

RESEARCH ARTICLE

Cytotoxic Activity of Biosynthesized Gold Nanoparticles with an Extract of the Red Seaweed *Corallina officinalis* on the MCF-7 Human Breast Cancer Cell Line

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Abstract

Background: Nano-biotechnology is recognized as offering revolutionary changes in the field of cancer therapy and biologically synthesized gold nanoparticles are known to have a wide range of medical applications. **Materials and Methods:** Gold nanoparticles (GNPs) were biosynthesized with an aqueous extract of the red alga *Corallina officinalis*, used as a reducing and stabilizing agent. GNPs were characterized using UV-Vis spectroscopy, transmission electron microscopy (TEM), energy dispersive analysis (EDX) and Fourier transform infra-red (FT-IR) spectroscopy and tested for cytotoxic activity against human breast cancer (MCF-7) cells cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, considering their cytotoxicity and effects on cellular DNA. **Results:** The biosynthesized GNPs were 14.6 ± 1 nm in diameter. FT-IR analysis showed that the hydroxyl functional group from polyphenols and carbonyl group from proteins could assist in formation and stabilization. The GNPs showed potent cytotoxic activity against MCF-7 cells, causing necrosis at high concentrations while lower concentrations were without effect as indicated by DNA fragmentation assay. **Conclusions:** The antitumor activity of the biosynthesized GNPs from the red alga *Corallina officinalis* against human breast cancer cells may be due to the cytotoxic effects of the gold nanoparticles and the polyphenol content of the algal extract.

Keywords: *Corallina officinalis* - cytotoxicity - gold nanoparticles- human breast cancer (MCF-7) cell line- seaweeds.

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Introduction

Breast cancer is the most common and the second leading cause of cancer death among women. Annually, more than 1 million cases and half million deaths are recorded worldwide (Ardebil et al., 2011). Age, family history, reproductive abnormalities, exogenous hormones (contraceptives or hormone replacement therapy) and geographical locations are some of the risk factors (American Cancer Society, 2007). Breast cancer is a heterogeneous disease which is characterized by the proliferation and abnormal differentiation of malignant immature cells that often carry aberrations that deregulate hundreds or even thousands of genes (Ghojzadeh et al., 2013).

The incidence of breast cancer is rising rapidly in the population group that used to enjoy low incidence of the disease (World Health Organization Cancer Control, 2007). Many challenges in treating breast cancer patients remain, including reducing treatment-related adverse events, managing triple-negative breast cancer despite poor outcomes and the lack of a therapeutic target and balancing treatment toxicity with quality of

life in patients with metastatic cancer who have already received extensive therapy. To overcome these obstacles, researchers have introduced the use of nanotechnology in breast cancer diagnosis and treatment (Yezhelyev et al., 2006).

Nanotechnology has large potential in detection and treatment of cancer in its incipient stage (Singh and Nehru, 2008). Nanotoxicology is a sub-specialty of particle toxicology. It addresses the toxicology of nanoparticles which appear to have toxicity effects that are unusual and not seen with larger particles. Due to the importance of this size class of particles, the term nanotoxicology has been coined that aims to establish their toxic potential (Oberdorster et al., 2005). Nanoparticles encapsulated with other agents may have potential for cancer control (Muthurilappan and Francis, 2013; Najar et al., 2013; Yin et al., 2013).

Gold nanoparticles may be helpful in overcoming some of the challenges inherent to current breast cancer treatment modalities (Selim and Hendi, 2012). The potential advantages include radiation dose enhancement for recurrent tumors in a previously radiated field or radio sensitization via hyper thermic treatment of similarly

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recurrent tumors (Lee et al., 2014).

Biological synthesis of gold nanoparticles (GNPs) is economical and environmentally benign. It has an outstanding numerous benefits without the use of high pressure, energy, temperature, and toxic chemicals that possess advancement over both physical and chemical methods (Mohanpuria et al., 2008) particularly in producing well-defined NPs with quite controllable shapes and sizes (Yuqing et al., 2009).

Algae are otherwise called bionanofactories because they synthesized nanoparticles with high stability, are easy to handle, and eliminate cell maintenance (Song and Kim, 2009). Recently, gold nanoparticle synthesized using the extract of seaweeds such as *Sargassum wightii* (Singaravelu et al., 2007), *Turbinaria conoides* (Vijayaraghavan et al., 2011), *Laminaria japonica* (Ghodake and Lee, 2011), and *Stoechospermum marginatum* (Rajathi et al., 2012) was reported. However, no reports on the biosynthesis of GNPs using the aqueous extract of *Corallina officinalis* were recorded up till now.

