RESEARCH ARTICLE

Bactericidal Application and Cytotoxic Activity of Biosynthesized Silver Nanoparticles with an Extract of the Red Seaweed *Pterocladiella capillacea* on the HepG$_2$ Cell Line

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Abstract

Background: Nano-biotechnology is recognized as offering revolutionary changes in various fields of medicine. Biologically synthesized silver nanoparticles have a wide range of applications. Materials and Methods: Silver nanoparticles (AgNPs) were biosynthesized with an aqueous extract of *Pterocladiella (Pterocladia) capillacea*, used as a reducing and stabilizing agent, and characterized using UV-VIS spectroscopy, Fourier Transform Infra red (FT-IR) spectroscopy, transmission electron microscopy (TEM) and energy dispersive analysis (EDX). The biosynthesized AgNPs were tested for cytotoxic activity in a human hepatocellular carcinoma (HepG$_2$) cell line cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 1% antibiotic and antimycotic solution and 2 mM glutamine. Bacterial susceptibility to AgNPs was assessed with *Staphylococcus aureus*, *Bacillus subtilis* [Gram+*ve*] and *Pseudomonas aeruginosa* and *Escherichia coli* [Gram-*ve*]. The agar well diffusion technique was adopted to evaluate the bactericidal activity of the biosynthesized AgNPs using Ampicillin and Gentamicin as gram+*ve* and gram-*ve* antibacterial standard drugs, respectively. Results: The biosynthesized AgNPs were 11.4±3.52 nm in diameter. FT-IR analysis showed that carbonyl groups from the amino acid residues and proteins could assist in formation and stabilization of AgNPs. The AgNPs showed potent cytotoxic activity against the human hepatocellular carcinoma (HepG$_2$) cell line at higher concentrations. The results also showed that the biosynthesized AgNPs inhibited the entire panel of tested bacteria with a marked specificity towards *Bacillus subtillus*. Conclusions: Cytotoxic activity of the biosynthesized AgNPs may be due to the presence of alkaloids present in the algal extract. Our AgNPs appear more bactericidal against gram-positive bacteria (*B. subtillus*).

Keywords: Cytotoxicity - human hepatocellular carcinoma - pathogenic bacteria - silver nanoparticles

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Introduction

Cancer is considered as one of the major death causes for humans. In 2008, it was responsible for 7.6 million deaths worldwide, particularly in the economically developing countries, and its incidence is continuously increasing due to aging, growth of the world's population and cancer-causing behaviors (Ahmedin et al., 2011).

Cancer is an uncontrolled cell growth with the ability to invade, metastasize and spread to distant sites. In the past decade, there has been increasing interest on the possible relationships between bacteria and different stages of cancer development (Rajeev et al., 2012). Proof of causality has proven more elusive but slowly the research support for implicating specific pathogens has emerged. The International Agency for Research on Caner has now confirmed 7 viral or bacterial agents as carcinogenic on humans (Herrera et al., 2005). Cancers of stomach, liver and cervix rank among the most prevalent ones with viral or bacterial origin (Hamilton and Aaltonen, 2000). Bacteria which got widespread attention are *Salmonella typhi* in gall bladder cancer (Dutta et al., 2000; Shukla et al., 2000; Lax and Thomas, 2002),Chlamydia trachomotis with increased risk of cervical cancer (Chocolatewala et al., 2010) and *Chlamydia pneumonia* and *Streptococcus bovis* linked with lung cancer and malignancies of colon subsequently (Anttila et al., 2003; Kocazeybek, 2003; Biarc et al., 2004; Gold et al., 2004; Littman et al., 2004).

Despite several modes of therapy-such as chemotherapy, immunotherapy, and radiotherapy, the therapy of cancer remains a challenge (Amiji, 2007). Nowadays, several nano-technological approaches have been used to improve delivery of chemotherapeutic agents to cancer cells with the goal of minimizing toxic effects on healthy tissues while maintaining antitumor efficacy (Raphael et al., 2008). The new age drugs like nanoparticles of metals which can combat conditions like cancer and fight human pathogens like bacteria are discovered (Gutierrez et al.,

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2010; Ratan et al., 2011).

