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Cytotoxic Effect of Extracts From The Moroccan Marine Sponge on Human Prostate Cancer Cell Line

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Abstract **- Marine sponges have been prominently featured in the area of cancer research. Here, we evaluated the cytotoxicity of aqueous and dichloromethane extracts of *Cliona viridis*, a marine sponge, collected from the Moroccan coast. Using the WST 1 assay, dichloromethane extract displayed significant cytotoxicity against human prostate cancer cell line PC3 with IC_{50} value of 150 µg /ml while the aqueous extract had no effect on the cell proliferation. These data highlight the potential of *C. viridis* for future drug discovery against major diseases, such as prostate cancer. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.**

***Index Terms*— *Cliona viridis*, Cytotoxicity, Marine sponge, Prostate Cancer, WST-1.**

I. INTRODUCTION

Natural products are considered as a great source of anticancer agents [1]. However, marine organisms have a shorter history of utilization in the treatment and/or prevention of human disease compared to the terrestrial plants that have been used for thousands of years [2].
The systematic investigation of oceans for probable medicines hasn't begun until the middle of the 20th century. Since then, marine sources such as sponges, fungus, alga, mollusks and some other have revealed more than 10,000 bioactive molecules, and they are still being discovered [3]. Numerous studies have demonstrated the antimicrobial [4], [5], antibacterial [6], [7], antiviral [8] antifungal [9], and cytotoxic [10], [11] activities.
Nowadays, sponges are taking the leading position in the marine environment; they have a potential to provide novel leads malaria, viral diseases and cancer [12]. The cytotoxic effect of marine sponges has been reported in different studies. The marine sponge Aurora globostellata showed a strong cytotoxic activity against different cancer cells lines [13]. H.K. Lim and al. showed that extracts of Hyrtios spp have a cytotoxic effect on colorectal carcinoma RKO cell line by the induction of p53 and p21 proteins [14].
In Morocco, many reports on screening Moroccan marine sponges for biological activities have been exposed [15], [16], [17], [18], [19] and some of them described the moderate cytotoxic effect of Fasciculatin, a furanosesterterpene, isolated from *Ircinia variabilis*, the marine sponge from the Atlantic Coast of Morocco [20].

In order to contribute to the search for new anticancer agents, the purpose of the present study was to explore the potential cytotoxicity of aqueous and organic extracts of the marine sponge *C. viridis*; collected from the Atlantic coast of Morocco against the human prostatic cancer cells PC3 .

II. MATERIAL AND METHODS

A. Sample collection and systematic identification

Cliona viridis (Fig.2) was collected during winter season, in January 2010 at low tide to a depth of 3-5 m below the waves of breezeblocks in the commercial port of Jorf Lasfar, El Jadida (Fig.2). The systematic identification of marine sponge was
performed by Dr. Maria Jesús Uriz, Research Professor at the Center for Advanced Studies of
Blanes (Centro de Estudios Avanzados of Blanes [BEAC]) and the Higher Council for Scientific Research (Consejo Superior Investigaciones Científicas of [CSIC]) in Spain.

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Figure 1: Morphology of *C. viridis*

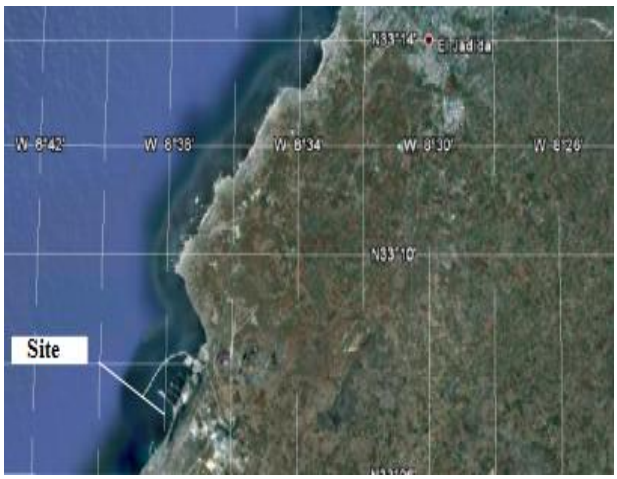


Figure 2: Map showing the collect site of the marine sponge *C. viridis*

B. Extracts preparation

Dichloromethane extract

The samples were washed and then frozen overnight at -30 °C for one night and placed in the freeze-drying chamber equipped with a tray heater. The freeze drying was set up at -70 ° C, at low pressure for several hours. The sponge (dried weight) was homogenized in 100 ml of ethanol (80%), kept in a dark for 24 h and filtered. The filtrate was again extracted in 2 x 100 ml of absolute ethanol. The ethanol extracts were combined and evaporated under reduced pressure until total evaporation. The suspension was completed with distilled water to 100 ml as final volume and extracted tree times with dichloromethane CH_2Cl_2 (100 ml). The CH2Cl2 extracts were combined, dried on anhydrous sodium sulfate (Na_2SO_4), filtered and concentrated under reduced pressure to give a crude dichloromethane extract.