Generally, marine organisms are attractive sources of novel, biologically-active compounds due to their tremendous biodiversity (Kwon et al., 2007). Much attention has been paid to the anticancer activity of seaweed constituents. Seaweeds possess cytotoxic compound such as fucoidans, laminarians and terpenoids, which have anticancer, antitumour and antiproliferative properties (Smit, 2004), so that it occupy a significant place as a source of biomedical compounds. Moreover, ethanolic extracts of *Corallina pilulifera* (EECP) showed cytotoxic activity against human cervical adenocarcinoma cell line, HeLa (Kwon et al., 2007). Nevertheless, the opportunities to discover new anticancer agents in seaweeds remain great.

Therefore, this investigation was designed to elucidate the fabrication and characterization of GNPs by the red alga *C. officinalis*. Its in-vitro cytotoxicity and effects on DNA was assessed against human breast cancer cell line (MCF-7).

Materials and Methods

Sample collection

The red seaweed *Corallina officinalis* sample was collected from the Rocky Bay of Abu Qir, (N 31° 19' E 030° 03') Mediterranean Sea, Alexandria, Egypt. The algae were brought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. Water was drained off and the seaweed was spread on blotting paper to remove excess water. The alga is classified as Phylum: Rhodophyta; Order: Corallinales; Family: Corallinaceae (Aleem, 1993). Algal aqueous extract was prepared according to the method of Rajeshkumar et al. (2013a).

Biosynthesis of GNPs

The algal aqueous extract was used as reducing and stabilizing agent for 1 mM H₂AuCl₄-3H₂O. Typically 10 ml of extract were added to 100 ml of 1 mM aqueous solution of gold chloride and kept at room temperature. A colour change to red of the surrounding medium was observed

by visual observation confirming the reduction of gold ions to GNPs. A stock solution of the lyophilized GNPs (5 mg/ml) was prepared in sterilized deionized water and kept at 4°C.

Characterization of GNPs

The biosynthesis of GNPs was confirmed by UV-Vis spectral analysis using UV-6800UV\Vis Spectrophotometer (JENWAY-Germany). The absorption maxima were scanned at the wavelength of 300-700 nm.

The particle size and the shape of GNPs were visualized by using transmission electron microscopy (JEOL TEM instrument).

The structure of GNPs were characterized by Energy-dispersive analysis X-ray (EDX) spectrum using X-ray micro-analyzer (Module Oxford 6587INCA X-sight) attached to JEOL JSM 5500 LV Scanning electron microscopy.

Fourier Transform Infra-Red (FT-IR) spectral analysis was carried out to identify the possible biomolecules responsible for the reduction of the gold chloride and the capping of the GNPs synthesized by seaweed extract. The spectrum was recorded in the range of 500 to 4000 cm⁻¹ (Tensor 27, Bruker Corp., USA) according to Rajeshkumar et al. (2013b).

Evaluation of the cytotoxic activity of GNPs on MCF-7 cells

Human breast cancer cell line (MCF-7) was cultured in Dulbecco's Modified Eagle Medium (DMEM). All culture media were supplemented with 10% fetal bovine serum (FBS), 1% antibiotic and antimycotic solution (50,000 units/L of penicillin and 50 mg/L of streptomycin) and 2 mM glutamine. Cultures were held in 75 cm culture flasks at 37°C, 5% CO₂ and 95% relative humidity, changing media at least twice a week (Devi and Valentin Bhimba, 2012).