The biologically synthesized silver nanoparticles have wide range of applications. Their characterization is an emerging field of nanotechnology because of their applications in many fields including biology and medicine (Begum et al., 2009). Metallic nanoparticles can be obtained by physical, chemical or biological methods. However, biological synthesis is reliable and eco-friendly, and has received particular attention (Duran et al., 2005; Kalishwaralal et al., 2008; Maliszewska and Sadowski, 2009).

Silver is the metal of choice in the field of biological system, living organisms and medicine (Parashar et al., 2009). Since the use of silver-based antiseptics may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics (Jones et al., 2004).

Sriram et al. (2010) demonstrated the efficacy of biologically synthesized AgNPs as an antitumor agent using Dalton's lymphoma ascites (DLA) cell lines *in vitro* and *in vivo*. They added that the histopathologic analysis of ascitic fluid showed a reduction in DLA cell count in tumor-bearing mice treated with AgNPs.

Valentin Bhimba et al. (2001) found that almost 60% of drugs approved for cancer treatment are of marine natural origin. Sithranga and Kathiresan (2010) stated that marine algal flora contributes by 65.63% to anticancer compound.

Further, isolation of cytotoxic anti-tumor substances and bioactive compounds having antibacterial activities from marine organisms has been reported in several references during the last 40 years. Till now, hundreds of biopotential anti-tumor agents have been isolated from marine origin especially from marine algae (Saifuddin et al., 2006; Chakraborty et al., 2010; Joel and Valentin Bhimba, 2010; Valentin Bhimba et al., 2011). In most cases, the evaluation of the anti-cancer potential of crude extracts from different sea organisms has been carried out by the *in vitro* cytotoxicity tests in malignant cell cultures (Valentin Bhimba et al., 2012).

Devi and Valentin Bhimba (2012) studied that the antibacterial activity of AgNPs against wound isolates and *in vitro* cytotoxic activity on human Caucasian colon adenocarcinoma. Duran et al. (2010) made a study on the potential use of AgNPs on pathogenic bacteria, their toxicity and possible mechanisms of action. The green synthesis of AgNPs is a cost-effective and environmentally benign method.

Therefore, this study was designed to describe a simple one step method for the synthesis and characterization of AgNPs by the reduction of aqueous $AgNO_3$ using *Pterocladiella (Pterocladia) capillacea* aqueous extract, investigating its cytotoxic activity on human hepatocellular carcinoma cell line ($HepG_2$) and bactericidal potentialities against two Gram+*ve* and two Gram-*ve* bacterial strains.

Materials and Methods

Sample collection

The red seaweed *Pterocladiella (Pterocladia) capillacea* 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: Gelidiales; Family: Gelidiaceae with the descriptive name fine red turf (Abbott and Hollenberg, 1976; Aleem, 1993). Algal aqueous extract was prepared according to the method of Vivek et al. (2011).

Green synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) (E.Marck) was used in this work and 10 ml of the aqueous extract of *P capillacea* was added to 90 ml of 1 mM aqueous $AgNO_3$ solution, and kept at room temperature for 48 hr at 120 rpm. The aqueous extract is used as reducing and stabilizing agent for 1mM of silver nitrate. Suitable controls were maintained throughout the conduct of experiments. Appearance of a brownish colour in solution is a clear indication of the formation of AgNPs in the reaction mixture (Vivek et al., 2011). The resulted AgNPs colloid was lyophilized. A stock solution of the lyophilized AgNPs (5 mg/ml) was prepared in sterilized deionized water and kept at 4°C.

Characterization of AgNPs

The biosynthesis of AgNPs 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 to visualize the shape of AgNPs were carried out by using transmission electron microscopy (JEOL TEM instrument).

The structure of AgNPs 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 Ag+ions and the capping of the AgNPs synthesized by seaweed extract. The spectrum was recorded in the range of 500-4000 cm-1 according to Kasthuri et al. (2009).

