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ORIGINAL ARTICLE **OPEN ACCESS**

Biosynthesis of gold nanoparticles, characterization and anticancer activity of three red seaweeds

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ABSTRACT
In the present study, three marine macro red algae viz., Gracilaria corticata, G. foliifera and G. kilakkaraiensis were used for the biosynthesis of gold nanoparticles (AuNPs). The colour change from pale yellow to red or deep purple indicates the reduction of gold ions. The AuNPs thus synthesized were characterized using UV-Vis spectrophotometer, Scanning Electron Microscopy (SEM) analysis and X-Ray Diffraction (XRD). UV-Vis spectrophotometer peak and XRD patterns corresponding to gold nanoparticles were observed. SEM analysis was used to analyze the shape and size of the nanoparticles. The cytotoxic activity of AuNPs synthesized by marine algae were tested against breast cancer cell line (MCF-7) and cervical cancer cell line (HeLa). The anticancer activity of the seaweeds was observed and compared using changes in the morphology of the treated and control cells and cell viability %. The cytotoxic assay against HeLa cancer cell line showed greater activity in G. foliifera with an IC value of 51.53% and MCF-7 cancer cell line showed greater activity in G. kilakkaraiensis with an IC value of 48.62%. However, other species also showed good activity against both the cell lines. The results show that these red seaweeds could serve as a good source for the biological synthesis of gold nanoparticles and for cancer therapies.
KEYWORDS: Gold nanoparticles, Gracilaria, HeLa, MCF-7, MTT, cancer cell line

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INTRODUCTION

Seaweeds are marine macro multicellular algae that are photosynthetic in nature. They are rich in proteins, minerals, vitamins, phenols, fibers, carbohydrates, alkaloids, flavonoids, tannins, terpenoids, steroids and polysaccharides [1, 2, 3]. They are used as food, fodder, fertilizer due to high nutritional contents; also in various industries, in treatment of effluents [4] and medicine in many coastal countries. A specific group of seaweed was used in traditional Chinese herbal medicine preparation to treat cancer [5]. The secondary metabolites of these seaweeds possess antibacterial, antifungal, antioxidant, antitumor [5, 6], hepatoprotective [7] and antiviral properties [8, 9, 10] which helps in identifying their role in pharmacological field especially in cancer therapy [11, 12, 13].

Cancer is the most deadly disease that spreads rapidly by multiplying cells uncontrollably in human body parts. Among women, the first common life threatening cancer is breast cancer and most of fatal cases are reported worldwide. The use of biological resource in cancer therapy has gained much interest among pharmacological society. Many drugs are produced by directly deriving natural compounds from plants including microorganisms such as algae, bacteria and fungi, or indirectly deriving from them [14]. Especially the nanotechnology field uses biological resource for the eco-friendly synthesize of nanoparticles for its varied applications.

Generally nanoparticles are synthesized by physical and chemical methods [15], electrochemical methods [16] that involve much energy and materials. On account of yield and cost, nowadays bionanotechnology has gained much interest where it involves plants or microorganisms as a source for the synthesis of nanoparticles which is ecofriendly, non-toxic and cost efficient. Several marine specimens have been used to synthesis different metallic nanoparticles *viz*., gold, silver, cadmium, silicon, germanium and lead [17]. and also various marine algae were used to synthesis gold and silver nanoparticles [18 - 29].

In the present study, three marine red algae *G. corticata*, *G. foliifera* and *G. kilakkaraiensis* have been used to synthesis AuNPs and characterization was done using UV-Vis spectrophotometer, X-ray diffraction, Scanning electron microscope. The nanoparticles are subjected to study the *in vitro* cytotoxic activity against two cancer cell lines MCF-7 (breast adenocarcinoma) and HeLa (cervical carcinoma).