Cell maintenance and culture procedures

MCF-7 cells were seeded in 96-well tissue culture plates. The stock solution was diluted using the cell culture medium during evaluation of cytotoxic activity of the GNPs on the cell line, using different concentrations of 6, 3, 1.5, 0.75 and 0.375 μl/ml. These appropriate concentrations were added to the cultures to obtain respective concentration of GNPs and incubated for 48 hrs at 37°C. Untreated cells were used as a negative control: 1) The cells were then passed 2 or 3 times as a monolayer in tissue culture grade flasks (e.g., 25 cm²) at 37°C±1°C, 90%±5% humidity, and 5.0%±1% CO₂/air before running the test. The cells were examined on a daily basis (i.e., on workdays) under a phase contrast microscope, and any changes in morphology or their adhesive properties were noted; 2) The biosynthesized GNPs were allowed to equilibrate to room temperature, and then it was subjected to 2 fold serial dilution (using DMEM as diluents). It began with 5 μl/ml of DMEM as the highest concentration and it was followed by 4 other dilutions (6, 3, 1.5, 0.75 and 0.375 μl/ml). Then Inoculation for each well with 4×10⁵ cell/well; 3) After cells attain almost 50% confluence, 125 μl of the previously prepared dilutions was added,

and the cells were incubated for $24\text{h}\pm 0.5\text{h}$ ($37^\circ\text{C}\pm 1^\circ\text{C}$, $90\% \pm 5\%$ humidity, and $5.0\% \pm 1\%$ CO_2/air); 4) After 24 hrs, each well was examined under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells; 5) After removal of the Routine Culture Medium, the cells were rinsed very carefully with $250\ \mu\text{L}$ pre-warmed D-PBS, then $250\ \mu\text{L}$ neutral red medium was added and incubated at $37^\circ\text{C}\pm 1^\circ\text{C}$, $90\% \pm 5\%$ humidity, and $5.0\% \pm 1\%$ CO_2/air , for 3 hrs; 6) The absorption of the resulting color solution was measured (within 60 minutes) of adding neutral red desorbed solution at 520 nm, of 10 nm in a micro titer plate reader (spectrophotometer). Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC_{50}) was determined according to the following: $\text{Cell viability (\%)} = \text{Mean OD/ control OD} \times 100$.

Following GNPs treatment, the plates were observed under an inverted microscope to detect morphological changes and photographed.

Isolation of total DNA from mammalian cells and DNA-fragmentation assay

Human breast cancer (MCF-7) cells were suspended in 0.5 ml lysis buffer, then incubated for 1.5h in 37°C incubator, centrifuged at 14,000 rpm/RT/5 min and the supernatant was transferred into new tube. Equal volume of isopropanol and 25ml 4M NaCl (100 mM final concentration) were added and incubated overnight at -20°C then centrifuged again for 14,000rpm/RT/20-25 min. DNA pellet was dissolved in 30-50ml double distilled H_2O , 1-2 ml RNAase was added and incubated at 37°C for 1h. Concentration of DNA was measured and 0.7mg DNA/lane was run on 1% agarose gel (Loannou and Chen, 1996).

Results

Biosynthesis of gold nanoparticles (GNPs)

Reduction of 1 mM gold chloride into gold nanoparticles (GNPs) during exposure to the aqueous extract of *Corallina officinalis* turned the colour to red (Figure 1), was observed by visual observation confirming the reduction of gold ions to GNPs.

UV-Visible (UV-Vis) spectral analysis

The biosynthesized GNPs using *C. officinalis* was confirmed by the UV-Vis spectral analysis at various nm. The colour changed into pink red was due to excitation of Surface Plasmon Vibration which indicated the formation of GNPs (Figure 2). The Surface Plasmon band

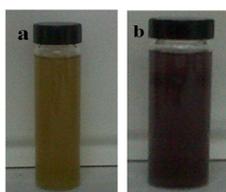


Figure 1. Biosynthesis of Capped GNPs by Reduction of AuCl_4^- Ions using Aqueous Extract of *Corallina officinalis*: A) Algal Extract and B) GNPs Colloid

was observed close to 530 nm throughout the reaction, indicating that the GNPs were dispersed in the aqueous solution with no evidence for aggregation.

Transmission electron microscope (TEM)

The morphological and structural features of the biosynthesized GNPs through reduction of *C. officinalis* aqueous extract was carried out by transmission electron microscopy. It seems to be spherical in shape and well distributed (Figure 3), with average size of $14.57 \pm 1\text{nm}$.