Evaluation of cytotoxic activity of the silver nanoparticles on human hepatocellular carcinoma cell lines ($HepG_2$) Cell culture

Human hepatocellular carcinoma ($HepG_2$) cell line 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% CO and 95% relative humidity, changing media at least twice a week (Devi and Valentin Bhimba, 2012).

Cell maintenance and culture procedures

Human hepatocellular carcinoma ($HepG_2$) cell line was seeded in 96-well tissue culture plates. The stock solution was diluted using the cell culture medium during

evaluation of cytotoxic activity of the biosynthesized AgNPs on $HepG_2$ cell lines, using different concentrations (5, 2.5, 1.25, 0.625 and 0.312 µl/ml). These appropriate concentrations were added to the cultures to obtain respective concentration of AgNPs and incubated for 48 hrs at 37ºC. Untreated cells were used as a negative control. *i*) $HepG_2$ cells are passed 2 or 3 times as a monolayer in tissue culture grade flasks (e.g., 25 cm^2) at 37ºC±1ºC, 90%±5% humidity, and 5.0%±1% CO_2/air before running the test. The cells should be examined on a daily basis (i.e., on workdays) under a phase contrast microscope, and any changes in morphology or their adhesive properties must be noted. *ii*) The synthesized AgNPs 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 µ/ml of DMEM as the highest concentration and it was followed by 4 other dilutions (2.5, 1.25, 0.625, and 0.312). Then Inoculation for each well with 4×10^5 cell/well. *iii*)After cells attain almost 50% confluence, 125 µl of the previously prepared dilutions was added, and the cells were incubated for 24 h±0.5 h (37ºC±1ºC, 90%±5% humidity, and 5.0%±1% CO_2/air). *iv*) 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. *v*) After the remove of the Routine Culture Medium, the cells were rinsed very carefully with 250 µL pre-warmed D-PBS, then 250 µL neutral red medium was added and incubated at 37ºC±1ºC, 90%±5% humidity, and 5.0%±1% CO_2/air, for 3 hrs. *vi*) 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. ***Cell viability (%)=Mean OD/ control OD×100***.

Bacterial susceptibility to AgNPs

Prior to each experiment, the stock solution of AgNPs was sonicated for 10 min using a sonicator (60 amplitude), vortexed for 1 min and then immediately used in the working concentrations of 5, 10 and 20 µg/ml for estimating the bacterial susceptibility.

Bacterial susceptibility to AgNPs was performed against cultures brought from the Regional Center of Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The four bacterial strains used named *Staphylococcus aureus* (RCMB010028), *Bacillus subtilis* (RCMB010067) [Gram+*ve*] and *Pseudomonas aeruginosa* (RCMB010043) and *Escherichia coli* (RCMB 010052) [Gram-*ve*]. Agar well diffusion technique was adopted to evaluate the bactericidal activity of the biosynthesized AgNPs on nutrient agar (Khan et al., 2011).

As a quantitative assay, four wells were bored in the agar plate. Different concentrations of AgNPs (5, 10 and 20 µg/ ml) were added to the respective wells and a control was maintained with the fourth well. Then the plates were incubated at 37ºC for 24 hrs. The experiment was done in triplicate for each pathogenic bacterium and compared with the control antibiotics using Ampicillin and Gentamicin as gram +*ve* and gram -*ve* antibacterial standard drugs, respectively. Mean zone of inhibition in mm±standard deviation was recorded.

Results

Green synthesis of silver nanoparticles

Reduction of 1mM silver nitrate into silver nanoparticles (AgNPs) during exposure to the aqueous seaweed extract of *P capillacea* turned the color to dark brown (Figure 1).

UV-visible (UV-VIS) spectral analysis

The synthesized AgNPs using *P capillacea* was confirmed by the UV-VIS spectral analysis at various nm. The color changed into brown was due to excitation of Surface Plasmon Vibration which indicated the formation of AgNPs (Figure 2). The Surface Plasmon band was observed close to 400 nm throughout the reaction, indicating that the AgNPs were dispersed in the aqueous solution with no evidence for aggregation.