MATERIAL AND METHODS

The seaweeds were collected from Kovalam, Chennai (GPS - 12° 47' 24.7596" N, 80° 15' 14.04" E), Tuticorin (GPS - 8° 44' 59.99" N, 78° 11' 60.00" E), Tiruchendur (GPS - 8° 29' 46.2635" N, 78° 7' 47.8433" E). The samples were identified using the monograph titled Rhodophyta Vol. II Part II-B [30]. They were washed and cleaned thoroughly to remove debris and epiphytes.

Synthesis of AuNPs

The cleaned specimens were shade dried for 3-5 days, powdered and sieved through a mesh size of <0.5mm. The dried powder was used for the synthesis and gold precursor used was hydrochloroauric acid ($HAuCl_4$-) (SRL). A stock solution of 10^{-3}M concentration was prepared for the study. About 30mg of seaweed powder was added to 10ml of gold stock solution in a test tube and incubated at room temperature for 2 to 24h. The colour change from pale yellow to red or purple indicates the reduction of gold ions and synthesis of AuNPs. Then the resultant was centrifuged at 5,000rpm for 15min at 4°C. The pellet was dried, powdered and used for further analysis.

Characterization of AuNPs

The optical property of AuNPs was determined by UV-Vis spectrophotometer at 200- 800nm range of spectra. The crystalline nature was analyzed using X-ray diffraction (XRD) patterns. The shape and size of the particles were analyzed using Scanning electron microscopy (SEM).

Cytotoxic studies of AuNPs

Further, the *in vitro* MTT assay was carried against two cancer cell lines MCF-7 and HeLa.

Cell line and culture

HeLa and MCF-7 cell lines were obtained from NCCS, Pune. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100U/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37°C.

In vitro assay for anticancer activity (MTT assay) [31]

Cells (1×10^5/well) were plated in 24-well plates and incubated at 37°C with 5% CO_2 condition. After the cell reached the confluence, the various concentrations of the samples were added and incubated for 24h. After incubation, the samples were removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4h. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC 50) was determined graphically. The % cell viability was calculated using the following formula:

$$\%\ cell\ viability = \frac{A570\ of\ treated\ cells}{A570\ of\ control\ cells} x100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

Characterization of AuNPs

The colour change from pale yellow to red or purple was the initial observation to confirm the synthesis by reduction of gold ions. UV-Vis spectrophotometer reading at 200-800nm spectra showed peak at 547nm for *G. corticata*, 556nm for *G. foliifera* and 559nm for *G. kilakkaraiensis* which are corresponding peaks for AuNPs (Figs. 1(a-c)). The crystalline nature of AuNPs was analyzed using XRD patterns and compared with the JCPDS database. The XRD graph showed corresponding three peaks in *G. corticata* – 37.92°, 44.01° and 64.13°; four peaks in *G. foliifera* – 38.28°, 44.58°, 64.3° and 77.99°; four peaks in *G. kilakkaraiensis* – 38.28°, 44.73°, 65.19° and 78.23° (Figs. 2(a-c)). The comparative results showed that the patterns of AuNPs synthesized by these algae were identical with the Bragg's reflections for gold.

SEM analysis showed AuNPs of size 65 to 150nm and hexagonal to spherical shaped in *G. corticata* (Fig. 3a), 65 to 80nm and spherical shaped in *G. foliifera* (Fig. 3b), 55 to 75nm and spherical shaped in *G. kilakkaraiensis* (Fig. 3c).

Cytotoxic assay

The cytotoxic activity was evaluated based on the growth inhibition assay. The inhibition assay was calculated based on the concentration of the synthesized AuNPs samples used and the cell death percentage. The growth inhibition activity against HeLa and MCF-7 cells were studied based on the IC_{50}

value. The proliferation of HeLa cells were inhibited and higher activity was observed in *G. foliifera* with an IC value of 51.53% and MCF-7 cell proliferation was inhibited and greater activity was observed in *G. kilakkaraiensis* with an IC value of 48.62%. For *G. corticata*, IC value of HeLa cell line was 52.9% and MCF-7 cell line was 54.99. For *G. foliifera*, IC value of MCF-7 cell line was 46.21% and for *G. kilakkaraiensis*, IC value of HeLa cell line was 52.62% (Table 1).