Energy-dispersive analysis X-ray (EDX) spectrum

The presence of gold element in the GNPs is further confirmed by EDX spectrum. The results presented in Figure 4 indicated the existence of gold in the GNPs sample in their corresponding EDX spectra at (1Kev). The EDX profile shows a strong gold signal along with other metals such as oxygen, carbon, magnesium, sodium, copper and zinc peaks, which may have derived from the biological and chemical molecules and bound to the

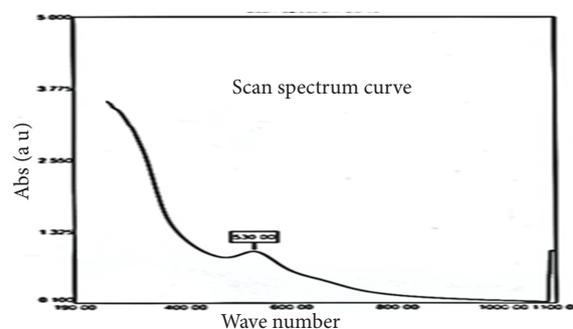


Figure 2. UV/Vis Absorption Spectrum of the Capped GNPs Synthesized using *Corallina officinalis* Aqueous Extract

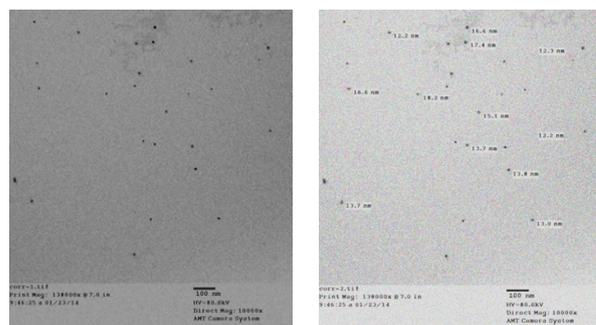


Figure 3. Electron Micrograph of the Capped GNPs Synthesized using *Corallina officinalis* Aqueous Extract

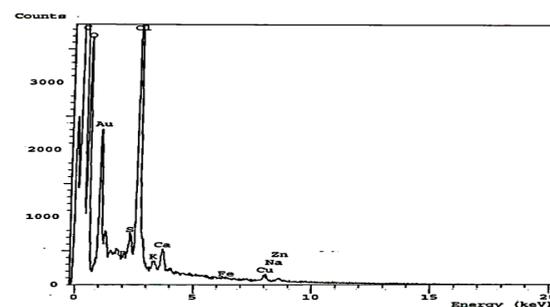


Figure 4. Energy-dispersive Analysis X-ray (EDX) Spectrum of the Capped GNPs Synthesized using *Corallina officinalis* Aqueous Extract

Fourier transform Infra-red (FT-IR) analysis

FT-IR analysis was used for the characterization of *C. officinalis* mediated GNPs. The spectrum reveals the presence of different functional groups (Figure 5). The major peak at 3444 cm⁻¹ may correspond to NH stretching vibrations of free NH group or OH stretching vibrations of hydroxyl group. Sharp peak 1634 cm⁻¹ is assigned to amide-I and amide-II bonding from the capped peptides. However weak peak at 508 cm⁻¹ was also observed.

The cytotoxic activity of the biosynthesized GNPs on MCF-7 cells

The cytotoxic effect of the GNPs was evaluated *in vitro* against MCF-7 cell line using different concentrations (6, 3, 1.5, 0.75 and 0.375 μl/ml). The results in Figure (6) showed that MCF-7 cells incubated with either 3 μl/ml or 6 μl/ml of the biosynthesized GNPs for 48 hrs had rounded, floating and clumped dead cells. Finally the plasma membrane had an inflated 'balloon-like appearance on its rupture site that could be caused by necrosis.

The viability of tumor cells was confirmed using neutral red assay. The results revealed that the concentration necessary to produce 50% of tumor cell death was 1.5 μl/ml of the biosynthesized GNPs (Figure 7). Moreover, there was a direct dose response relationship; cytotoxicity

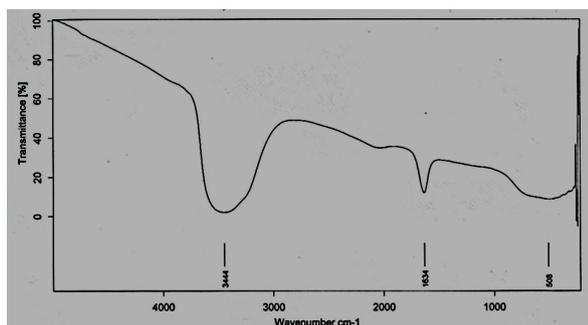


Figure 5. FT-IR Spectrum of the Capped GNPs Synthesized using *Corallina officinalis* Aqueous Extract

