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Evaluation of Cytotoxic Activity of *Sargassum vulgare* From the Lebanese Coast Against Jurkat Cancer Cell Line

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ABSTRACT

The discovery of cancer drugs that effectively destroy cancer cells or stop their growth without toxicity to normal cells is a challenge to enhance the therapeutic effects and reduce side effects. Many papers have highlighted the implication of marine algae that show anticancer activity. In this report, we assessed the cytotoxic activity of two different crude extracts from *Sargassum vulgare* (Sargassaceae), a marine brown algae collected from the Lebanese coast, against Jurkat human cancer cell line using trypan blue exclusion test. Both extracts, water: ethanol extract and chloroform: ethanol extract, showed cytotoxic activity against Jurkat cancer cell line with IC50 values of 136.907 µg/ mL and 49.056 µg/ mL, respectively after 72 hours of treatment. Further research designed to isolate and identify novel and efficient anticancer drug candidates from these seaweed extracts need to be explored.

INTRODUCTION

Cancer is a leading cause of death worldwide. Leukemia, also called blood cancer, is a part of malignancies usually characterized by abnormal and excessive proliferation of precursors of the white blood cells, blocked at a stage of differentiation (Pui *et al.*, 2008). In this case, the bone marrow produces white blood cells more than the body needs. These cells, in turn, begin to invade the healthy blood cells, resulting thereafter anemia, bleeding, and infections. Leukemia remains a serious public health problem with an estimated mortality rate of death 24,450 and an estimated incidence rate of 54,270 new cases of leukemia in 2015 (Siegel *et al.*, 2015). Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and which might cause several undesirable side effects (Prucker *et al.*, 2009). The discovery of new anticancer drug with less side effects and toxicity has become a need for many researchers. In this context, natural products derived from plants, marine organism and micro-organism have interested many scientists (Cragg and Newman, 2005). Over the past years, nature has played an important role as a source of new drugs. The marine ecosystem appears today as a substantial source of new natural molecules with unique biological activity and original chemical structure, which can be useful while looking for effective drugs for the treatment of human diseases (Haefner, 2003). Among these marine organisms, marine algae (also called seaweed) have been one of the natural sources of bioactive compounds (Smit, 2004). Seaweed is classified into three broad groups based on pigmentation- brown, red and green seaweed.

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They are ubiquitous in our daily lives, and their uses are many and varied. Besides food uses, they take an important place in agriculture (fertilizers), in the pharmaceutical industry (coating of medications) and in the field of cosmetics (soap, cream, toothpaste), and products cleaning, etc. (Person, 2011). The algae consist of proteins, a small amount of lipids compared to the high percentage of carbohydrates that are in the form of polysaccharides such as alginates, carrageenan and ulvans. They also contain high contents of minerals and vitamins. In addition, they synthesize and accumulate small amounts of secondary metabolites which have promising pharmacological and biological activities. Recent findings showed that seaweeds had anticancer, antibacterial, antioxidant, and many other effects (El Gamal, 2010; Smit, 2004). *Sargassum* is a genus of brown algae of *Phaeophyceae* class, *Fucales* order, and *Sargassaceae* family, and which contains about 400 different species identified so far (Mattio and Payri, 2011). This type of algae is consumed as food and medicine in many cultures. The treatment of thyroid diseases such as goiter is one of the important traditional uses to *Sargassum* (Liu *et al.*, 2012). The iodine presence in such algae appears to play an important role in the therapy. A wide range of pharmacological properties of crude extracts or isolated components of *Sargassum* such as phlorotannin, fucoidan, sterol and glycolipid, was discovered. It's about anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, hypoglycemic, and hypolipidemic activities, etc. (Mattio and Payri, 2011). Much work has been devoted to assessing the anticancer activity of the crude extracts or isolated compounds of the brown algae of the genus *Sargassum*. De Sousa *et al.* (2007) provide that alginate-type polysaccharide extracts of *Sargassum vulgare* inhibit *in vivo* the rate of proliferation of sarcoma 180 cells transplanted in mice, as observed by the reduction of the expression of Ki67 (De Sousa *et al.*, 2007). Also, another type of polysaccharide heterofucan (1.5 mg/ mL) isolated from *S. filipendula* shows a decrease in cell proliferation of HeLa (cervical cancer) (Silva Costa *et al.*, 2011). Similarly, De la Mare *et al.* (2012) isolate a plastoquinone from *S. heterophyllum* with anti-proliferative action against MDA-MB-231 (human breast carcinoma) after 96 hours, at an inhibitory concentration IC50 of 67.4±5.9 µM (De la Mare *et al.*, 2012). However, Khanavi *et al.* (2010) show the cytotoxicity of the hexane fraction and of the methanolic extract total *S. swartzii* against the proliferation of cancer cells of Caco-2 (colon adenocarcinoma) at an IC 50 of 99.9 ± 19.38 µg/mL, and T47D (breast carcinoma) at an IC 50 of 205.21 ± 84.1 µg/mL, respectively (Khanavi *et al.*, 2010). On the other hand, Namvar *et al.* (2013) exhibit the inhibition of the growth of both tumor cells, MCF-7 and MDA-MB-231, in a dose and time-dependent manner, after being treated for 24 h with the methanol extract of *S. muticum* at an IC 50 of 22 µg/mL and 55 µg/mL, respectively (Namvar *et al.*, 2013). Crude extracts and various compounds such as sulfated polysaccharide of *S. filipendula* (Silva Costa *et al.*, 2011), the sargaquinoic acid of *S. heterophyllum* (De la Mare *et al.*, 2012), a kind of chromene derived from *S. siliquastrum* (Heo *et al.*, 2011), the hexane fraction of *S. swartzii* (Khanavi *et al.*, 2010), and the methanol extract of *S. muticum* (Namvar *et al.*, 2013) showed a pro-apoptotic action of the HeLa, MDA-MB-231, HL-60, Caco-2 and MCF-7 cells respectively. By decreasing the size and number of vessels, the methanolic extract of *Sargassum muticum* (50 and 100 µg/mL) implanted *in vivo* into fertilized eggs, was able to induce an anti-angiogenic activity in the chorioallantoic membrane (Namvar *et al.*, 2013). The work presented above is not an exhaustive list. *Sargassum vulgare*, a marine brown algae, was found abundantly at the Lebanese coast. This study aims at assessing the cytotoxic activity of ethanol: water extracts (50: 50, V/V) and ethanol: chloroform extracts (50: 50, V/V) prepared from *Sargassum vulgare* against the human cancer cell line Jurkat (acute lymphoblastic leukemia) using trypan blue exclusion method. Since no researcher in Lebanon and abroad examined the cytotoxic activity of the crude extracts or the bioactive compounds derived from *Sargassum vulgare*, a brown seaweed species collected on the Lebanese coast against human leukemia cell lines, we decided to select the Jurkat cell line for this study.

