

In Vitro Cytotoxic Activity of *Laurencia papillosa*, Marine Red Algae from the Lebanese Coast

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ABSTRACT

Marine algae are an excellent source of bioactive compounds with a variety of biological activities. This paper presents a study on the cytotoxic activity of *Laurencia papillosa* (*Rhodomelaceae*), marine red algae collected at the Lebanese coast against the human cancer cell line Jurkat (acute lymphoblastic leukemia) through the trypan blue exclusion assay. The results show that the percentage of cell viability was reduced by increasing the concentration of both algal extracts: ethanol/ water extract, and ethanol/ chloroform extract. The ethanol/ chloroform extract exhibits the highest levels of cytotoxicity with IC50 value of 57.77 µg/ mL against Jurkat cancer cell line especially after 72 hours of treatment compared with the ethanol/water extract with IC50 value of 121.642 µg/ mL. Further research should be explored in order to study the bioactive compounds of *Laurencia papillosa* that could be potentially useful therapeutic agents for patients with acute lymphoblastic leukemia.

INTRODUCTION

Cancer is one of the most serious diseases that threaten the human health all over the world. According to the type of abnormal white blood cells involved, and depending on the rate of proliferation of leukemia cells, leukemia, also known as blood cancer, is divided into four main groups: acute lymphocytic, chronic lymphocytic, acute myeloid, and chronic myeloid (Pieters *et al.*, 2008). Acute lymphoblastic leukemia which is a malignant disorder of lymphoid progenitor cells, presents the most common type of childhood cancer. *Jurkat* cells may be used to study T-lymphocyte behavior, cell signaling, and expression of chemokine receptors. They are also important in producing interleukin 2. The *Jurkat* cell line was established in the late 1970s from the peripheral blood of a 14- year- old boy with T cell leukemia. Different derivatives of the *Jurkat* cell line can be

now obtained from cell culture banks that have been mutated to lack certain genes (Hu *et al.*, 2015). Regarding the resistance of cancer cells to currently used antitumor drugs, finding new, safe, and effective anticancer drugs with fewer side effects has become a need for many scientists (Ross, 2000). There has been a lot of attention to natural compounds obtained from marine algae to discover their medicinal and pharmaceutical properties such as anticancer, antibacterial, and antioxidant effects (Smit, 2004). Long time ago, and in many Asian countries especially China and Japan, the marine algae were used as functional foods and medicinal herbs because of their richness in lipids, minerals, vitamins, and bioactive substances like polysaccharide, protein, and polyphenol (Namvar *et al.*, 2013). Because some types of algae were used to treat cancer, many crude extract and compounds derived from various algae have been tested for their antitumor activities (Mohamed *et al.*, 2012). These studies have pointed out that marine algae form a promising source of bioactive compounds as human therapeutic agents. *Laurencia* is a genus of marine red algae, *Rhodophyta* division, *Ceramiales* order, and *Rhodomelaceae* family, that mostly lives in tropical oceans (Cabioc'h, 1992).

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The marine red algae *Laurencia* is a more diverse group with about 140 species of algae (Kim *et al.*, 2013). This genus is a prolific synthesizer of halogenated metabolites, capable of producing more than 250 different compounds (Kim *et al.*, 2013). They are among the most prolific producers of secondary metabolites in the marine environment (Vieira *et al.*, 2015), including sesquiterpenes, diterpenes, triterpenes and many other types. Those compounds have various biological activities such as antifungal, antibacterial, and anticancer activities (Kladi *et al.*, 2006). Several researchers have studied the antiproliferative as well as the cytotoxicity action of the crude extracts and compounds derived from *Laurencia* vis a vis different cancer cell lines. A sesquiterpene, the elatol (0.1-100 μM) isolated from *Laurencia microcladia* by Campos *et al.* (2012) shows an anticancer effect by reducing cell viability of B16F10 (melanoma murine), A549 (human lung carcinoma), DU145 (carcinoma of human prostate), L929 (mouse fibroblasts), and MCF-7 (human breast adenocarcinoma). In addition, treatment with elatol was able to reduce tumor growth *in vivo* in mice inoculated by B16F10 cells (Campos *et al.*, 2012). Another study indicates that the polyether of *Laurencia viridis* has cytotoxic activity against human cancer cell lines Jurkat (acute T cell leukemia), MM144 (myeloma), HeLa (cervix adenocarcinoma), and CADO-ES1 (Ewing sarcoma human). It is worth noting here that leukemic Jurkat cells were more sensitive to polyether compounds tested with an IC 50 values of 2.0-3.5 μM (Pacheco *et al.*, 2011). These two compounds, the sesquiterpene of *L. microcladia* (Campos *et al.*, 2012) and the polyether of *L. viridis* (Pacheco *et al.*, 2011), also show a pro-apoptotic effect to the cells B16F10 and Jurkat, respectively. This effect is triggered by various changes such as a decreased expression of anti-apoptotic proteins Bcl-xL, an increased expression of pro-apoptotic proteins Bak, and an activation of caspase 9 (Campos *et al.*, 2012). The increase in apoptotic population sub-G0 phase was another indication of the induction of apoptosis (Pacheco *et al.*, 2011; Campos *et al.*, 2012). In addition, the sesquiterpene isolated from *Laurencia microcladia* increases the expression of p53 tumor suppressor gene in the B16F10 cells (Campos *et al.*, 2012).