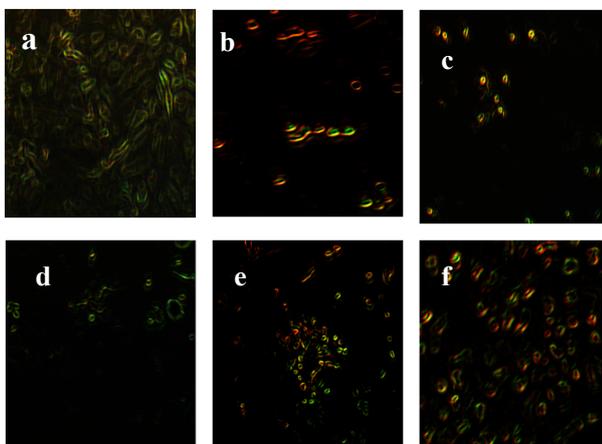


Figure 6. The Effects of Capped GNPs in Recipient MCF7 Cells. (a) MCF7 Cells were Treated with or without GNPs, for 48 h, and then Observed under Microscopy. (a): Microscopy Image of Normal MCF7 Cells, (b; c; d; e; f): Microscopy Image of MCF7 Cells Treated with GNPs at Concentrations of 0.375; 0.75; 1.5; 3 and 6 μl/ml, respectively

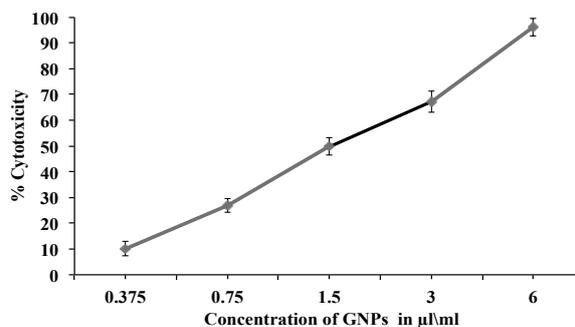


Figure 7. *In vitro* Cytotoxic Activity of the Capped GNPs Synthesized using *Corallina officinalis* Aqueous Extract Against Human Breast Cancer (MCF7) Cell Line

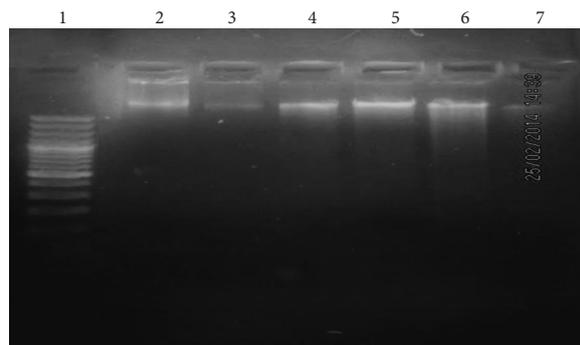


Figure 8. Agarose Gel Electrophoretic Analysis of DNA Isolated from MCF7 Cells Incubated with Different Concentrations Synthesized using *Corallina officinalis* Aqueous Extract. Lane1: DNA ladder; Lane 2: control and Lane 3: 0.75 μl/ml; Lane 4: 0.375 μl/ml; Lane 5: 1.5 μl/ml; Lane 6: 3 μl/ml and Lane 7: 6 μl/ml, respectively

was increased at higher concentration indicating that 1.5 μl/ml of GNPs significantly inhibited the cell's growth. However, the lowest tested concentration (0.375 μl/ml) of GNPs was also able to inhibit the cell line's growth.

DNA fragmentation assay

To detect the mechanism of action of the biosynthesized GNPs against MCF-7 cell line, DNA fragmentation assay was carried out (Figure 8). DNA gel electrophoresis clearly showed that the biosynthesized GNPs at Lanes 6 and 7 (concentrations 3 and 6 μl/ml respectively) appeared as a mild DNA smear which might be considered as mild necrosis, however, no DNA changes were observed at Lanes 3, 4, and 5 (concentrations 0.75, 0.375, 1.5 μl/ml) respectively.

