Production, characterization and cytotoxicity effects of silver nanoparticles from *Cystseira myrica*

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**Production, characterization and cytotoxicity effects of silver
nanoparticles from *Cystseira myrica***

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Abstract:

Background: A green, eco-friendly approach to biosynthesizing silver nanoparticles has been reported for marine macroalga *Cystoseira myrica* extract as a reducing agent.

Methods: Algal extract was prepared from the marine brown seaweed *Cystoseira myrica*. Different pH and temperature impact the green synthesis of silver nanoparticles suggesting that the synthesis depends greatly on pH and temperature. Silver nanoparticles have been detected by modifying the color of the algal extract and verified by the use of UV-vis and other characterizations. The Structure and characters of synthesized nanoparticles were confirmed using TEM, DLS, XRD, and FTIR. Cytotoxicity of the biosynthesized nanoparticles using provided cell lines of breast carcinoma cells (MCF-7) and human hepatocellular carcinoma cells (HepG2).

Results: Shape of silver nanoparticles at pH 9, 75°C for 30 min and was found to be suitable for the biosynthesis process and the AgNPs exhibited a characteristic absorption peak at 434nm. High resolution Electron Microscope Transmission (HR-TEM) reported polydisperse and spherical shapes ranging from 8 to 15 nm. High attractive and repulsive forces between each nanoparticle were recorded with an average zeta potential value of approximately -29.3 mV for *C. myrica* extract NPs. The X-ray diffraction study revealed the crystalline structure of silver nanoparticles. FT-IR has shown the bioreduction of silver ions to silver nanoparticles through biomolecules found in *C. myrica* extract. Silver nanoparticles have been found to have anticancer activity. The cytotoxicity assay was studied against MCF-7 and HepG2 at various concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.2 and 0.1 µg/mL). By increasing the concentration of AgNPs from 0.1 to 100 µg/mL the maximum percentage of viability against MCF-7 and HepG2 cell line decreased from 94.55 ± 7.55 to 19.879 ± 0.503 and from 78.56 ± 11.36 to 25.81 ± 2.66 after time exposure respectively.

Conclusions: The silver nanoparticles from *Cystoseira myrica* have cytotoxicity activity against MCF-7 breast carcinoma cell line and HepG2 human hepato cellular carcinoma cell line.

Keywords: Silver nanoparticles, *Cystoseira myrica*; pH; Cytotoxicity; MCF-7; HepG2.

Background

In this article, to date, more than 2,400 natural marine products have been extracted from seaweed, so the biological synthesis of semiconductor and metal nanoparticles offers several advantages over traditional physical and chemical methods, such as being a quick, simple and environmentally friendly alternative that does not require expensive tools or hazardous chemicals [1]. Many biologically active compounds are found in marine algae, which are employed as a source of feed, food, and as an anticancer agent in medicine [2]. Minerals, polysaccharides, polyunsaturated fatty acids, and vitamins are abundant in Phaeophyceae algae [3]. Furthermore, these organisms exhibit significant quantities of secondary metabolites with pharmacological interest, such as phenolic compounds, terpenoids, and alkaloids, which have been associated to interesting biological activities, such as anticancer and neuroprotective properties [2,4].

The family of Phaeophyceae (fucales), the *Cystoseira* has a significant number of species found throughout the Mediterranean Seas and Atlantic [5]. Sterols, phlorotannins, sesquiterpenoids and meroditerpenoids are abundant in species belonging to this genus, according to phytochemical investigations [6], some of which include antioxidant, antifouling, antitumoral, and/or antimicrobial properties with potential medicinal and pharmaceutical use [2]. *Padina sanctae-crucis*, *Petalonia fascia*, *Dictyota ciliolata* and *Turbinaria tricostata* are examples of Phaeophyceae algae that have exhibited antiproliferative activity in cancer cell lines [7].