The brominated diterpenes isolated from the organic extract of *Laurencia obtusa* exhibited cytotoxicity against five human cell lines: MCF7 (breast), PC3 (prostate), HeLa (cervix), A431 (epidermis), and K562 (myelogenic) (Iliopoulou *et al.*, 2003). Moreover, the laurene-type sesquiterpenes isolated from the organic extract of *Laurencia obtusa* were evaluated for their antitumor activity in an *in vitro* model of Ehrlich ascites Carcinoma (Alarif *et al.*, 2012). The Ehrlich ascites *in vitro* assay shows that this laurene sesquiterpene had a cytotoxic activity.

Furthermore, Zaleta-Pinet *et al.* (2014) reported that some compounds isolated from ethanol extract of *Laurencia pacifica* (25 μM) were cytotoxic against a panel of cancer cell lines HT29 (colon), U87, SJ-G2 (glioblastoma), MCF-7 (breast), A2780 (ovary), H460 (lung), A431 (skin), DU145 (prostate), BE2-C (neuroblastoma), MIA (pancreas), ADM (murine glioblastoma) and MCF10A (normal breast cell line) (Zaleta-Pinet *et al.*, 2014).

In addition, Kladi *et al.* (2006) highlight the cytotoxicity of isolated metabolites from *Laurencia obtusa* and *Laurencia microcladia* against various human tumor cell lines: K562 (chronic myelogenous leukemia), MCF7 (breast adenocarcinoma), PC3 (prostate adenocarcinoma), HeLa (carcinoma of the cervix), A431 (carcinoma squamous), A549 NSCLC-N6 (lung cancer), and CHO cells which are a cell line derived from the ovaries of a Chinese hamster, by a MTT test (Kladi *et al.*, 2006).

In this study, we prepared ethanol: water (50: 50; V/V) and ethanol: chloroform (50: 50; V/V) crude extracts from the marine red algae *Laurencia papillosa* collected from the Lebanese coast. The main objective is to evaluate their cytotoxic activity on the human cancer cell line Jurkat through the trypan blue exclusion assay.

MATERIALS 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

The marine red algae *L. papillosa* was 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 - 80°C 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 (2g) of *L. papillosa* 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 *L. papillosa* 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 % CO₂ (37°C). Liquid Nitrogen Storage: Complete growth medium supplemented with 5% (v/v) DMSO in 1 mL aliquots of approximately 5 x 10⁶ cells. RPMI 1640 medium for suspension cells with fetal bovine serum (FBS) (Lampronti *et al.*, 2006; Saab *et al.*, 2011).

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 *L. papillosa* at different concentrations were compared with untreated controls. Besides, *Jurkat* cells were treated with ethanol: water *L. papillosa* extract at different concentrations as well as chloroform: ethanol: *L. papillosa* extract. The plates were incubated for 3 days. The cytotoxicity was determined by trypan blue staining. The cell suspension (20 mL) was taken from the well and mixed with 20 mL 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. Then, 10 mL of this mixture were used for cell counting (Esseily *et al.*, 2012).

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 (IC₅₀) was calculated by nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows [GraphPad Software, San Diego, CA, USA (Hwww.graphpad.com)H].

RESULTS AND DISCUSSION

The yields of ethanol: water and ethanol: chloroform extracts of *Laurencia papillosa* relative to the initial amount of dry seaweed were determined. The result shows that the best yield is for the water: ethanol extract with a value of 27.386 %, and the low yield of 3.433 % is for the ethanol: chloroform extract.

