Silver Nanoparticles: Anti-Bacterial and *invitro* Cytotoxic Activity

J. Saraniya Devi and B. Valentin Bhimba

***Abstract*—**The present study was aimed to evaluate the anti bacterial and anticancer properties of the silver nanoparticles synthesized using the macro algae-Gelidiella sp. The antibacterial activity was investigated against pathogenic bacteria viz., Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Bacillus sp. by disc diffusion method. The anticancer activity of nanoparticles has been assessed in vitro using (4,3,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT assay) on (HT29) colon cell line. The potency of silver nanoparticles to inhibit the cancerous growth was recorded in terms of decrease in viable cell count as compared to the control value. The inhibition of the growth of the human colon cell line (HT 29) has been found to be dose dependent.

***Keywords*—**Antibaterial activity, Cytotoxicity, HT29 colon cell lines, Marine macroalgae – Gelidiella sp, Silver nanoparticles.

I. Introduction

NANOTECHNOLOGY presents potential opportunities to create better materials and products. Silver nanoparticles (AgNPs) have been extensively used in various areas like medical instruments, personal care products, solar energy conversion, food service, building materials, water treatment, catalysis and textiles because of their antibacterial effect. Nowadays microorganisms become resistant to many drugs. Therefore there is a need to develop new antimicrobial drugs for the treatment of infectious diseases. Antimicrobials of marine origin have enormous therapeutic potential. Cancer is an abnormal type of tissue growth in which the cells exhibit and uncontrolled division leading the increase in the number of dividing cells [1]. Several studies have reported that silver nanoparticles have significantly induced cell necrosis or apoptosis in many cell types [2]. There is increasing demands for anticancer therapy [3]. Invitro cytotoxicity testing procedures reduces the use of laboratory animals [4] and hence use of cultured tissues and cells have increased [5]. The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno-pharmacology [6]. It is a challenge to find drugs for the effective treatment of various types of cancers. HT29 cells are human intestinal epithelial cells which produce the secretory component of immunoglobulin A and carcinogenic antigen. The incidence of colon cancer is rising in every country of the world. With this aim, many attentions have been paid to natural compounds in marine plants, microorganisms and plants.

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II. Materials And Methods

The macroalgae-Gelidiella sp. was collected from Mandapam coastal regions, Tamil Nadu, Southeast coast of India. Sample was washed thoroughly with sterile water to remove debris and salt on the surface and ground to fine powder. The powder was extracted aqueous and the filtrate was used for the synthesis of silver nanoparticles. The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium. The interaction between protein-silver nanoparticles was analyzed by FT-IR. XRD, SEM and TEM analysis was employed to visualize the size and shape of silver nanoparticles.

Antibacterial activities of the synthesized silver nanoparticles were tested against human pathogenic bacteria viz., Escherichia coli, Bacillus sp, Klebsiella pneumonia and Staphylococcus aureus by disc diffusion method. All microorganisms were obtained from the MTCC (Microbial Type Cell Culture), India and were maintained at 4ºC on nutrient agar.

The HT29 cell line was seeded in 96-well tissue culture plates. Stock solutions of nanoparticles (5 mg/ml.) were prepared in sterile distilled water and diluted to the required concentrations using the cell culture medium. Appropriate concentrations of Ag-NP stock solution were added to the cultures to obtain respective concentration of Ag-NP and incubated for 48 hrs at 37˚C. Non-treated cells were used as control. Incubated cultured cell was then subjected to MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole) colorimetric assay. Following Ag-NP treatment, the plates were observed under a light microscope to detect morphological changes and photographed.

III. Results

It is well known that silver nanoparticles exhibit brown colour in water [7], this colour arises due to excitation of surface Plasmon vibrations in the metal nanoparticles [8]. The peak at IR spectroscopic study confirmed that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal 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 silver nanoparticles in the aqueous medium. SEM and TEM observation reveals that the silver nanoparticles formed were predominantly cubical with uniform shape.

It was well known that silver nanoparticles exhibit strong antibacterial activity due to their well developed surface which provides maximum contact with the environment. Silver nanoparticles of Gelidiella sp. were fairly toxic to Bacillus sp. and Staphylococcus aureus with the inhibition zone of 25 and 24 mm. However, nanoparticles exhibited low toxicity against Klebsiella pneumonia and Escherichia coli with the zone of 17 and 19 mm [Figure 2]. Zone of inhibition around silver nanoparticles for individual bacterial culture with standard antibiotic ampicillin of 50µl is shown in the figure-1.