Silver nanoparticles (AgNPs) are highly attractive to researchers because of their unique properties and application in a wide range of biochemical and biomedical fields. The production of silver nanoparticles is highly attractive because of the metal's nobility and its wide variety of uses. Their applications include antimicrobial activity, drug development, antiviral activity [1] as well as cancer treatments [2], their uses in the field of enzyme-released systems, drug delivery, biosensor designing, and ultrasound medical imaging has increased because of these features [8]. Chemotherapy drugs, especially alkylating metabolites, are accessible for cancer treatment, however, they have limited specificity for targeting cancer cells, causing side effects in normal cells. Finding drugs and therapies for the treatments of different types of cancer is difficult. As a result, traditional methods require the combination of targeted drug delivery and controlled released technologies

which are more harmful and effective. Nanoparticles are reported to be anticancer agents for cancer cells such as human liver carcinoma cells (HepG2) [9], breast cancer cell line (MCF-7) [10] and cervical cancer cell line (HeLa) [4]. In this study, we used brown algae as a model biological system for their ability to synthesize AgNPs. In addition, their antitumor activity of synthesized AgNPs was investigated for anticancer efficacy against two cancer cell lines.

Materials and methods

Preparation of algal extract

The brown algae *Cystoseira myrica* used in this work is a member of Phaeophyta. *Cystoseira myrica* was collected from Hurghada coastal along the Red sea, Egypt. The marine brown seaweed *Cystoseira myrica* was cleaned with seawater to remove impurities. The seaweed was carried in sterile polythene bags to the laboratory. In the laboratory, the sample was washed with tap water triplicate to remove dirt, sand, epiphytes and then with deionized water three times, to remove any metallic compounds until the pH of the wash solution was equal to deionized water. It was dried in the shade at room temperature until constant weight. The powdered dried algal materials were ground in an electric mortar to get the powder form and passed through 0.2 mm sieve. About 5 gm of powdered alga was added with 50 ml double distilled water in 250 ml conical flask, mixed well on a rotary shaker for 1 hour and then boiled from 5-10 min about 60-80°C [11], the crude extract obtained was filtered and used as reducing and stabilizer agent (stored at 4°C for experimental use).

Optimization of reaction conditions for silver nanoparticles biosynthesis

To study the effect of different conditions on the synthesis of silver nanoparticles, the reaction solution was incubated at 30,60,90,120 and 150 min. To study pH effect, the pH of the reaction solution was adjusted to (5, 7 and 9) in order to study the experiments [12] by using NaOH (0.1N) or HCL (0.1N) [13] after adjusting pH value at 9. The reaction temperature was carried at 25°C and by heating in the water bath at 50 and 75°C [11].

The algal extract of *Cystoseira myrica* was mixed with 1mM silver nitrate (AgNO_3) solution at a mixing ratio (1:9) according to Hashemi et al, [11]. For studying each factor, the absorbance of the resultant solution

was recorded spectrophotometrically from 200 to 700 nm using UV-vis spectroscopy at the absorption range 400-450 nm [14].

Estimation and Characterization of silver nanoparticles

Visual Observations

Algal extract solution and 1mM silver nitrate (AgNO_3) solution were mixed and the reaction of the color change was noticed visually. The color was changed from pale yellow to reddish-brown appearance was indicated of the silver nanoparticles biosynthesis.

Ultra Violet Visible Spectroscopy

Silver nanoparticles formations by the extract of *Cytoseira myrica* were further examined by UV-visible spectra. Measuring sharp peaks given by UV-visible spectrum confirm surface plasmon resonance (SPR) of AgNPs at the absorption range between 200 and 700 nm. The UV-visual spectra of the samples were recorded using a spectrophotometer (Nicolet evolution 100, Cambridge) with digital data capture, wavelength range 200 to 700 nm in chemistry Laboratory of faculty of education, Ain Shams University.

Transmission Electron Microscope

By using High resolution Electron Microscope Transmission (HR-TEM) Analysis (JEOL JEM-2100) at the Egyptian Petroleum Research Institute, Egypt. Characterization of scale, shape, and silver nanoparticle assembly status was monitored. Two drops of silver nanoparticle solutions were placed on carbon-coated TEM grids to prepare samples for TEM research. Before the analysis, the film on the TEM grid was dried.

DLS and Zeta-potential

Zeta potential of AgNPs from an algal extract of *Cytoseira myrica* was evaluated using a Zeta sizer Nano-ZS, MALVERN, United Kingdom), Nanotechnology Centre, Egyptian Petroleum Research Institute (EPRI), Egypt JEOL JEM- 2100 electron microscope, Japan).