Transmission electron microscope (TEM)

The structural characterization of the synthesized AgNPs through reduction of *P capillacea* aqueous extract was carried out by transmission electron microscopy. The biosynthesized AgNPs seem to be spherical in shape and well distributed with a statistic average size of 11.38±3.52 nm. As shown in Figure 3, the edges of the particles were lighter than the centers.

Energy-dispersive analysis X-ray (EDX) spectrum

The presence of elemental silver is further confirmed by EDX spectrum with the absorption peak in the range of 3 to 4 keV (Figure 4).

Fourier Transform Infra red (FT-IR) analysis

The typical appearances of absorption spectra had 6 clear bands (peaks) over the wave number range 600 to

Figure 1. Photograph Showing Color Changing of $AgNO_3$ into Dark Brown by *P capillacea* Extract

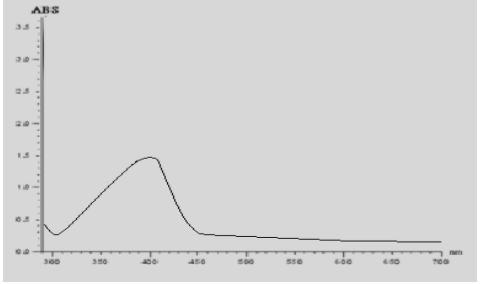

Figure 2. UV/VIS Absorption Spectrum of Reduction of $AgNO_3$ using *P capillacea* Extract

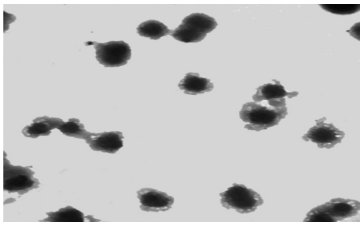

Figure 3. Electron Micrograph of the capped AgNPs Synthesized using *P capillacea* extract; Note that the edges of the Particles are lighter than the Centers, Suggesting the Presence of Protein Shells

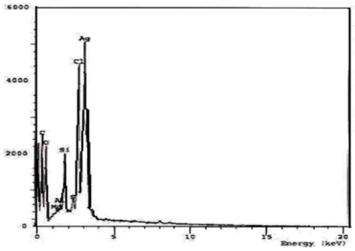

Figure 4. EDX Spectrum of the Biosynthesized AgNPs by *P capillacea* extracts Showing Strong Signals of Silver Element

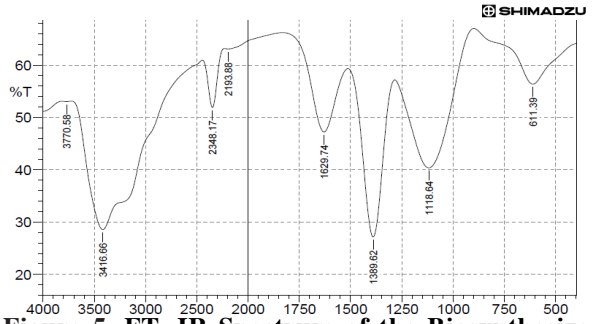

Figure 5. FT- IR Spectrum of the Biosynthesized AgNPs using *P capillacea* extract

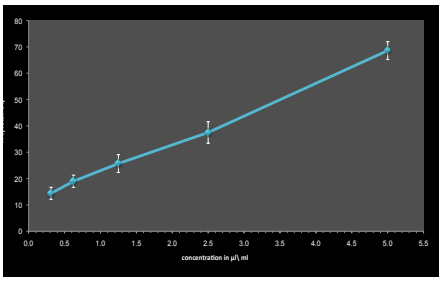

Figure 6. *In vitro* Cytotoxic Activity of the Biosynthesized Ag NPs using the *P capillacea* Aqueous Extract Against Human Carcinoma ($HepG_2$) Cell Line