Aqueous extract

The aqueous phase was evaporated, dissolved twice in absolute ethanol; then filtered and concentrated under reduced pressure to obtain crude aqueous. Dichloromethane and aqueous extracts of *C. viridis* were evaluated for their cytotoxic effects.

C. Cytotoxicity screening

Cell lines and cell cultures

Human prostate cancer cell line PC3 is used as a model. Cancerous cells were cultured in DMEM supplemented with 10% heat-inactivated fetal calf serum, 1% Glutamine, and 1% antibiotics. The cells were grown at 37°C in a humidified atmosphere and 5% of CO_2. The cells were fed until confluent and expanded by trypsinization, then, sub-cultured at lower numbers in new culture flasks.

Cytotoxic effect of the aqueous and dichloromethane extracts from *C. viridis* against PC3 human prostate cancer cell line was estimated on the basis of mitochondrial metabolic activity using WST1 (disodium mono{4-[3- (4-iodophenyl)-2-(4-nitrophenyl)-2H-tetrazol]-3-ium-5-yl] benzene-1,3-disulfonate}) assay as described elsewhere [21].

Exponentially growing PC3 cells were seeded on 96-well microplates at a density of 8000 cells per well. After 24 h, the culture medium was replaced by an experimental one with different concentrations of extract (initially dissolved in DMSO), ranging from 9.3 to 300 µg/ ml of aqueous and dichloromethane extracts, in duplicate, and re-incubated for 72h. Following incubation, 100 µl of the medium was aspirated and 10 µl of WST1 reagent was added and the plate was re-incubated for further 4 h. Cell viability was assessed by absorbance reading of each well at 450 nm using a Wallac Victor X3 multiplate reader. Data are expressed as percentages of absorbance between treated and control wells. Mitomycin was used as positive control.

Eventually, the viability was calculated via the formula below (1):

$$Viability\% = \frac{Optical\ density\ of\ sample}{optical\ density\ of\ control} \times 100 \qquad (1)$$

Additionally, cytotoxicity of the aqueous and dichloromethane extracts was defined by plotting of the percent cytotoxicity index CI%, versus concentrations of the sponge extracts (2):

$$CI\ \% = \left[1 - \left(\frac{Optical\ density\ of\ sample}{optical\ density\ of\ control}\right) \times 100\right] \qquad (2)$$

III. RESULTS AND DISCUSSION

A. Systematic identification

The morphological and anatomical characteristics of the collected sponge were analyzed and the species was identified through microscopic and macroscopic comparative analyses.

The typical characteristics taken into account include color, size, shape and internal structure and then the sponge was compared with the existing photographs and data [22].

The details of taxonomic scientific classification of the marine sponge are given below (Table1):

Table1: Taxonomical classification of *C. viridis*

Kingdom	Animalia
Phylum	Porifera
Class	Demospongiae
Order	Hadromerida
Family	Clionaidae
Genus	*Cliona*
Species	*Cliona viridis*

Cliona viridis is an excavating sponge, and found to be papillae sticking out of calcareous substrates and covering a massive surface which has completely overgrown and eroded the substrate; it is easily distinguished from the rather similar yellow *Cliona celata* by having a green color.

Sponges are morphologically identified using four main criteria viz: color, shape, skeletal features and spicule types. Traditional taxonomy methods mainly follow the investigation on skeletal elements.

Recent investigation methods by means of electron microscopy, chemistry and/or molecular techniques have demonstrated that some species are actually comprised of more than one species [23], [24]. Molecular characteristics analysis provides more precise classification criteria for species that lack taxonomically important morphological features. By using the molecular data, one can study all the aspects of sponge evolution [25].

The phylogeny of Demospongiae was revisited recently and congruent results were thereby obtained with ribosomal DNA, mitochondrial DNA and nuclear housekeeping genes mitochondrial cytochrome c oxidase subunit.

3.2 *In vitro* Cytotoxicity Assay

Cytotoxic activity of the aqueous and dichloromethane extracts of *C. viridis* against the PC3 cell line was evaluated by measuring cell viability. Cells were treated for 72 h with the extracts at different concentrations ranging from 9.3 to 300 µg/ml, and IC_{50} values were calculated from the dose-response curves obtained by plotting percentage of cell growth as a function of extracts concentrations (Fig. 3).