X-ray diffraction

The prepared AgNPs sample was analyzed by XRD diffractometer (JED-2300T) Cu-K α X-rays of wavelength 1.54060 Å and data were taken for the range of 5° to 80° with a step of 0.026°. Three drops of silver nanoparticle solution were placed on a microscopic slide and left for two hours at 35° C until air-dried. With Cu kal radiation at a 2 angle, the X-ray generator was operated at a voltage of 45kv and a current of 30mA.

Fourier-transform infrared (FTIR)

The spectra were measured using an FT-IR spectrometer (FT/IR-6100 type A) in the wavelength range of 4000 to 400 nm⁻¹. The biomolecules in the algal extract of *Cystoseira myrica* that are responsible for the reduction of silver ions to create nanoparticles are identified using FT-IR.

Evaluation of antitumor of silver nanoparticles

Cytotoxicity

The tissue culture unit of the holding company for Biological Products and Vaccines (VACSERA), Giza, Egypt, provided cell lines of breast carcinoma cells (MCF-7) and human hepatocellular carcinoma (HepG2). The cytotoxic effect of the biosynthesized silver nanoparticles was investigated against breast carcinoma cells (MCF-7) and human hepatocellular carcinoma cells (HepG2) according to Saintigny et al, [15].

Cytotoxicity of Ag-NPs (MTT assay)

The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was used to test the viability of breast carcinoma cells (MCF-7) and human hepatocellular carcinoma (HepG2) cell lines as described by Mosmann [16]. Cell cultures were carried out in sterilized 96-well microtiter plates either alone (negative control) or with different concentrations of AgNPs (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.2, and 0.1 g/ml) [17]. Data were collected from three independent experiments [18]. The percentage of viable cells was calculated as follows:

Cell viability percentage = (OD of treated cells / OD of untreated cells) X 100 [19].

Statistical analysis

Minitab 19 was used to analyze the experimental data. Before any statistical analysis, all assays were cleaned. Missing data and mistyping errors have been checked. To compare the results of different groups, inferential statistics were used. All parametric assumptions for all variables have been tested. Under the fit General linear model, different comparisons were examined using One-way analysis of variance (ANOVA). Tukey test for pairwise comparisons was used to conduct post hoc analyses of all group interactions. P values were determined to be significant at 0.05.

Results and Discussion

The present study was proposed to assess the ability of marine macroalgae to synthesis silver nanoparticles and their cytotoxic effect against tumor cells. The cytotoxic effects of silver nanoparticles may be attributed to the fact that silver nanoparticles may interfere with the proper functioning of cellular proteins, causing subsequent changes in cellular chemistry [20]. Organic substances found in marine algae are responsible for the release and redact of silver ions in nanoparticles [21]. The color of the aqueous extract of seaweed after exposure to silver nitrate solution (while keeping the entire reaction in room light) changed to brown, indicating the formation of silver nanoparticles [22,23]. The peaks at 400-450 nm in the UV spectrum confirmed the formation of AgNPs. The band registered a low wavelength at the start of the reaction; therefore it was accelerated. Due to the aggregation of nanoparticles generating greater sizes of nanoparticles that required less energy and thus longer wavelength due to polydisperse of nanoparticles, after 150 minutes of reaction, the band was at a high wavelength [24]. So, the rate of reaction is directly proportional to reaction time until 150 min of synthesis because after 150 min, the activity of AgNPs in the solution was stable for two months [25]. The result showed that AgNPs biosynthesized by *Cystoseira myrica* had the highest cytotoxic activity so that we discussed its characterization as followed:

Optimization of biosynthesis of silver nanoparticles

The change in color of the algal extract with the addition of silver nitrate is the first visual indication of silver nanoparticle formation by algae, these findings resemble those documented by Saber et al, [22].

pH

The pH of the solution is one of the most important parameters in the synthesis of silver nanoparticles. The value of pH greatly affected the morphology and size of the biologically synthesized nanoparticles. The electrical charges of biomolecules and capping agents were dramatically affected by pH, affecting their capacity to reduce and bind ions of metal [26]. In this study, the AgNPs formation was recorded at these values of pH 5, 7 and 9. The color of silver nanoparticles synthesized by extract of *Cystoseira myrica* at various pH over different periods changed from colorless to slightly brown, dark brown, and finally dark brown colloidal