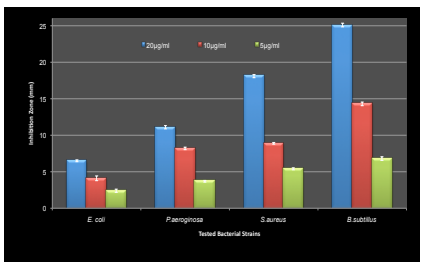

Figure 7. Inhibition Zone of the Biosynthesized AgNPs using *P capillacea* Extract Against Four Bacterial Strains

Table 1. FT-IR Spectrum of AgNPs Synthesized using *P capillacea* extract Showing the Functional Groups of the Protein

Frequency (cm^{-1})	Bond/stretching
3416.66	Protein *v*(N-H) stretching (amide A)
2348.17	P-H stretching of phospholipids, or Combination C-H stretching, or CN stretching. Or stretching vibration of $-NH^{2+}$ as well as $-NH^{3+}$
1629.74	Protein amide I band, mainly *V* (C=O) stretching
1389.62	Protein *v* (CH_2) and *v* (CH_3) bending of methyl carboxylic Acid *v* (C-O) of COO- groups of carboxylates lipid *v* (N (CH_3)$_3$) bending of methyl.
1118.64	Aromatic C-H in-plane bend
611.39	Alkynes C-H bend

4000 cm^{-1} were detected. These bands in published FT-IR spectra are related to specific functional groups. The strong band at 3416.66 cm^{-1} corresponds to Protein v (N-H) (amide A) stretching of carboxylic acids. The peak at ~ 2300 cm^{-1} may be attributed to one of the following: P-H stretching of phospholipids; combination C-H stretching; CN stretching or stretching vibration of $-NH^{2+}$ as well as $-NH^{3+}$. The peak at 1629.74 cm^{-1} correspond to protein amide I band, mainly *V*(C=O) stretching, 1389.62 cm-1 was assigned to protein *V* (CH_2) and *V* (CH_3) stretching of methyl carboxylic acid *vs* (C-O) of COO^-groups of carboxylates lipid V ($N(CH_3)_3$) bending of methyl, 1118.64 cm^{-1} correspond to aromatic C-H in-plane bending and 611.39 cm^{-1} correspond to alkyne C-H bend (Figure 5 and Table 1).

The activity of the the biosynthesized AgNPs on human carcinoma ($HepG_2$) cell line

The cytotoxic effect of the silver nanoparticles was evaluated *in vitro* against human carcinoma ($HepG_2$) cell line at different five concentrations (5, 2.5, 1.25, 0.625, and 0.312 µg/mlL). The viability of tumour cells was confirmed using neutral red assay. The results revealed that the concentration necessary to produce 50% of tumor cell death was 3.7 µg/mlL of the biosynthesized AgNPs (Figure 6). Further, there was a considerable direct dose response relationship; cytotoxicity increased at higher concentration indicating that 5 µg/mlL of AgNPs significantly inhibited the cell lines. However, the lowest tested concentration (0.312 µg/mlL) of AgNPs was able to inhibit the cell line's growth. Cytotoxcicty increased at higher concentration.

Bacterial susceptibility to the biosynthesized AgNPs

The bactericidal activity of AgNPs using *P capillacea* extract was performed against four prominent pathogenic strains. In the preliminary qualitative antibacterial assay, good zones of inhibition were observed; however, the algal extract showed no effect.

In the quantitative assay, the biosynthesized AgNPs inhibited the whole panel of the tested bacteria, which was of dose dependent. On using 20 µg/ml of AgNPs, the results showed a serious range of specificity towards B. subtillus 010067 followed by S. aureus 010028. The values of inhibition zones were 25.1±0.25 mm and 18.1±0.22 mm as respective (Figure 7). This was confirmed by the TEM, where there was an appearance of the bacterial cell

