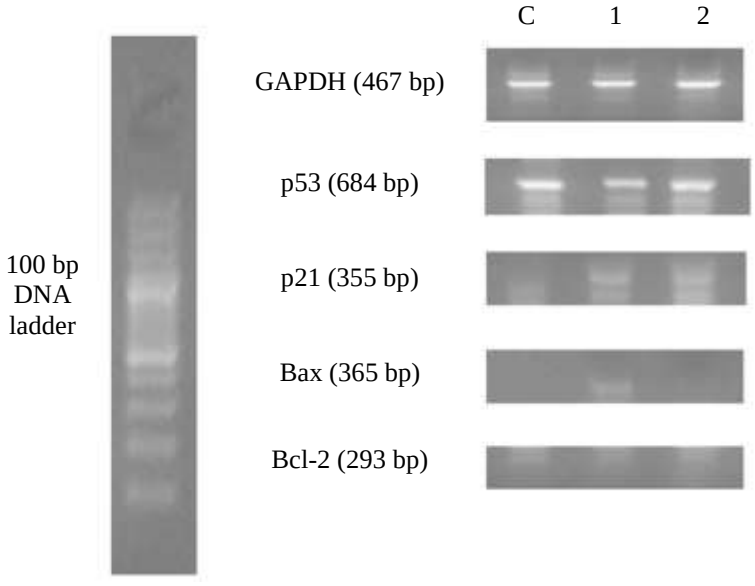


**Fig. 6:** Apoptotic genes expression in Caco-2 cells treated with $IC_{50}$ of tested samples for 48h; where: C is control cells;1 is cells treated with 205.32 ug/mL of Ulvan extract; and 2 is cells treated with 26.445 ug/mL of Ag-NPs extract; (365 bp band of Bax gene was expressed in sample 1, while Bcl-2 do not expressed in all samples)

### *Statistical Analysis*

SPSS (version 20) was used for propit analysis to calculate the medium inhibition concentration ($IC_{50}$) for determining cytotoxic activity (Francis *et al*., 2006).

## Results and Discussion

### *Results*

### *Characterization of Biosynthesis of Silver Nanoparticles using Ulva lactuca (Ag-NPs)*

UV-VIS, SEM and TEM were used to prove the success of the bio-reduction of silver nitrate by aqueous extract of *Ulva lactuca* into silver nanoparticles (Fig. 1-3) (Amin, 2020b).

The absorption spectrum of UV-VIS was shown at 446 nm (Fig. 1); the SEM of silver nanoparticles was spherical with an average size 3.89:55 nm (Fig. 2) and in TEM showed the success of the reaction (Fig. 3).

### *Discussion*

Homo sapiens organism, epithelial human Cell and adherent culture were used.

Ulvan is a polysaccharide composed of sulfate, rhamnose, xylose, glucuronic acid and iduronic acid (Lahaye and Robic, 2007) and was extracted and purified by water-extraction and ethanol-precipitation method with yield 5:10 g/100g of dry weight of *Ulva lactuca* and its sulfate content was 3.998% (Shalaby and Amin, 2019).

### *Characterization of Green Synthesized Ag-NPs*

Product color was brown and the absorption spectrum of UV-VIS was shown at 446 nm (Fig. 1). This was due to the surface plasmon of various silver nanoparticles which also proved the success of the reaction (Khalifa *et al*., 2016). The SEM of silver nanoparticles was spherical with an average size 3.89:55 nm (Fig. 2). While in TEM showed the success of the reaction between silver nitrate and phytochemical constituents of *Ulva lactuca*; bio-reduction; and the product biosynthesized silver nanoparticles were spherical shape with 3.89:55 nm (Fig. 3).