Discussion

Breast cancer is the most common cancer for females all over the world. It accounts for 30% incidence rate in the new female cancers (Siegel et al., 2012). Crude seaweeds or their organic extracts have antiproliferative activity in human cancer cell lines *in vitro*, as well as inhibitive activity in tumors growing in mice (Nagumo et al., 1988; Noda et al., 1989; Deslandes et al., 2000). Gold is non-toxic, inert and stable which has a high binding capacity, thereafter GNPs is considered potential anti-cancer drug carrier (Paciotti et al., 2004).

In the present study, reduction of H₂AuCl₄·3H₂O into

(GNPs) during exposure to the aqueous extract of *C. officinalis* turned the colour to red. In case of negative control (seaweed extract alone), no change in the color was observed. The distinct red colour was observed due to the phenomenon of surface plasmon resonance as reported by (Link and El-Sayed, 2000). The surface plasmon band in the GNPs solution remains close to 530 nm throughout the reaction period indicating that the particles are dispersed in the aqueous solution, with no evidence for aggregation. Similarly, Venkatesan et al. (2014) showed that the UV-Vis spectra recorded at 532 nm correspond to the formation of GNPs by a novel *Ecklonia cava*, while Rajathi et al. (2012) reported the formation of gold nanoparticles using *Stoechospermum marginatum* confirmed by the presence of an absorption peak at 550 nm.

As observed by TEM herein, the algae mediated GNPs seemed to be spherical in shape, well distributed and within the range reported by Ghodake and Lee (2011), who found that GNPs synthesized using the aqueous extract of the brown alga *Laminaria japonica* ranged from 15 to 20 nm. The shape of metal nanoparticles considerably changes their optical and electronic properties (Chandran et al., 2006).

EDX is an excellent technique for identifying atomic compositions, and readily available on most electron microscopes. In the present experiment, gold existed in the biosynthesized GNPs was found in its corresponding spectra. Similar findings were reported by Jadhav et al., (2009).

Algal-mediated GNPs synthesis mechanism was proposed to involve electrostatic interactions between gold anions and functional groups present in the algal extract. Thus, the algal phytochemicals include hydroxyl, and amino functional groups, which can serve both as effective metal-reducing and as capping agents to provide a robust coating on the metal nanoparticles in a single step (Rajeshkumar, 2013b).

In the FT-IR analysis, the bands were tentatively identified on the basis of reference standards, and published FT-IR spectra in relation to specific molecular groups (Sigee et al., 2002; Krishnan and Maru, 2006; Xie et al., 2007). The results of this study confirm that the hydroxyl functional from polyphenols might be involved in the bioreduction of Au (III) ions into Au (0) and carbonyl group from proteins has stronger ability to bind metal so that the proteins or enzymes could most possibly cap the GNPs to prevent the agglomeration of the particles. In this respect Singaravelu et al. (2007) reported that the hydroxyl groups present on the secondary metabolites of the algal material might be involved in the synthesis of GNPs. Similarly, Vijayaraghavan et al. (2011) revealed that Hydroxyl groups present in the brown algal polysaccharides were involved in the bioreduction of Au (III) ions into Au (0). In addition Rajeshkumar et al. (2013b) reported that protein biomolecules in the algae extract do a dual function as reducing the gold ions and stabilizing the GNPs in the aqueous medium. The AuCl₄ anion can bind to positively charged functional groups, such as amino groups (NH₂). Generally, Srivastava et al. (2013) stated that, in the case of biogenic synthesis, the presence of active chemical groups plays a key role in

reduction of metallic ions and subsequent formation of nano/microparticles.

Based on the above mentioned GNPs' surface chemistry, polyphenols and the peptides and/or proteins of *C. officinalis* extract were found to be the key biomolecules engaged in the dual function of Au (III) reduction and healthy capping of the GNPs. The positively charged groups of the *C. officinalis* biomolecules might have regulated the surface mediated process by means of electrostatic interaction, which could have begun with the nucleation of Au⁰ to Au atoms and could have finally formed into GNPs. The study results suggested that molecules attached with GNPs have free and bound amide group. These amide groups may also be in the aromatic rings. This concludes that these compounds could be polyphenols with aromatic ring and bound amide region. Therefore the capping ligand of the GNPs may be an aromatic compound or amines. In accordance with these suggestions, the literature survey found that the marine red algae are rich sources of phenolic compounds especially bromophenols. The biological properties of polyphenols include antioxidant (Bhattacharya et al., 2010) and anticancer (Borchardt et al., 2008) effects. Furthermore, tannis and flavonoids are defined as naturally occurring seaweed polyphenolic compounds which have been found only in marine algae (Li et al., 2010). The seaweeds also contain high amounts of polyphenols such as catechin, epicatechin, epigallocatechin gallate, and gallic acid (Yoshie et al., 2002). These algal natural products demonstrated a broad range of biological activities including antimutagenic, and cytotoxic activities (Naqvi et al., 1980).