In order to evaluate the ethanol: chloroform extract and the ethanol: water extract prepared from the red algae *Laurencia papillosa* collected from the Lebanese coast, *Jurkat* 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 *L. papillosa* extracts at concentrations ranging from 25-1000µg/mL.

The trypan blue exclusion assay, intended to count the viable cells after exposure to different concentrations of extract, is summarized in (Fig. 1 and 2). Based on these data, it was shown that the number of *Jurkat* cells in the negative control wells (without extract) increased after 24, 48, and 72 hours of treatment. Besides, the number of the viable cells is decreased with the increase in concentration of both algal extracts, ethanol: water and ethanol: chloroform extracts, from 25 to 1000 µg/mL, after 24, 48 and 72 hours of treatment. Thus, the cytotoxicity of the two *L.*

papillosa crudes extracts against *Jurkat* cells depends on time and dose. The ethanol: chloroform extract with IC₅₀ value of 57.77 µg/ mL is more cytotoxic than the ethanol: water extract with IC₅₀ value of 121.642 µg/ mL, after 72 h of treatment. According to this result, we notice that the cytotoxicity of *Laurencia papillosa* crude extracts also depends on the choice of the extraction solvent against human leukemic cell lines *Jurkat*. Indeed, many studies have assessed the cytotoxic activity of other algae species of the genus *Laurencia*. A comparative study was carried out in order to examine the cytotoxic properties of extracts obtained from a group of *Laurencia* (*L. aldingensis*, *L. catarinensis*, *L. dendroidea*, *L. intricata*, *L. translucida*, *L. sp.*, and *Palisada flagellifera*) against MES-SA cells (human uterine sarcoma) (Stein *et al.*, 2011).

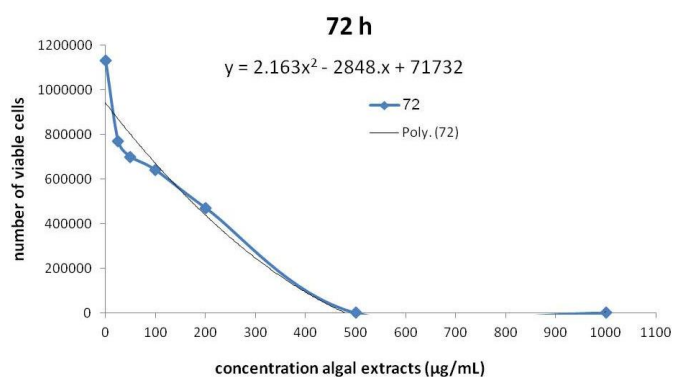


Fig. 1: Treatment with ethanol: chloroform extract (V: V) after 72 hours of treatment.

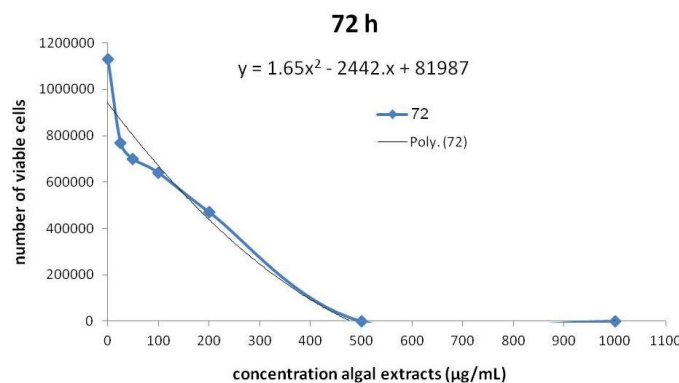


Fig. 2: Treatment with ethanol: water extract (V: V) after 72 hours of treatment.

The high level of cytotoxicity was marked for the hexane extract (hexane being a non polar solvent) with over 80% of cell mortality, while the low level of cytotoxicity was recorded for the methanol extract (methanol being a polar solvent) with 30% of cell death. Many halogenated metabolites isolated from the organic extract of *Laurencia catarinensis* were evaluated for their in vitro cytotoxic activity against HT29, MCF7, and A431 human tumor cell lines. Certain compounds were found to be much more active among all evaluated metabolites with IC₅₀ values lower than 20 µM (Lhullier *et al.*, 2010). Furthermore, an arabinopyranoside isolated from *Laurencia majuscula* had an antitumor activity in

LOVO (human colon adenocarcinoma) and Bel-7402 (hepatocellular carcinoma) cell lines (Du *et al.*, 2010). The IC₅₀ of arabinopyranoside treatment on LOVO and Bel-7402 cells was 15.82 µg/ mL and 11.5 µg/ mL, respectively. Western blotting and Flow cytometric analysis demonstrated that the arabinopyranoside was also able to arrest the cell cycle in the G2/M phase.

