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ANTIPROLIFERATIVE POTENTIAL OF EXTRACT FROM *HYPNEA FLAGELLIFORMIS* ON HeLa CANCER CELL LINES

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ABSTRACT

Marine algae are having a potential source of phytochemicals which would produce a wide variety of biologically active compounds for pharmaceutically important. Seaweeds are used as a raw material for many biotechnological industries because of the large number of bioactive compounds recently discovered from them. The present study was focused on to evaluate the anticancer activity of ethyl acetate extract from the red seaweed Hypnea flagelliformis collected from Tuticorin coastal waters at a depth of 7 to 10 meters by Scuba diving. Ethyl acetate extract showed anticancer activity with IC_{50} value of about 138.321µg/ml. The biological activity observed in this study creates a path for further chemical analyses such as the isolation of the active compounds responsible for this activity, in order to contribute to the discovery of new drugs.

KEY WORDS

Antiproliferation, MTT, Hypnea flagelliformis, HeLa cell lines, Gulf of Mannar.

INTRODUCTION

Now-a-days peoples are suffering from lot of physical and mental illnesses. So, the biotechnological industries has the responsibility to overcome the problems in the form of drug discovery. Natural products play an important role in drug development. The analysis has been made for bioactive secondary metabolites from plants, microbes and marine animals [1]. Marine organism plays an important role for the production of pharmacologically important diverse group [2]. Marine algae such as harbor endophytes like their terrestrial counterparts are a potential source of new secondary metabolites [3]. This offers an abundance of highly potent secondary metabolites for exploitation as bioactive compounds. Moreover, it is widely accepted that marine natural products provide unusual and unique chemical structures upon which molecular modeling and chemical synthesis of new drugs can be based with greater efficacy and specificity for the treatment of many human diseases [4,5]. Despite this, the number of seaweed species studied and identified corresponds to only 2% of the 150,000-known species worldwide [6]. From the species identified, approximately two thousand chemical compounds have been characterized [7].

More recently, seaweeds are reported to be a rich source of antioxidant compounds [8-10]. For example, chlorophylls, carotenoids, tocopherol derivatives such as vitamin E and related isoprenoids, which are structurally related to plant-derived antioxidants, were

found in some marine organisms including seaweeds [11]. Antioxidants in biological systems have multiple functions, including defense against oxidative damage and participating in the major signaling pathways of cells. Besides, some compounds from the seaweeds have antibacterial activities with potential use as mosquito control agents. Isolation of cytotoxic anti-tumor substances from marine organisms has been reported in several references for the last four decades [12]. Several species of seaweeds are rich sources of polysaccharides and glycoproteins with immune-stimulant, anticancer or antiviral activity [13-16]. Certain algae have long been used in traditional Chinese herbal medicine in cancer treatment [17]. Red and green algae have been shown to demonstrate protective effects against mammary, intestinal and skin carcinogenesis [18]. Zandi *et al*., [19] reported that cold water extract of red alga, *Gracilaria corticata*, possessed biological activity against tumor cells replication. In recent years, much attention has been focused on fucoidan, a sulphated polysaccharide derived from brown seaweeds. Recent studies evidenced that fucoidan has strong antitumor activity and exhibited important roles against human cancer cell lines [20,21]. The red seaweed species, *Hypnea flagelliformis* which is extensively distributed in Tuticorin south east coast of India, have been uncovered as a novel source for a variety of compounds. However, there is limited information about their biological activity on cancer cell growth inhibition. The objectives of this study were to screen and evaluate the anti-proliferative activities of crude ethyl acetate extract of *Hypnea flagelliformis.* The information compiled during the course of this study would pave the way for further development of cancer therapy.

MATERIALS AND METHODS

Collection of Seaweeds

Seaweed (*Hypnea flagelliformis*) was collected by Scuba diving at a depth of 7-10 meter, at Tuticorin coast of Gulf of Mannar during September 2014 to March 2015 (Lat 8°50'19.60835"N Long 78°15'22.19003"E). The taxonomic identification of species was done by Dr. Barrett Brooks, National Museum of Natural History, Washington expert in Algae identification.

Preparation of the extracts

Collected seaweeds were transported to the laboratory in polyethylene bags filled with sea water. Seaweed sample was washed thoroughly with running water to remove epiphytes, animal castings, attached debris and sand particles and the final washings were done using distilled water and dried under shade. After that the samples were cut into small pieces with the help of chopper and powdered in an electric blender. The seaweed powder was successively extracted using solvents of increasing polarity according to Arokiyaraj et al., [22] with slight modifications. Approximately 100 g of the powdered materials were extracted with 500 ml of ethyl acetate in a Soxhlet apparatus for 8 h at room temperature not exceeding the boiling point of the solvents. The extracts were filtered through Whatman no 1 filter paper and then concentrated in vacuum at 40 °C by using hot air oven. The residues obtained were stored in a freezer at -20 °C until further tests. The seaweed extracts were further subjected for Cytotoxicity assay.