During the last few years nanotechnology has witnessed breakthrough in different fields of medicine. Nanoparticles has opened a new arena in the field of cancer therapy because of its unique properties such as the small size, controlled release of drugs and reduced toxic side-effects (Jana et al., 2001; Sun and Xia, 2002). Metallic nanoparticles can be obtained by physical, chemical or biological methods. However, biological synthesis is reliable and eco-friendly, and has received particular attention. We have recently reported the cytotoxic activity of biosynthesized silver nanoparticles with an extract of the red seaweed *Pterocladia capillacea* on the liver cell cancer (HepG2) cell line (El-Kassas and Attia, 2014).

The research scenario of this study extended to estimate the *in vitro* cytotoxic activity of the biosynthesized GNPs against human breast cancer (MCF-7) cell line. The biosynthesized GNPs induce a concentration dependent inhibition against MCF-7 cell line. In this respect, Chithrani et al. (2006) & Chithrani and Chan (2007) investigated the toxicity of GNPs at the cellular level. They concluded that GNPs enter cells in a size and shapedependent manner. The smaller a particle, the greater it's surface area to volume ratio and the higher its chemical reactivity and biological activity. Because of their large surface area, nanoparticles will, on exposure to tissue and fluids, immediately adsorb onto their surface some of the macromolecules they encounter. This may, for instance, affect the regulatory mechanisms of enzymes and other proteins (Ramakrishna and Rao, 2011). Moreover, the

cytotoxic effects of nanomaterials like quantum dots (Hardman, 2006) or carbon nanotubes (Magrez et al., 2006) extend even to normal, noncancerous cells, and some debates have been raised on their unrestricted use. In contrast, with GNPs such issues do not arise and specific cell targeting is achieved using specific surface functionalization of the GNPs (El-Sayed et al., 2006).

The mechanism of cytotoxicity induced by GNPs is also of scientific interest. In order to understand the cytotoxic mechanism of GNPs, DNA fragmentation assay was done and the results showed that the prepared nanoparticles at high concentrations had the potential to un-orderly fragment the DNA which is a hall mark of necrosis. The results suggest that GNPs doesn't induce any inflammatory change at lower concentrations in contrast to higher ones. Here, GNPs of diameter 14.57 ± 1 nm capped with aromatic compound or amines may not affect cell viability in short term but affect cell proliferation and cause DNA damage. Cell response was quick and long lasting. Cells internalized the particles and mounted a robust stress response on the level of DNA. Pan et al. (2009), indicated that the cell death predominantly by necrosis as the prime cause. They suggested that GNPs apart from the mitochondrial membrane may also damage multiple targets along their cellular trajectory, including lipids of the cell membrane, components of the endocytic pathway, newly synthesized proteins, and DNA. Pan et al. (2007) explain why many compounds, including Au1.4MS, induce necrosis at high doses and apoptosis at lower, subnecrotic doses (Kroemer, 1995). However, Selim and Hendi (2012) suggested that gold nanoparticles may induce apoptosis in MCF-7 cells and this warrants further investigation to determine if *in vivo* exposure consequences may exist for gold nanoparticles application.

Nanoparticles exhibit similar size range as many biomolecules such as proteins and DNA, and thus offer great possibilities for the integration of nanotechnology into biotechnology (Niemeyer and Ceyhan, 2001). Currently gold nanoparticles (GNPs) are used in different biomedical applications, including chemotherapy and drug delivery (Han et al., 2007a; 2007b).

Thus, the study results allow us to predict the potential cytotoxic effect of the biosynthesized GNPs by the aqueous extract of *C. officinalis* against human breast cancer (MCF-7) cell line. Thus, they can be used *in vitro* at gradient concentrations up to 1.5 μ l/ml. However further *in vivo* studies are needed to fully characterize the antitumor potential of the biosynthesized GNPs and to examine whether this would prove it to be a novel approach for anticancer therapy.

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