MATERIAL AND METHODS

Chemicals

Chemicals and reagents used to study anti-proliferative activities were purchased from Sigma-Aldrich Co. (Beirut, Lebanon) while the other chemicals, solvents, and reagents were purchased from Alpha Co. (Beirut, Lebanon). The fetal bovine serum was the only chemical obtained from CELBIO (Milano, Italy).

Plant materials

Algae were manually collected from El Barbara beach in Lebanon at the end of May 2015. The collected samples were transported to the laboratory where they were first washed thoroughly with fresh water to remove slats, sand, and epiphytes, then with hydrochloric acid 10% for a few seconds to remove the organic matter, and finally with distilled water. Then, the algae were frozen at - 80ºC and lyophilized. After drying, the algae were ground to powder in a blender. Voucher specimen No 700 was botanically authenticated by Dr Mona Tannoury, Biology Department, Faculty of Sciences II, Lebanese University and deposited in the Biology Department Herbarium, Faculty of Sciences II, Lebanese University.

Sample extraction

The dried and ground sample (2 gms) of *Sargassum vulgare* was dissolved in 40 mL of ethanol: water (v: v) by maceration during 24 hrs. The material was filtered by using Whatman paper No 1, whereas the extract plant was transferred to 50 ml -round-bottom flask, and then taken with rotary vacuum evaporator. Moreover, these crude extracts were freeze-dried by lyophilization. While another dried and ground sample (2 gms) of *S. vulgare* was extracted with chloroform: ethanol by maceration similar to the extraction of ethanol: water protocol (Choi *et al.*, 2004).

Cell lines and culture condition

Jurkat cells (T lymphocyte cells): The cells are suspended lymphoblasts. The growth medium was Advanced RPMI 1640 (Gibco/Invitrogen), 10% fetal bovine serum (Hyclone), 10 mM Hepes 100 U/mL penicillin, and 100 µg/mL streptomycin, 5 % CO_2 (37˚C). Liquid Nitrogen Storage: Complete growth medium supplemented with 5% (v/v) DMSO in 1 mL aliquots of approximately 5 x 10^6 cells. RPMI 1640 medium for suspension cells with fetal bovine serum (FBS) (Saab *et al.*, 2011; Lampronti *et al.*, 2006).