The morphology of the cells were observed under inverted microscope (Biolink) and the morphological changes were recorded (Fig. 4 - 11). The growth inhibition of HeLa and MCF-7 cells proliferation was observed in all the three samples. Comparatively, *G. foliifera* and *G. kilakkaraiensis* showed good inhibitory activity. *G. corticata* has also showed inhibitory activity.

Seaweeds are widely used in the study of cytotoxic activity against cancer cell lines. Researchers have conducted experiments to study the bioactivities of marine algae and contributed for cancer therapies [32, 33]. Many researchers had studied the anti-proliferative activities using different solvent extracts of marine algae [3, 24, 25, 26, 34-36] and using AuNPs synthesized by marine algae [27, 28, 29]. The present study investigated the cytotoxic activity of AuNPs synthesized from three marine red algae and showed great inhibitory activity against two cancer cell lines. These AuNPs act as capping agent and used in drug delivery. The whole experiment was conducted in a eco-friendly manner.

Table 1: Anticancer activity of samples on HeLa and MCF-7 cell line

Samples	HeLa cell line		MCF-7 cell line	
	Concentration (µg/ml)	Cell Viability (%)	Concentration (µg/ml)	Cell Viability (%)
G. corticata	1000	36.86	1000	35.34
	125	52.90	500	54.99
G. foliifera	1000	24.91	1000	28.75
	62.5	51.53	500	46.21
G. kilakkaraiensis	1000	7.80	1000	6.96
	7.8	52.62	7.8	48.62

*Concentration (µg/ml) – Concentration of AuNPs sample used for growth inhibition
*Cell viability (%) – Cells viability at different concentration of AuNPs

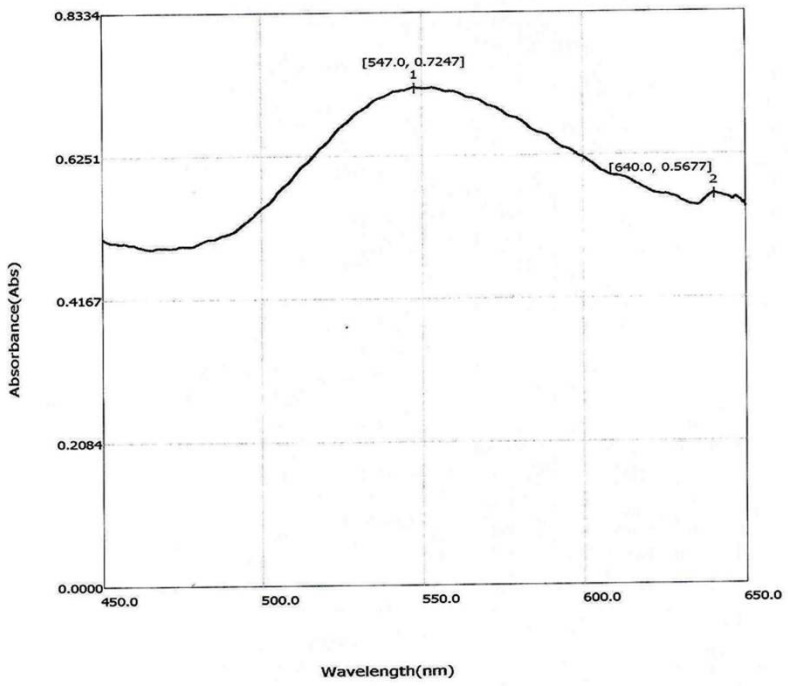

Fig. 1a: UV-Vis spectrophotometer spectra of *G. corticata* showing peak for AuNPs