On the other hand, a diterpene and several sesquiterpenes were isolated from the organic extract of *Laurencia obtusa* and their cytotoxicity were evaluated against mammalian cell lines KB, HepG2, and MCF-7 (Angawi *et al.*, 2014). First, the MTT test showed a moderate activity of the diterpene towards KB and MCF-7 cells with IC₅₀ values of 0.171 and 0.184 µM, respectively. One of the isolated sesquiterpenes also had a moderate activity against KB at a concentration of 0.213 µg/ mL, while another sesquiterpene exhibited a broad range of cytotoxic activity against KB, HepG2, and MCF-7 with IC₅₀ of 0.100, 0.057, and 0.054 µM, respectively.

Ethno botanicals and medical uses

Marine algae open numerous perspectives for research and for many economic sectors. They are primarily consumed as vegetables, mainly in Asian countries (Kandale *et al.*, 2011). They usually consist of proteins, a small amount of fat compared to the percentage of carbohydrates, which are mainly in the form of polysaccharides such as alginates, carrageenan and ulvans. In addition they contain high levels of minerals, vitamins and pigments (Makkar *et al.*, 2016). They are a potential source of food supplements due to the diversity of molecules which gives them multiple nutritional qualities. Alginates, carrageenans and agars are the major industrial products derived from algae. The extraction of these phycocolloids is used in the food industry (gelling agents), pharmaceuticals (dressings, coatings of medicaments) and biotechnology (culture medium, the Petri dishes). They are also found in cosmetics (body lotions, soaps, shampoos, toothpaste), and even in industrial products (adhesives, paints for building) (Person, 2011). Marine algae have traditionally been used in animal feed. Today, algae are found in the form of flour, and constitute a primary source of nutrients for many animal species. They are also used in agriculture (Kandale *et al.*, 2011; Makkar *et al.*, 2016) (fertilizer / bio-fertilizers, phytosanitary treatments to promote the growth of leaves and stems, plant protection against certain diseases), and as well as a bio-based materials (home textiles, adhesives, cements and mortars, anti-fouling paints for ships, paper and cardboard industry, etc.). Algal biomass can be used in bioenergy because of their low fat content, which excludes the production of biodiesel (Person, 2011). In addition, marine algae are used in cosmetics for their moisturizing, nourishing, regenerating, slimming, soothing, remineralising, and other properties. Algal baths, with their nerve and muscle relaxing properties, anti-fatigue, tonic, invigorating and soothing properties are very effective in the medical and aesthetic field. For all these reasons, they are a natural therapy of choice to maintain the beauty of the skin, to significantly delay skin aging, etc. (Person, 2011).

Recently, much interest has been developed for the isolation of new bioactive compounds from marine algae because of their many beneficial effects on health (Oumaskour *et al.*, 2013). Numerous reports show that marine algae present a broad range of biological activities such as antibacterial (Kavita *et al.*, 2014), antifungal (Morales *et al.*, 2006), antiviral (Plouguerne *et al.*, 2013), anti-inflammatory and antioxidant (Mhadhebi *et al.*, 2014), and anticancer (Duc Thinh *et al.*, 2013). They are in fact a rich source of sulfated polysaccharides, which are known to have many biological activities including anticoagulant, gastroprotective, anti-inflammatory activities, and many others which give them an interest in pharmaceutical uses (Chaves *et al.*, 2013).

CONSLUSION

The present study revealed that the two crude extracts prepared from the marine red algae *Laurencia papillosa* collected from the Lebanese coast are cytotoxic against the Jurkat human cancer cell line in a time and dose dependent manner, via the trypan blue exclusion assay. The IC₅₀ values are 121.642 µg/ mL and 57.77 µg/ mL for the ethanol: water extract and the ethanol: chloroform extract, respectively after 72 hours of treatment.

L. papillosa appears to be an interesting and profitable red seaweed due to its anticancer activity against human leukemia cell lines Jurkat. Regarding this preliminary result, more studies are needed to demonstrate that *L. papillosa* crude extracts exhibit no cytotoxicity towards normal cells. Furthermore, the isolation and purification of new natural antitumor compounds of those algal extracts should be explored, and the examination of the mechanism of action of these compounds as well, (their effect on the cell cycle and their ability to induce apoptosis).

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