solution. The color variations indicate a reduction in Ag^+ to Ag^0 [27]. UV spectroscopy was used to detect the formation of AgNPs throughout time (fig1). At low pH (5) the broad band was recorded and the presence of broad band indicated AgNPs aggregation (fig.1.a.). The neutral pH 7 was also reported to support the phycofabrication of the nanoparticles. The present study reported that an increase in pH accelerates the time of reduction of silver ions and stabilizes the silver nanoparticles by adsorbing on them. In the alkaline medium, the stabilizing and reducing ability of *Cystoseira myrica* extract is increased. The absorbance increased as the pH values ranged from 5 to 9, and a narrow SPR band at a lower wavelength was seen (434nm). Haglan et al, [28] reported that decreasing particle size results in an absorbance peak because the surface plasmon resonance (SPR) of metal nanoparticles records the blue shift with decreasing particle size. The produced nanoparticles morphology, shape, composition, size, and dielectric environment influence the surface plasmon resonance (SPR) band [29]. The SPR band recorded around 434nm suggested that the AgNPs were spherical which has been confirmed by the result of TEM of this study. The spherical AgNPs were recorded at the absorption band at around 400nm in the UV-vis spectra [30]. It is clear from fig. (1) that pH 9 was found to be better for the synthesis of silver nanoparticles because it accelerates the rate of the reaction and it reduces the size of nanoparticles as compared to other pH values [31].

Temperature

Temperature is one of the key influential factors in reactions. In order to report the influence of the heating process in the synthesis of silver nanoparticles, the solution temperature was heated from 25 to 50 and 75°C respectively. It is observed that increasing the temperature was accompanied by a decrease in the time required for the silver nanoparticles biosynthesis fig. (2). The color of the silver nitrate on the addition of *Cystoseira myrica* extract changed from pale yellow to dark brown during the different incubation periods per minute with different temperatures [4]. The change of color of the extract was revealed by the visual observation. The change of color was due to the activation of the surface plasmon resonance (SPR) due to silver nitrates reduction in the extract with temperature [32]. The results showed a parallel increase of nanoparticle content and elevation of the temperature at different incubation periods so that a continuous decrease in increasing the incubation time was recorded until it reached few minutes at 75°C. Optical spectroscopy demonstrated that the effect of varying temperatures on the rate of synthesis of silver nanoparticles increased the intensity of SPR. The UV-Visible

spectrum recorded revealed that at 25°C, after adding silver nitrate (1mM) to *Cystoseira myrica* extract solution (1:9), no change in color was showed and the UV–vis data observe no absorbance peak. A broad absorption peak appears at 415nm after 120 and 150 min as shown in Fig. 2(a). After 120 and 150 minutes, a broad absorption peak appears at 415nm, as illustrated in Fig. 2 (a). The absorbance peak of silver nanoparticles becomes stronger at 50°C, as seen in Fig. 2 (b), and the position blue shifts from 429 nm to 426.5 nm. Results recorded showed that with increasing temperature, after 30 minutes at 75°C, the typical absorbance peaks became stronger and blue shifted, with a peak at 413.5nm. As the reaction time increases, a broad absorbance band forms on the higher wavelength side, corresponding to the agglomeration of some silver nanoparticles. The synthesis rate increased with an increase in reaction temperature up to 75 °C at the optimal AgNO₃ concentration of 1 mM, suggesting maximum synthesis and staying stable for longer periods, showing stabilized synthesis. Despite a large number of studies on AgNPs synthesis, there are just a few studies on optimization. For *E. coli*-mediated AgNPs synthesis, Gurunathan et al, [33] observed that 5 mM AgNO₃, 60°C temperature, and pH 10 provided optimum conditions for the maximum production of small-sized nanoparticles. The increased rate of AgNPs synthesis at optimum circumstances could be a direct result of the effect of pH and temperature on the main biomolecule responsible for the decrease in the *Cystoseira myrica* extract, according to the present study.