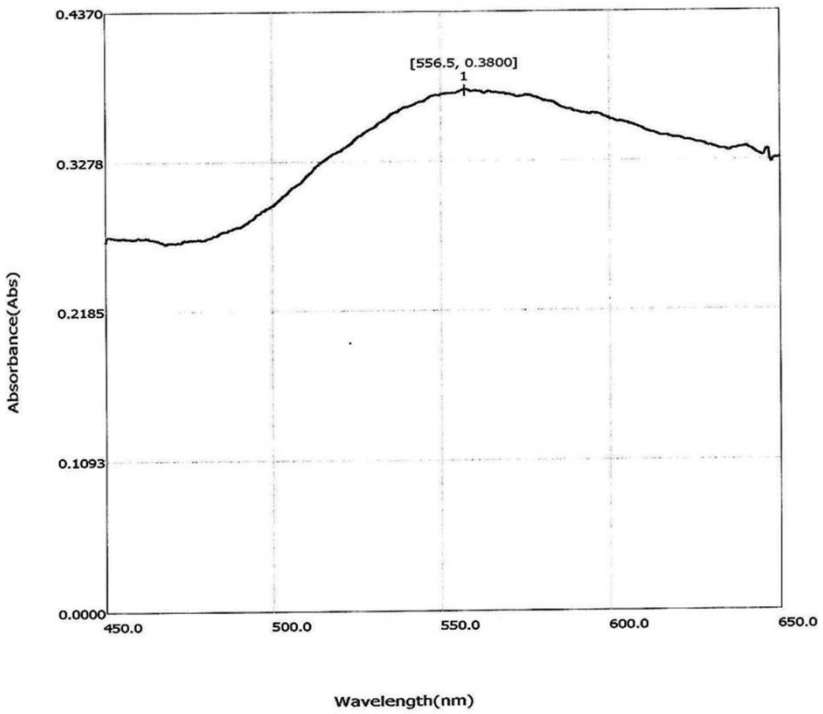

Fig. 1b: UV-Vis spectrophotometer spectra of *G. foliifera* showing peak for AuNPs

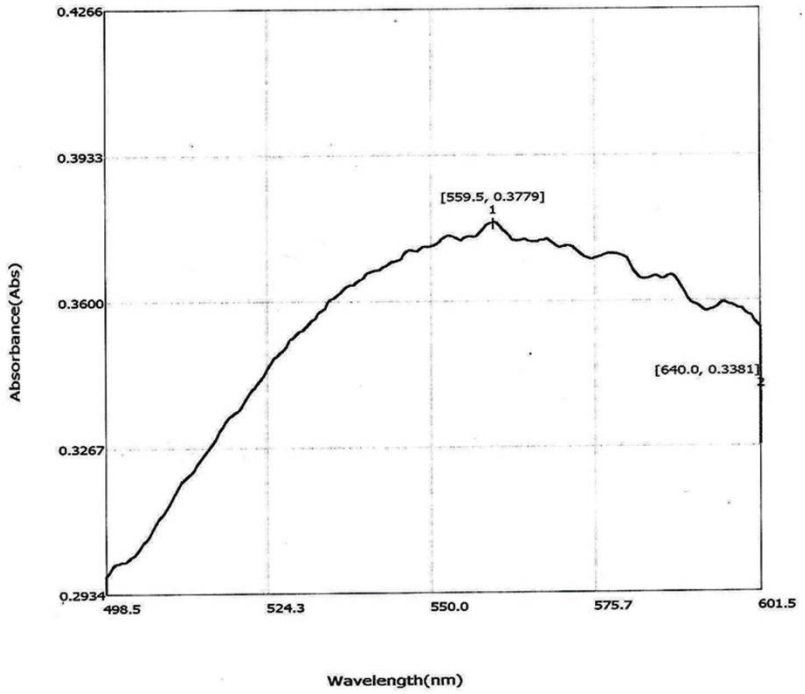

Fig. 1c: UV-Vis spectrophotometer spectra of *G. kilakkaraiensis* showing peak for AuNPs