The cytotoxicity of the silver nanoparticles was evaluated in vitro against HT29 cell lines at different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 µg/ml). Our cytotoxicity analysis of the sample shows a direct dose-response relationship; cytotoxicity increased at higher concentrations (see fig. 3). The sample demonstrated a considerable cytotoxicity against the HT29 cell lines. The concentration necessary to produce 50% cell death was 39.5 µg/ml. The presence of 31.25 µg/ml of silver nanoparticles significantly inhibited the cell line's growth (> 60%). The plates were observed under a light microscope to detect morphological changes and photographed.

IV. Discussion

Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as source of food, feed and medicine. Until now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations [9]. From this study, it is clear that the synthesized silver nanoparticles were found to be bactericidal. It has been reported the greater the zone of inhibition, greater the antibacterial properties of the silver nanoparticles and that antibacterial effect was dose dependent [10, 11]. The AgNPs synthesized using the marine microalgae was reported for its antibacterial agent by Devina Merin et al [12]. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also aggravates patient's recovery. The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology [13]. Certain algae have long been used in traditional Chinese herbal medicine in the treatment of cancer [14]. Marine macro-algae contain a number of biodynamic compounds of therapeutic value. These compounds provides valuable ideas for the development of new drugs against microbial infections, inflammations and cancer [15]. The invitro cytotoxicity of the AgNPs was evaluated against HT29 cell line at different concentrations. The invitro screening of the silver nanoparticles showed potential cytotoxic activity against the colon cancer cell lines. The IC50 value was plotted by taking the concentration of AgNPs on X-axis versus percentage of cell viability on Y- axis.

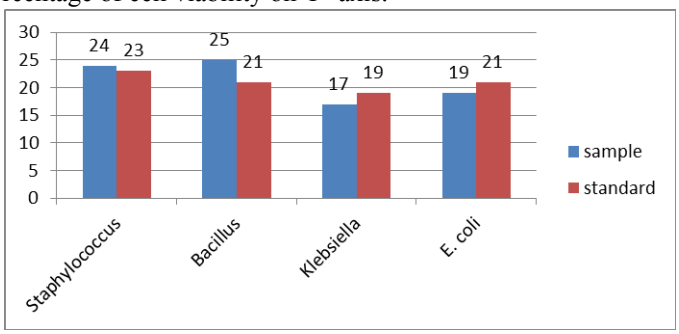

Fig. 1 Screening of antibacterial activity of *Gelidiella sp.* with standard

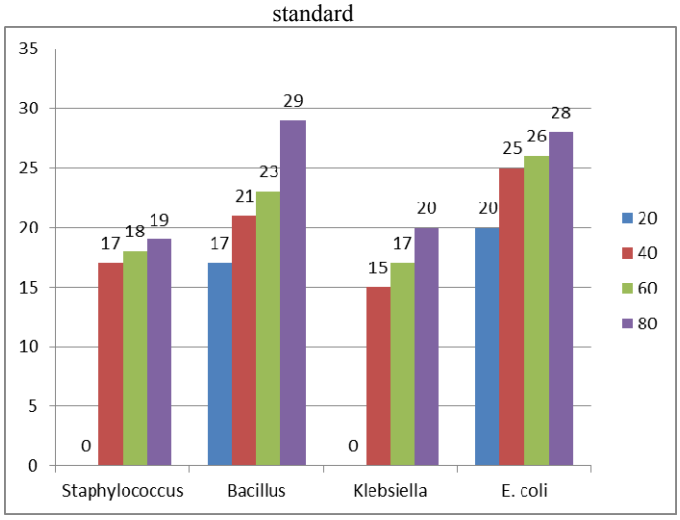

Fig. 2 Screening of antibacterial activity with different concentrations of silver nanoparticles

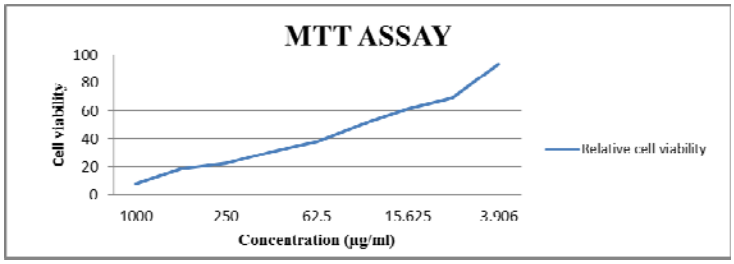

Fig. 3 Effect of silver nanoparticles on HT29 cell viability

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