Preparation of cell suspension

A subculture of HeLa cells in Dulbecco's Modified Eagle's Medium (DMEM) was trypsinized separately, after discarding the culture medium. To the disaggregated cells in the flask 25 mL of DMEM with 10% FCS was added. The cells suspended in the medium by gentle passage with the pipette and the cells homogenized.

Seeding of cells

One mL of the homogenized cell suspension was added to each well of a 24 well culture plate along with different concentration of ethyl acetate extract (0 to 300 g/mL) and incubated at 37°C in a humidified CO_2 incubator with 5% CO_2. After 48 hrs incubation, the cells were observed under an inverted tissue culture microscope. With 80% confluence of cells proliferative assay was carried out.

Cytotoxicity assay

The assay was carried out using (3-(4, 5-dimethyl thiazol-2yl)-2, 5- diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial succinate dehydrogenase and reductase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. After 48 h incubation the wells were added with MTT and the same was left for 3 h in room temperature. All wells were removed the content using pipette and 100µl SDS in DMSO were added to dissolve the formazan crystals, absorbance's were read in Lark LIPR-9608 micro plate reader at 540 nm [23].

RESULT AND DISCUSSION

In vitro cytotoxic effect of ethyl acetate extract on HeLa cell line

To examine potential cytotoxic effects of *Hypnea flagelliformis* extract on human cervical cancer cell lines (HeLa cell line), they were cultured for 48 hours and various concentrations of ethyl acetate extract was analysed by MTT assay. The activity against cancer cell lines is one of the most important specificities of marine algae and many algae have shown cytotoxic and antitumor activities [24]. In this study, the extracts of *Hypnea flagelliformis* showed a significant number of cell death of HeLa cells. The percentage of viable cells were calculated using the formula based on which the IC_{50} value of ethyl acetate extract was found to be 138.321 µg/ml (Table: 1 and Figure: 2). Morphological changes were determined by inverted tissue culture microscope (Figure: 1). It has previously been reported that *Sargassum wightii, Ulva fasciata* and *Gracillaria corticata* has shown an inhibitory effect against on HeLa, K-562 and MDA-MB cell lines [25]). As per the results red seaweed *Gracillaria corticata* extract (200 µg/ml) showed greater activity than Sargassum *wightii* and *Ulva fasciata*. Similarly, Lau *et al*., [26] studied the antiproliferative potential of extracts from red seaweed *Kappaphycus alvarezii* and *Kappaphycus striatum* against HeLa cell lines. The report showed that both 500 µg/mL of aqueous and methanolic extracts from *Kappaphycus striatum* showed highest anti-proliferative activity against HeLa cells with cell growth inhibition of 53.5 and 43.7%, respectively. According to previous studies fucoidans, laminarians, Dactylone, Gliotoxin and terpenoids stated to possess anticancer, antitumor, and antibacterial and antiproliferative properties are reported to be abundant in seaweeds [27,28].

Morphology Study

The treatment with seaweed extracts, a morphological observation of the HeLa cell lines shows that the onset of shrinkage of cells. It is increased progressively with dosage and time, and this cell shrinkage may be due to the growth inhibitory effect of seaweed extract.

CONCLUSION

Cancer is a disease which is characterized by uncontrolled growth with the potential to spread to other parts of the body. The spread of these abnormal cells must be controlled, failure may lead to death. According to the World Health Organization report, Cancer is the second leading cause of death globally and was responsible for 8.8 million deaths in 2015, nearly 1 in 6 death reports due to cancer [29]. This study revealed that the effect of ethyl acetate extract from red seaweed *Hypnea flagelliformis* as a novel therapeutic agent, which was characterized for their cytotoxic effects against HeLa (human cervical cancer) cell lines. This study also concluded that the seaweed *Hypnea Flagelliformis* has potential compounds for cancer therapy. So further studies on purification of targeted compound is required.