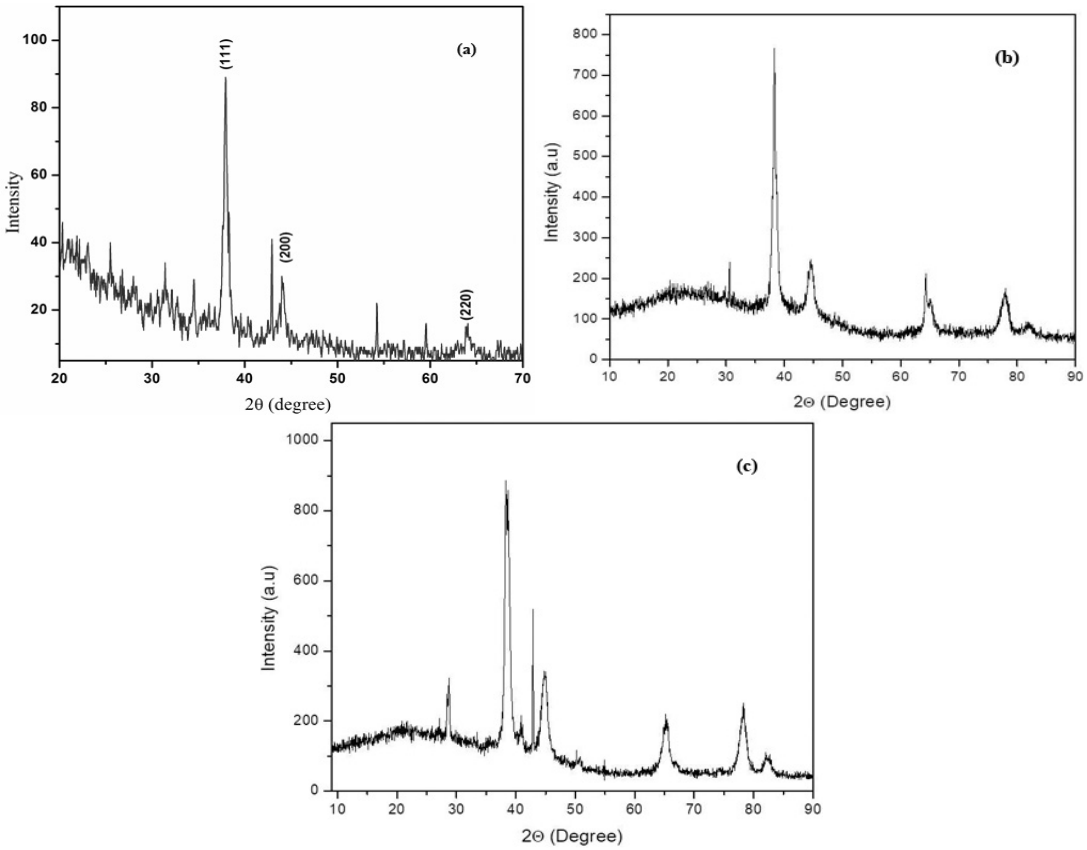

Fig. 2: XRD patterns of AuNPs from (a) *G. corticata* (b) *G. foliifera* (c) *G. kilakkaraiensis*