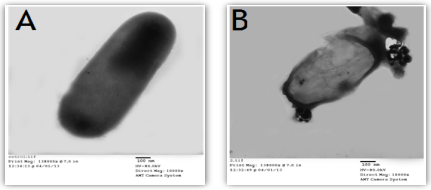

Figure 8. Transmission Electron Micrograph Showing Morphology of *B subtillus*. A) Normal bacterial cell; B) Bacterial cell in the presence of 20μg/ml AgNPs. X=10,000

death and aggregates composed of biosynthesized AgNPs when used against the Gram+*ve B. subtillus* (Figure 8). Further, the control was maintained, where a smaller zone of inhibition was observed. Weaker activities were reported against the Gram-*ve* pathogen of *P. aeroginosa* 01004 as well as of E. coli 010052 with values of inhibition zones of 11.1±0.22 mm and 6.5±0.12 mm, respectively. Therefore, it was observed that the antibacterial potency of AgNPs showed to be higher against Gram+*ve* strains than those of Gram-*ve*.

Discussion

At present, cancer claims the lives of approximately seven million people worldwide on annual basis. Therefore, the research goal of this study was to assist the green synthesis silver nanoparticles (AgNPs) using the seaweed *P capillacea* to estimate *in vitro* its cytotoxic activity against human hepatocellular carcinoma ($HepG_2$) cell line, as well as its antibacterial potentialities against human pathogenic bacteria .

P capillacea is abundantly growing seaweed in coastal areas of the south of Egypt. This plant contains Phycocolloids family (carrageenans) that have physicochemical gelling or stabilizing effects as well as considerable values of sulfated galactan that showed great antioxidant activity and anticoagulant power (Sebaaly et al., 2012).

The use of AgNPs can be exploited in various fields, particularly medical and pharmaceutical due to their low toxicity to human cells, high thermal stability and low volatility (Silver, 2003). In the present study, reduction of $AgNO_3$ into AgNPs during exposure to the aqueous extracts of *P capillacea* changed its color into brown. In case of negative control (seaweed extract alone), no change in the color was observed. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within 48 hours of reaction making it one of the fastest bioreducing methods to produced AgNPs (Kim et al., 2007).

The formation of AgNPs may be attributed to hydrophilic-hydrophobic interactions resulting in intermolecular forces (Medina-Ramirez et al., 2009). This result is similar to those described by many investigators (Ahmad et al., 2002; Shankar et al., 2004; Jain et al., 2009; Guangquan et al., 2012) who explained that the change in color is due to excitation of surface Plasmon vibrations in the metal nanoparticles. The surface plasmon band in the silver nanoparticles solution remains close to 400 nm throughout the reaction period indicating that the particles are dispersed in the aqueous solution, with no evidence for aggregation.

With the TEM, the biosynthesized AgNPs were mostly spherical, well distributed with average size of 11.38±3.52 nm without aggregation in solution. It is known that the shape of metal nanoparticles considerably changes their optical and electronic properties (Xu and Kall, 2002; Chandran et al., 2006).

The present investigation is in consistence with the results of Devi et al. (2013) who revealed that the edges of the particles were lighter than the centers, suggesting that biomolecules, such as proteins in *P capillacea* were capped these nanoparticles.

Further, in the current study, the elemental silver is further confirmed by EDX spectra with the absorption peak in the range of 3 to 4 keV. This result is in accordance with the study of Magudapathy et al. (2001), who reported that the presence of optical absorption peak in the range of 3 to 4 keV is typical for the absorption of metallic silver nano-crystallites. While weaker peaks like C, O_2 and Cl as well as Si are likely due to X ray emission from proteins\ sugars present in the seaweeds Devi et al. (2013). Presence of silicon in the *P capillacea* extract was recorded by Sebaaly et al. (2012).

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 (Dean et al., 1999; Sigee et al., 2002). The present results revealed that the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups of carboxylic acids. These results are confirmed by Sebaaly et al. (2012) who found these terpenoids in the *P capillacea* extract. The carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of AgNPs in the aqueous medium (Krishnaraj et al., 2010). Gole et al. (2001) reported that proteins can bind to nanoparticles either through free amine groups or cysteine residues, and therefore, stabilization of the AgNPs protein is possible.

In this study, we have employed a dose dependent approach to evaluate the toxicity of the biosynthesized AgNPs against the human hepatocellular carcinoma ($HepG_2$) cell line.The use of AgNPs should emerge as one of the novel approaches in cancer therapy and when the molecular mechanism of targeting is better understood, the applications of AgNPs are likely to expand further (Vaidyanathan et al., 2009).