Morphological and particle size distribution analyses

Because particles of less than 100-nanometer range have the ability to accumulate in tumor cells, the cytotoxic effect of silver nanoparticles is directly proportional to their size [34]. Fig .3(a) demonstrate the morphology of AgNPs developed by *Cystoseira myrica* extract using (HR-TEM) to be spherical crystalline in shape within the size range is 8-15 nm and the electronic, optical properties of metal nanoparticles are known to depend on their physical shape representing their antitumor activity [35]. The most common instrument for determining the distribution of size profile of small particles (nano-scale) in suspension is the dynamic light scattering (DLS). Therefore, the DLS curves of the silver nanoparticles are shown in Fig. 3(b). The size distribution curves of the prepared silver nanomaterial showed that the average size is less than 100 nm on an average of 8-15nm as confirmed with the HRTEM. Moreover, zeta potential is a measure of the magnitude of the charge repulsion/attraction, known to affect stability, between the particles in solution. Therefore, its

determination brings detailed insight into the reasons for materials aggregation, coagulation, or flocculation in suspensions, which is critically important during water treatment processes. In this work, zeta potential measurement shows that silver nanoparticle has a zeta potential value around -29.3 mV. These zeta potential values indicate that these Silver NPs have higher colloidal stability in the aqueous solution, which is probably due to a stronger repulsion behavior between single particles in polar solution (i.e., water) [36].

Crystal characterization: X-Ray analysis

The X-ray diffraction analysis using *Cystoseira myrica* extract for silver nanoparticles synthesis is showed in Fig.4. The diffracted intensities from 5° to 80° at 2θ theta angles were 26.092° , 32.098° , 37.233° and 46.2° which can be indexed to the lattice planes (56.87 %), (100%), (42.4%), and (30.65 %) respectively, confirming the face-centered spherical structure of the formed AgNPs by *Cystoseira myrica* extract and denoted the presence of crystalline particles. A peak at $2\theta = 37.23$ represents the pure silver formation of the reaction and it is in agreement with Khalifa et al, [37]. Due to a small percentage of salts (as chloride) in brown-marine algae or other biomolecules acting as reducing agents, such as the (O-H) group in *Cystoseira myrica*, number of weak unassigned Bragg reflections could be found [37].

Surface functionalities: FTIR analysis

FTIR spectrum is a tool that provides structural information, i.e., fingerprint, about the presence of certain functional groups that are present in the prepared samples and to determine the active groups in biomolecules which are responsible for the stability and coating of silver biosynthesized nanoparticles. In this work, the FTIR spectra of silver nanoparticles biosynthesized by *Cystoseira myrica* are shown in Fig. 5. Generally, the broad absorption band at 3400 cm^{-1} and the band at 1600 cm^{-1} for the prepared nanomaterial are due to the $-\text{OH}$ groups of the phenol compounds and N-H stretching vibration (presence of phenols or secondary amide) [38]. The presence of NO_2 was indicated by absorption peaks at 1300 cm^{-1} , which could be from AgNO_3 . C-H stretching mode peaks reported at 2300 cm^{-1} revealed a prominent and very sharp peak at 1600 cm^{-1} , which was attributed to the presence of nitrate ions, indicating the presence of protein [39]. Molecules containing NO_2 groups, such as nitro compounds, nitro amines, and nitrates commonly exhibited symmetric and asymmetric stretching vibrations of the NO_2 group at 1660 to 1500 and 1390 to 1260 cm^{-1} regions [38].

Cytotoxicity of silver nanoparticles.

Cancer is one of the major reasons for mortality worldwide [4]. As a result, finding drugs that are help treat various types of cancer is difficult. Silver nanoparticles have a biological role in the bioremediation and diagnosis of human cancers and gained great interest nowadays. Bioactive substances in algae and marine plants have been documented against different cancer cell lines. In this work, the percentage of cytotoxic response of silver nanoparticles against two human cancer cell line using MTT assay *in vitro* breast carcinoma cell line (MCF-7) and human hepatocellular carcinoma cell line (HepG2) treated with various concentration (from 0.1 to 100 µg/mL) investigated after 48 h time exposure was evaluated Fig.6. By increasing the concentration of AgNPs from 0.1 to 100 µg/mL the maximum percentage of viability against MCF-7 and HepG2 cell line decreased from 94.55 ± 7.55 to 19.879 ± 0.503 and from 78.56 ± 11.36 to 25.81 ± 2.66 after time exposure respectively. In general, data reported that AgNPs have a high significant inhibitory effect on MCF-7 and HepG2 cell line which was generally compared to control cells. The current findings are in agreement with Ranjitham et al, [10] who showed that the observed cytotoxic effect against the MCF-7 cell line was dose-responsive to AgNPs at various doses. Devi et al, [9] found that the synthesis of biogenic AgNPs by aqueous extracts of *Sargassum longifolium* and its effect against HepG2 cell line. Balan et al, [4] demonstrated the cytotoxic effects of Ag-NPs on MCF-7 breast cancer cell lines. Nanoparticles penetrate the mammalian cells through endocytosis or phagocytosis significantly dependent on AgNPs size [40]. The small size and surface charge of AgNPs encouraged good dispersion and diffusion into the tumor matrix [41]. Devi and Bhimba [42] have demonstrated that AgNPs synthesized by the seaweed *Hypnea* sp. are affected by particle dimensions. The smaller the particles, the greater the effects. Numerous studies have been reported to elucidate the mechanism of action of cytotoxicity of nanoparticles through the manufacture of free radicals. Venkatesan et al, [43] investigated the anticancer potentiality of AgNPs through their ability to scavenge free radicals. So, the increase in synthesis and the accumulation of damaging radicals which attack protein causing oxidative stress would result in permanent or partial damage of protein functionality and integrity. Dos santos et al, [44] and Slavin et al, [45] suggested that AgNPs regulate the activity of DNA-dependent kinase which takes place in repairing the damage of DNA and this indicates that when exposed to metal NPs, DNA gets damaged, but various pathways