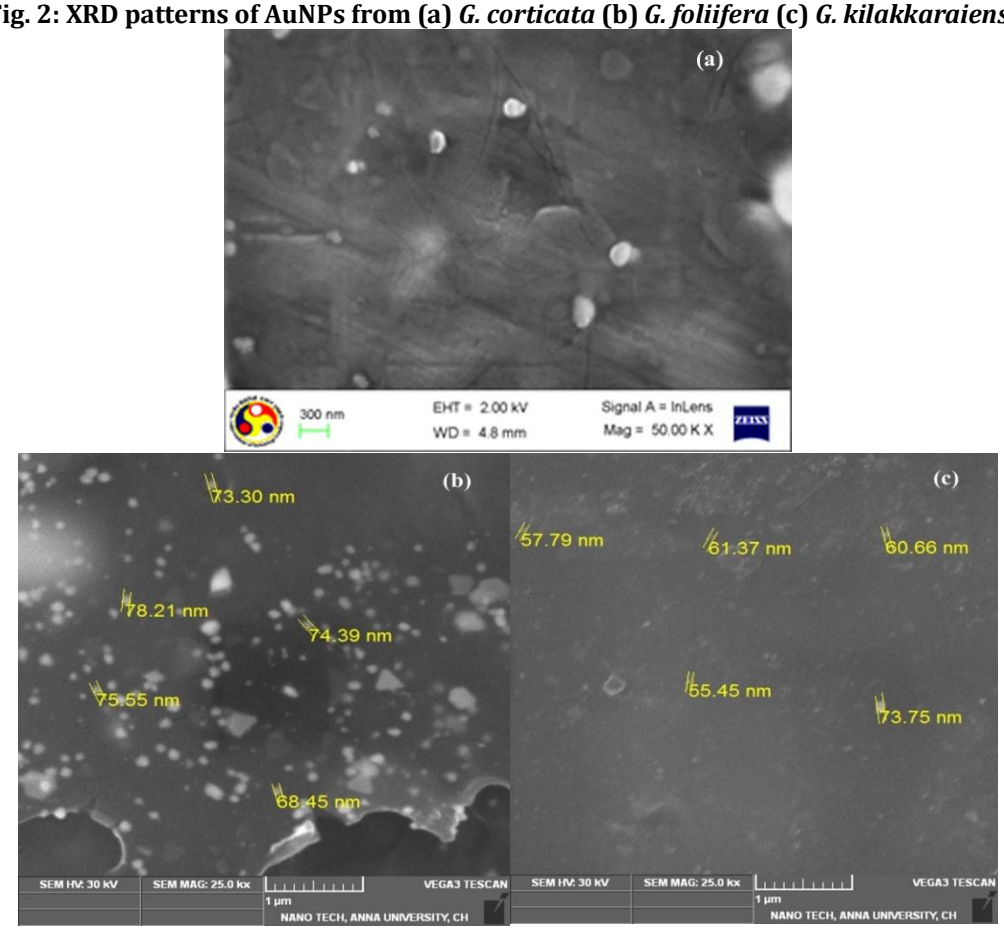

Fig. 3: SEM image showing AuNPs of (a) *G. corticata* (b) *G. foliifera* (c) *G. kilakkaraiensis*

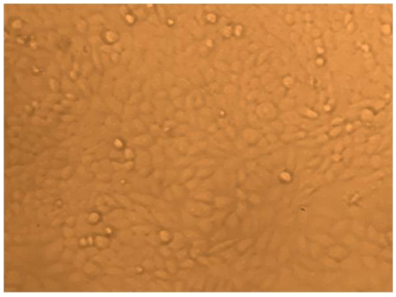

Fig. 4: Untreated normal HeLa cells

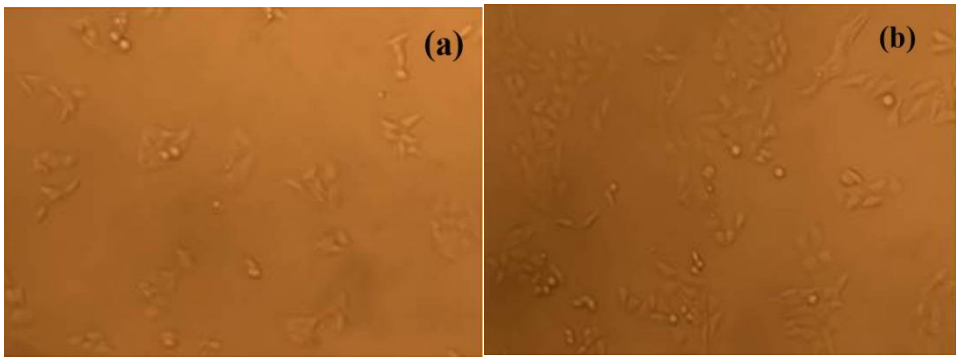

Fig. 5: HeLa cell growth inhibition by AuNPs from *G. corticata* at (a) 1000µg/ml conc. (b) 125µg/ml conc. at IC_{50}