Further, in the current results, the biosynthesized AgNPs induce a concentration dependent inhibition when evaluated *in vitro* against the human hepatocellular carcinoma cell line. Devi and Valentin Bhimba (2012) reported that the cytotoxic effect is inversely proportional to the size of AgNPs.

Martins et al. (2010) explained that the cytotoxic effect of silver is the result of active physicochemical interaction of silver atoms with the functional groups of intracellular proteins as well as with the nitrogen bases

and phosphate groups in DNA. Sriram et al. (2010) demonstrated that AgNPs serve as antitumor agents by decreasing progressive development of tumor cells. They suggested that AgNPs can induce cytotoxic effects on DLA cells, inhibiting tumor progression and thereby effectively controlling disease progression without toxicity to normal cells.

Bacterial and viral infections leading to chronic inflammation play a major role in gastrointestinal carcinogenesis (Hamilton and Aaltonen, 2000). A range of gastrointestinal cancers arise from inflammation and are preceded by a lengthy precancerous process, developing via multiple sequential steps. Substance-or disease-related chronic inflammation or oxidative stress results in the initiation of continual regenerative processes, where the replacement of lost and injured cells through cell division offers an opportunity for the accumulation of genetic damage (Orlando, 2002). Characteristic for the progression to invasive cancer, the appearance of abnormal cells (dysplasia) is followed by the development of preneoplastic lesions, and then, finally, the emergence of a carcinoma (Raza, 2000).

The current results proved that the AgNPs synthesized using *P capillacea* seem to be promising and effective antibacterial agent against the pathogenic strains especially for B. subtilues. It was observed that antibacterial potency of the biosynthesized AgNPs is dose-dependent assay, where the maximum zone of inhibition was detected at 20 μg/ ml compared to 10 and 5μg/ml AgNPs. In contrast, algal aqueous extract showed no zone of inhibition against any of the pathogenic strains used.

In the present scenario, the biosynthesized AgNPs have emerged up as novel antibacterial agents owing to their high surface area to volume ratio and its unique chemical and physical properties. The biogenic AgNPs synthesized using green seaweed, exhibited good antibacterial activity against many clinical pathogens (Raja et al., 2012).

Morones et al. (2005) explained that the bactericidal effect of AgNPs could be attributed to either their interaction with the surface of membrane or to their penetration inside the bacteria. Number of studies suggested that silver ions react with SH groups of proteins and play an essential role in bacterial inactivation. Silver ion binds to functional groups of proteins, resulting in protein denaturation, in addition to that DNA loses its replication ability and cellular proteins become inactivated and finally, cell death. Sondi and Salopek-Sondi (2007) suggested that the antibacterial activity of AgNPs on gram *–ve* bacteria was dependent on its concentration, and closely associated with the formation of 'pits' in the cell wall of bacteria.

Inside a bacterium, nanoparticles can interact with DNA, thus failing its ability to replicate which may lead to the cell death (Raja et al., 2001). Thus, AgNPs accumulated in the bacterial membrane, increasing its permeability and hence degradation, resulting in cell death. Kvitek et al. (2008) explained that the attachment of AgNPs to the surface of the cell membrane disturbing permeability and respiration functions of the cell.

From this study, the observed formation of aggregates composed of biosynthesized AgNPs and dead bacterial cells by performing the TEM analysis, helps us to conclude that the bactericidal activity of AgNPs predicted to be occurred as these nanoparticles somehow interact with "building elements" of the bacterial membrane, causing structural changes and degradation and hence increase in the cell permeability.

Thus, the obtained results allow us to predict the potential effect of the biosynthesized AgNPs using the extract of *P capillacea*, not only because of its bactericidal effect but also due to its cytotoxicity. However, further *in vivo* studies are needed to fully characterize the antiproliferative potential of the biosynthesized AgNPs to examine whether this would prove to be a novel approach for accelerating anticancer potentiality.

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