Cell viability assay

Jurkat cells (20,000 cells/ dish) were seeded in 24-well plates. Two wells were prepared for each concentration. The cells treated with *S. vulgare* at different concentrations were compared with untreated controls. Besides, *Jurkat* cells were treated with ethanol: water *S. vulgare* extract at different concentrations as well as chloroform: ethanol *S. vulgare* extract. The plates were incubated for 3 days. The cytotoxicity was determined by trypan blue staining. The cell suspension (20 µL) was taken from the well and mixed with 20 µL trypan blue. The living cells weren't stained and can be easily distinguished from the blue dead cells which absorbed the dye because of their damaged membrane. After 24, 48 and 72 h of incubation, 10 µL of cell suspension and an equal volume of trypan blue were mixed. Then, the mixture was loaded on a Neubauer hemocytometer and examined under a microscope to count the viable cells (clear, not blue) (Zandi *et al.*, 2010).

Statistical analysis

All experiments were carried out in triplicate. The data were expressed as means ± SD and the differences were evaluated by one-way analysis of variance (ANOVA) test completed by Dunnett's test. The differences were considered significant at $^{**}p < 0.01$. The 50% inhibitory concentration (IC_{50}) was calculated by nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows [GraphPad Software, San Diego, CA, USA].

RESULTS AND DISCUSSION

Different solvents were chosen to prepare extracts of algae and used as a mixture - water which is the most common polar solvent, ethanol which is known as an extraction solvent for polar compounds, and chloroform which is a non-polar solvent for the extraction of non-polar compounds. The crude extraction of dried brown seaweed *Sargassum vulgare* prepared by two different solvent mixtures was used to calculate the yield of each extract expressed as a percentage for 15 g of dry and ground plant material. The highest yield is marked for the water: ethanol extract with a value of 20.039 % w/w while the low yield of 3.533 % w/w is recorded for the ethanol: chloroform extract. In order to evaluate the Lebanese ethanol: chloroform *S. vulgare* extract and ethanol: water *S. vulgare* extract, Jurkat human cancer cells were seeded at initial cell concentration 20,000 cells/mL and then cultured for 3 days either in the presence or absence of *S. vulgare* extracts at concentrations ranging from 25-1000 µg/mL. The trypan blue exclusion assay showed that with an increase in concentrations of algal extract and in the duration of treatment (24, 48 and 72 hours), Jurkat cell viability was significantly decreased according to the negative control free extract. This infers the existence of time and dose dependent properties of *Sargassum vulgare* extracts against Jurkat tumor cell lines. Consequently, the ethanol: chloroform extract and ethanol: water extract prepared from *S. vulgare* were able to exert anti-proliferative activities against Jurkat human cancer cells with IC50 values 49.056±3.2 µg/mL and 136.907±5.2 µg/mL, respectively after 72 hours of treatment (Figs.1 & 2). The result shows that the chloroform: ethanol extracts have better IC 50 values compared to the ethanol: water extracts.

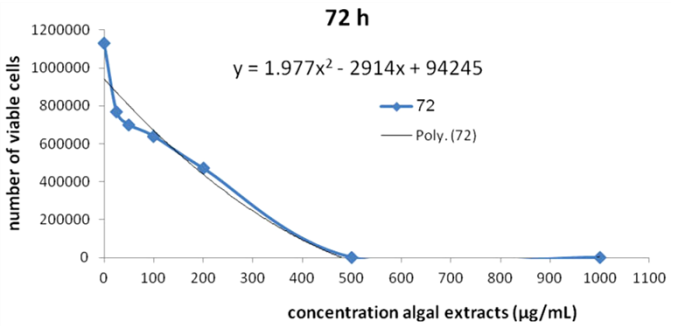

Fig. 1: treatment with ethanol: water extract (V:V)after 72 hours of treatment.

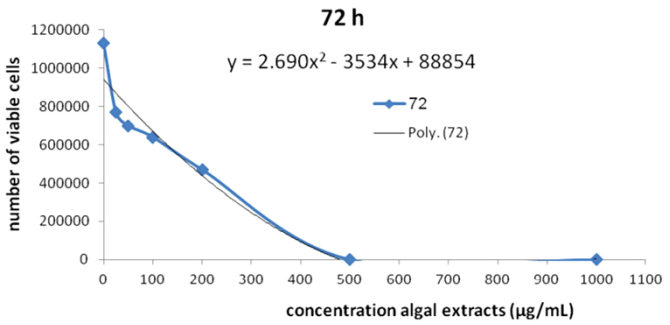

Fig. 2: treatment with ethanol: chloroform extract (V:V)after 72 hours of treatment.