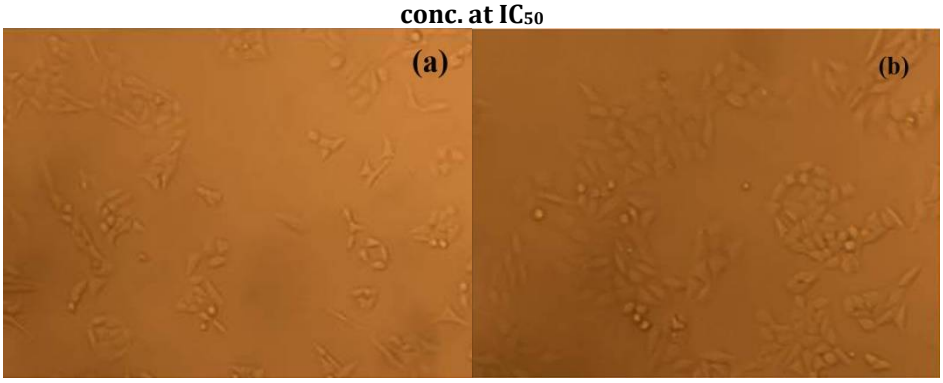

Fig. 6: HeLa cell growth inhibition by AuNPs from *G. foliifera* at (a) 1000µg/ml conc. (b) 62.5µg/ml conc. at IC_{50}

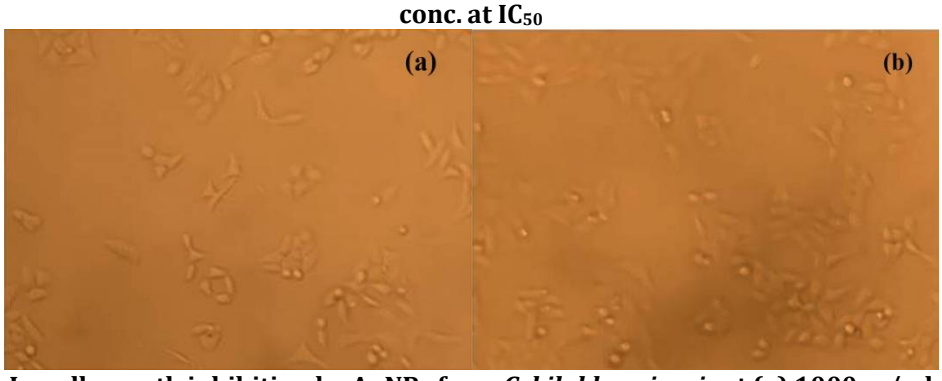

Fig. 7: HeLa cell growth inhibition by AuNPs from *G. kilakkaraiensis* at (a) 1000µg/ml conc. (b) 7.8µg/ml conc. at IC_{50}