### *Cytotoxicity Effects of Ulvan and Ag-NP$_S$ on Different Cancer Cell Lines*

*In vitro* cell lines of seven types of cancer cells (Caco2 cells, A549 cells, Mcf7 cells, Hep2 cells, HepG2 cells, PC3 cells, HELA cells) were used and their cytotoxic effects were studied (Table 2), (Fig. 4). The cytotoxic activity (as $IC_{50}$) of both Ulvan and Ag-NPs, against seven cell lines was mentioned in Table 2. *In vitro* cytotoxic concentrations were from 4.687:150 µg/ml for Ag-NPs, but from 78.12:10000 µg/ml for Ulvan. The cytotoxicity of cancer cells was increased with increasing the concentrations of both Ulvan and Ag-NPs, cell viability was decreased by increasing concentrations of both of them. Polysaccharides extracted from different marine organisms showed anticancer activity may be rely on inhibition of tumor cell, induction of apoptosis and inhibition of angiogenesis (Fedorov *et al*., 2013) and the higher antitumor activity of Ulvan may be due to its content of sulfate groups (Suresh *et al*., 2013). The effect of Ag-NPs is only on cancer cells, but not on normal cells (Satyavani *et al*., 2012). The cytotoxic effects of Ag-NPs may be due to its interfering with cellular proteins and inducing changes in their chemistry which is definite due to Ag-NPs very small size (3.89:55 nm). where the size of particles less than 100 nm has the possibility of accumulation in tumor cells, so the cytotoxic activity of Ag-NPs is directly proportional to their size (Khalifa *et al*., 2016; Devi and Bhimba, 2012) Table 2 indicates that biosynthesized Ag-NPs were more effective than Ulvan polysaccharides, because Ag-NPs can penetrate tissues and deliver to the target cells due to their small size.

### *Effect of Ulvan and Ag-NPs on Expression of Some Apoptosis Related Genes in Different Cancer Cell Lines*

In Fig. 5 and 6 effect of both of Ulvan and Ag-NPs may be due to the induction of apoptosis based on morphologic or phenotypic signs of cancer cells which rely to the balance between proteins that cause apoptosis e.g. P53, p21 and Bax and proteins that induce the cell viability e.g. Bcl-2. 365 bp band of Bax gene was expressed in Ulvan treatment, while Bcl-2 do not expressed in both treatments. These data indicated that both treatments induce down-regulation of Bcl-2, which also induce up regulation of pro-apoptotic and cell cycle arrest protein p53. It was previously reported that the induction effect on p53 may be due to the inhibition of cyclin dependent kinases. Protein p53 may up regulate the pro apoptotic protein Bax on one and may be cause growth arrest involving p21 as a major effector (Chinni *et al*., 2001; Giannakakou *et al*., 2001).

From previous results we confirmed that Ag-NPs and Ulvan were effective against different cell lines of cancer cells and cause programmed cell death; apoptosis; on cancer cells, but Ag-NPs were more effective than Ulvan at very lower concentrations.

**Table 2:** $IC_{50}$ of different cell lines treated with Ulvan and (Ag-NPs)

| Cancer cell line | Ulvan extracted from *Ulva lactuca* | Biosynthesized silver nanoparticles using *Ulva lactuca* (Ag NPs) |
|---|---|---|
| Caco2 cells | 205.32 | 26.4450 |
| A549 cells | 124.21 | 46.4255 |
| Mcf7 cells | 122.69 | 49.7690 |
| Hep2 cells | 120.36 | 20.7200 |
| HepG2 cells | 67.58 | 36.8260 |
| PC3 cells | 270.25 | 25.2205 |
| HELA cells | 194.65 | 18.3470 |

## Conclusion

Antitumor activities of both biosynthesized silver nanoparticles using *Ulva lactuca*, characterized by UV-VIS, SEM and TEM that indicated Ag-NPs shape is spherical with average size of 3.89-55 and Ulvan extracted from *Ulva lactuca* were achieved in this study and showed significant effects on many types of cancer cells (Caco2 cells, A549 cells, Mcf7 cells, Hep2 cells, HepG2 cells, PC3 cells, HELA cells) *in vitro*. RTPCR was carried out to study the genes expression of GAPDH, Bcl-2, Bax, P21 and P53 in treated cell lines. Both Ulvan and Ag-NPs have activities against the investigated cancer cell types and Ag-NPs is more effective than Ulvan at very lower concentrations. *In vivo* further investigations are needed to be conducted for both Ag-NPs and Ulvan since they are natural, non-toxic,available, cheap, safe and ecofriendly.

## Acknowledgement

The author wants to express deep appreciation to a Professor of Microbiology and Phycology at the Faculty of Science at the University of Zagazig, who helped me in the identification and classification of *Ulva lactuca*.

## Funding Information

The corresponding author is responsible of funding.

## Author's Contributions

All paper was made by corresponding author, while the second author revise the writing part only.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

## Differences of this Paper

Make a comparison and studying the effect of both natural polysaccharides obtained from available and safe green algal called *Ulva Lactuca* and biosynthesized Silver Nanoparticles using the same algal on different cancer cell lines and studying gene expression of both of them and introducing available, safe and ecofriendly sources for fighting cancer.

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