are activated to respond to the damage. It is unclear if Ag⁺ downregulates the gene directly to inhibit DNA repair or if it is due to additional toxicity mechanisms.

Conclusion

The extract of brown algae *Cystoseira myrica* is a source for eco-friendly, cost effective, nonhazardous stable nanoparticles. Silver nanoparticles (AgNPs) from the algal extract were observed within 30 min at 75°C. Rapid synthesis of AgNPs were obtained by increase of pH and temperature. The silver nanoparticles from *Cystoseira myrica* have cytotoxicity activity against two human cancer cell line using MTT assay *in vitro* MCF-7 breast carcinoma cell line and HepG2 human hepato cellular carcinoma cell line.

Abbreviations

AgNPs : Silver nanoparticles.

HepG2: Human liver carcinoma cells.

MCF-7: Breast cancer cell.

HeLa: Cervical cancer cell line.

SPR: The surface plasmon resonance.

DLS: Dynamic light scattering.

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Declarations

- Ethics approval and consent to participate:** This article does not contain any studies with animals performed by any of the authors.

- **Consent for publication:** Not applicable.
- **Availability of data and material:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The authors declare that they have no competing interests.
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- **Authors' contributions:** Rehab M. Mohamed conceived and designed research. Rehab M. Mohamed and Rabea A. Shehab contributed analytical tools. Hesham M. Abd El Fatah and M. O. Abdel-Salam analyzed data. Rehab M. Mohamed, M. O. Abdel-Salam and Hesham M. Abd El Fatah wrote the manuscript. Eman M. Fawzy and Rawheya A. Salah El Din revised the manuscript. All authors read and approved the manuscript.

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Fig. 1. : UV-Vis-Absorption spectra of the silver nanoparticles biosynthesized by *Cystoseira myrica* at different pH ; (a)pH=5, (b)pH=7 and (c)pH=9 at different time intervals minutes (zero time, 30,60,90,120 and150).

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Fig. 2. : UV-Vis Absorption spectra of the silver nanoparticles biosynthesized by *Cystoseira myrica* at different Temperature; (a) T=25°C, (b) T=50°C, (c) T=75°C and at different time intervals minutes (zero time, 30, 60, 90, 120 and 150).

Fig. 3. : Silver nanoparticles biosynthesized by *Cystoseira myrica* a) HR-TEM images. b) DLS Curve.

Fig. 4. : X-ray diffraction analysis of silver nanoparticles biosynthesized by *Cystoseira myrica*.

Fig. 5. : Fourier transform infrared spectroscopic data of silver nanoparticles biosynthesized by *Cystoseira myrica*.

Fig.6. Cytotoxic effect on breast carcinoma cell line MCF-7 (a) and human hepatocellular carcinoma cell line HepG2 (b) treated with various concentration of silver nanoparticles (from 0.1 to 100 µg/ml) synthesized by extract of *Cystoseira myrica* compared to breast carcinoma cell line and HepG2 human hepatocellular carcinoma cell line free from any treatment (Control) and free from any treatment (Control) after 48 hours of time exposure [different letters represent the significance between different groups and same letters express the non-significance between different groups].