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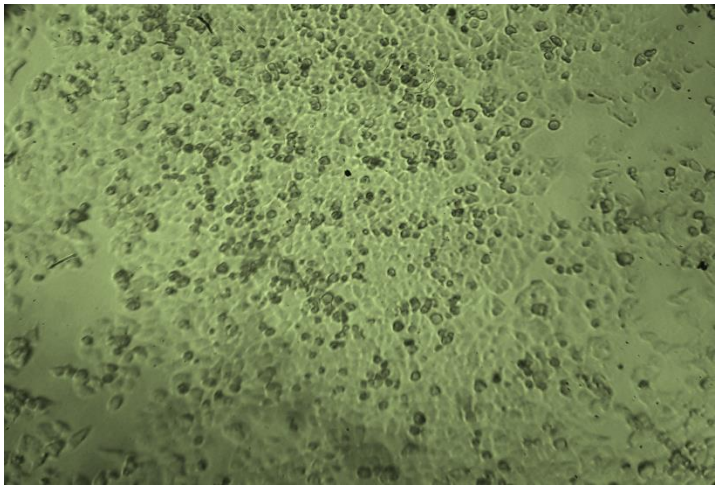
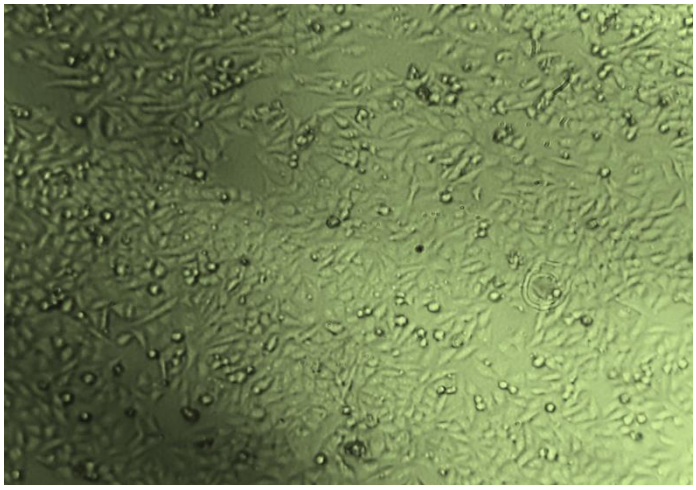
Table: 1 *In vitro* cytotoxicity effect of test drug against Hela Cell lines

Sample Concentration (µg/ml)	% Cell viability of Hela cells on MTT Assay
0	100
12.5	84.18
25	71.47
50	63.23
75	53.44
100	49.22
150	40.27
200	34.63
250	27.63
300	24.32

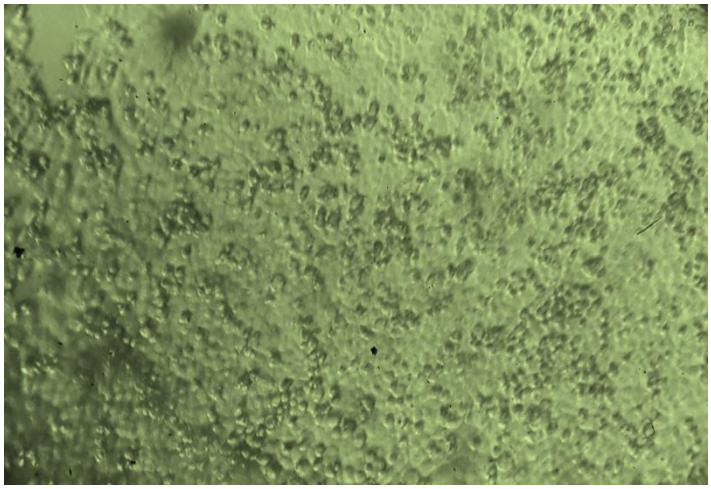
Figure 1: Anticancer activity of ethyl acetate extract against the HeLa cell lines



IC_{50} 100 µg/mL

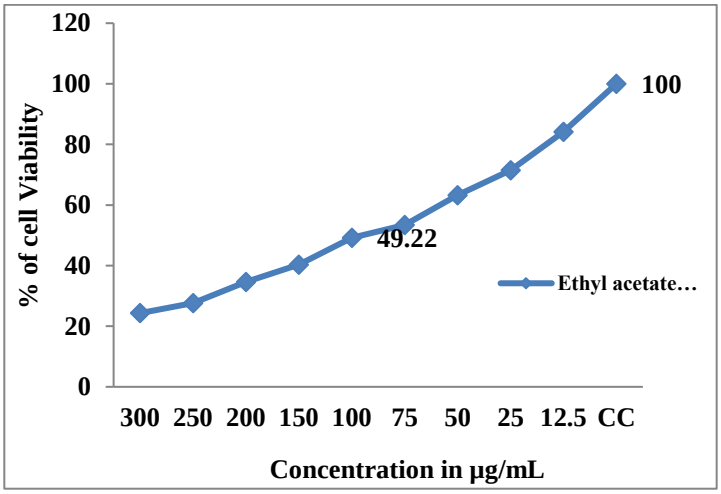


Control



Conc. 300 µg/mL

Figure 2: Anticancer activity of Ethyl acetate extract against the HeLa cell lines (IC_{50} value 138.321µg/ml)



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