Therefore, the ethanol: chloroform extracts of *S. vulgare* are more cytotoxic than the ethanol: water extracts. Hence, this result emphasizes the influence of the choice of solvent extraction on the cytotoxic activity of seaweed extracts against cancer cell lines.Some literature highlight the potential implications of marine algae which exhibit antitumor activity (Guedes *et al.*, 2013). Our preliminary results show the cytotoxic activity of the crude extracts of the marine brown algae *Sargassum vulgare* collected from the Lebanese coast against Jurkat cancer cell line using trypan blue exclusion assay. The cell viability was decreased in a time and dose dependent manner. Similar results were also observed by Zubia *et al.* (2009) who emphasize a strong cytotoxicity of the crude extracts on Jurkat cancer cell line (Zubia *et al.*, 2009). Jurkat cell viability decreased significantly after a treatment of 24 h with extracts prepared from marine red algae

Asparagopsis armata, Brongniartella byssoides, Heterosiphonia plumose, and *Calliblepharis jubata.* Similarly, the results obtained by Guedes *et al.* (2013) show that the aqueous extract of *Sargassum vulgare* was significantly cytotoxic against Hep-2 with IC50 of 18.7 ± 3.8 µg/mL (Guedes *et al.*, 2013). Many other studies have evaluated the cytotoxic effect of various algae species of the genus *Sargassum* as *Sargassum swartzii* and *Sargassum sp.* which showed similar results in terms of their cytotoxic effects. The fraction of hexane and the total methanol extract of *S. swartzii* exhibit cytotoxic activity against Caco-2 with IC50 of 99.9 ± 19.38 µg/mL, and T47D with IC50 of 205.21 ± 84.1 µg/mL, respectively (Khanavi *et al.*, 2010). Moreover, Mary *et al.* (2012) reported that the treatment with a concentration range of 100 to 300 µg/mL of ethanol extract of *Sargassum sp.* reduces the cell viability of both Hep-2 and MCF-7 cell lines in a dose dependent manner with an IC 50 value of 200 µg/mL and 250 µg/mL, respectively (Mary *et al.*, 2012). In addition, various types of lipids, steroids and sterols isolated from *S. carpophyllum* also have cytotoxic activity against various cancer cell lines, including HL-60 (human promyelocytic leukemia), P388 (murine leukemia), MCF-7 (breast cancer), HCT-8 (colon cancer), 1A9 (carcinoma human ovary), HOS (human osteosarcoma), and PC-3 (prostate adenocarcinoma) (Tang *et al.*, 2002). Likewise, some plastoquinones isolated from the methanol extract of *S. micracanthum* exhibit cytotoxic activity against cancer cell line of colon adenocarcinoma 26-L5 (high cytotoxicity with IC50 of 1.51 and 1.69 µg/mL, and moderate activity with IC50 of 17.5 µg/mL) (Mori *et al.*, 2005). An MTT test shows that the polysaccharide fractions from *S. pallidum* (1 mg/ mL) have a high antitumor activity against cancer cells HepG2 (hepatocellular human liver carcinoma), A549 (human lung carcinoma), and GC-803 (stomach cancer) (Ye *et al.*, 2008). On the other hand, the MTT test and the trypan blue exclusion assay show a decrease in viable cells following exposure lines K562 and Daudi to the extract by cold distilled water of *S. oligocystum* at a concentration of 400 and 500 µg/mL (Zandi *et al.*, 2010). The methanolic extract prepared from *Sargassum muticum* inhibits the growth of both cell lines MCF-7 and MDA-MB-231 in a dose and time-dependent manner, with IC50 values of 22 µg/mL and 55 µg/mL, respectively, after a 24 hour treatment (Namvar *et al.*, 2013). While the methanolic extract of *Sargassum ilicifolium* inhibits the proliferation of five lines of human cancer cells- MCF-7, HeLa, MDA-MB-231, HepG2, and HT-29- in a time and dose dependent manner after 24 h treatment, for IC50 values 37, 60, 62, 120, 300 µg/mL, respectively (Namvar *et al.*, 2014).

CONCLUSION

Our study indicates that the brown marine algae *Sargassum vulgare* collected from the Lebanese coast have promising anticancer activity. Depending on the extraction solvent used, the ethanol: chloroform extract gave the best result, i.e. high cytotoxic activity against Jurkat human leukemic cell line. This result highlights how the marine algae collected from the Lebanese coast can be an important source to discover new effective antitumor compounds. Further research is needed to isolate and identify the bioactive compound of *Sargassum vulgare* collected on the Lebanese coast, and also to elucidate the mechanism procedure by which the compound produces the cytotoxic effect - the compound's effect on the cell cycle and their ability to induce apoptosis.

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