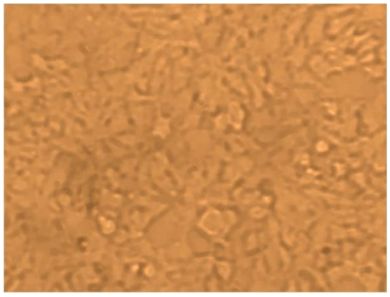

Fig. 8: Untreated normal MCF-7 cells

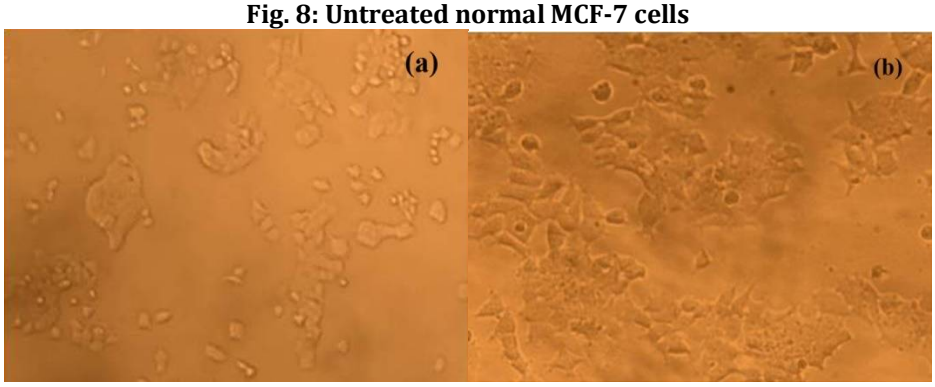

Fig. 9: MCF-7 cell growth inhibition by AuNPs from *G. corticata* at (a) 1000µg/ml conc. (b) 500µg/ml conc. at IC_{50}

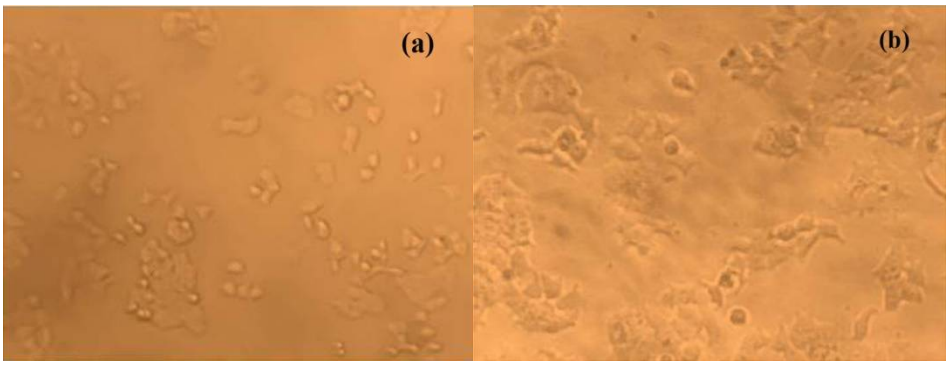

Fig. 10: MCF-7 cell growth inhibition by AuNPs from *G. foliifera* at (a) 1000µg/ml conc. (b) 500µg/ml conc. at IC_{50}

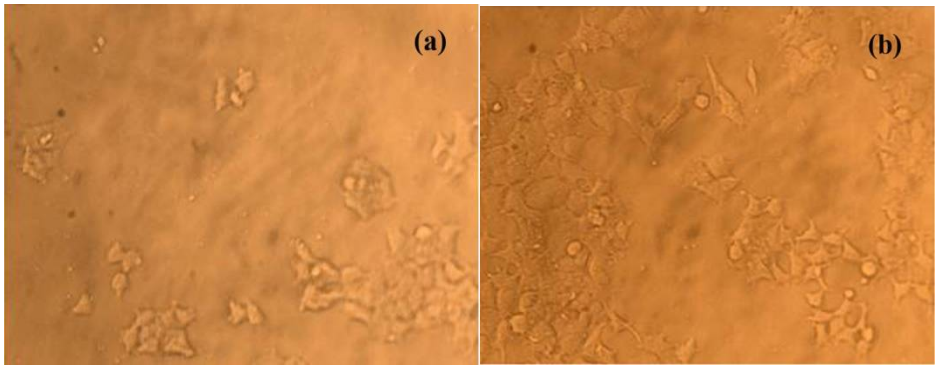

Fig. 11: MCF-7 cell growth inhibition by AuNPs from *G. kilakkaraiensis* at (a) 1000µg/ml conc. (b) 7.8µg/ml conc. at IC_{50}

CONCLUSION

The bioactive compounds from marine algae are responsible for various biological processes and play an important role in pharmaceutical, food and other related fields. It also serves as a good biological resource in inter-disciplinary fields that aim to conduct eco-friendly and non-toxic experiments. The current findings

could help the pharmaceutical field in finding a renewable bioresource for cancer therapies and other related industries to acquire knowledge about these natural sources for their upgradation to develop eco-friendly and non-toxic products.

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