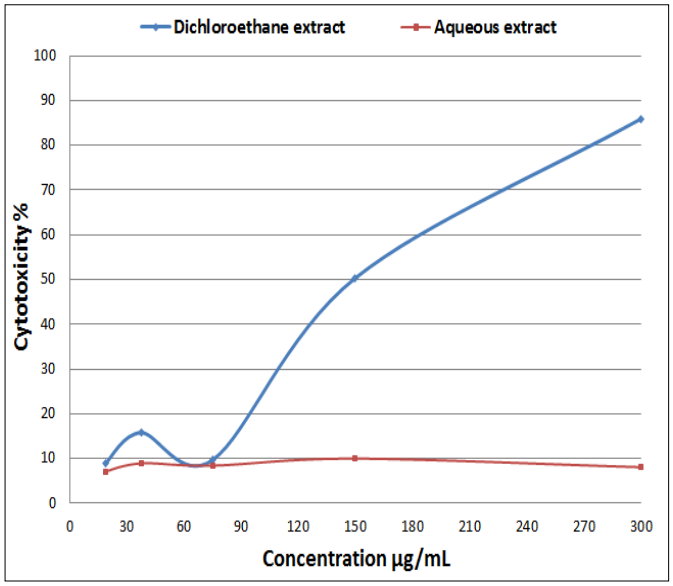
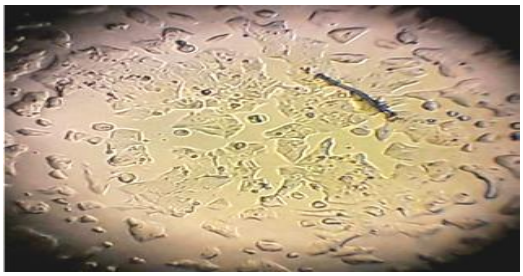


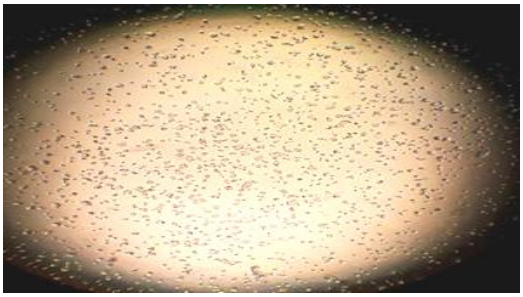
Figure 3: Cytotoxic effect of dichloromethane and aqueous extracts of *C. viridis* against PC3 cell line.

As displayed in **Figure 3**, dichloromethane extract of *C. viridis* exhibited a highly potent inhibition against the growth of a human prostate cell line PC3 in a dose-dependant manner with an IC_{50} of 150 µg/ ml. In contrast, aqueous extract had no effect on cells proliferation.

Furthermore, compared to untreated cells number of PC_3 cells was decreased and their morphologies have changed after treatment with dichloromethane extract (Fig. 4a). At high concentration such as 150 µg /ml (Fig.4b) the cytotoxic effect results in floated and spherical cells. This effect is pronounced with a treatment at 300 µg /ml (Fig.4c).



(a)



(b)

(c)

Figure 4: Morphological alterations of PC3 cells, induced after 72 hours of treatment by dichloromethane extract of *C. viridi*, observed under inverted microscope. A: Untreated cells (40 x), B (10x) and C (10x): cells treated with 150 and 300 µg/ml, respectively.

The results obtained in this preliminary study indicate that the dichloromethane extract of the sponge C. viridis has a remarkable cytotoxic effect on human prostate cell line PC3. However, PC3 cells remains refractory to the action of the aqueous extract which does not induce a cytotoxic effect even at the highest concentrations.

Sponges are currently considered promising sources of new bioactive compounds. The cytotoxic effect that bioactive metabolites of sponges can have on cell lines, attracts researchers whose interest is focused on discovering new anticancer molecules. Indeed several compounds, such as terpenoids and alkaloids, isolated from sponges have shown in vitro cytotoxicity and pro-apoptotic activities against various cancer cell lines [26].

Several marine compounds seem to possess anti-cancer activity, and many mechanisms of action of cytotoxic agents have been identified. Aragusterol A, a potent antitumor marine steroid isolated from the Okinawan sponge of the genus, *Xestospongia* induces antitumor effect on a large variety of human cancer cell by targeting the G1/S cell cycle phase [27]. Discodermolide, a polyketide isolated from *Diskodermia dissoluta* inhibits the proliferation of human and murine tumor cell lines and apoptosis is the main mechanism of action involved [28]. Fascaplysin-originally isolated from the sponge *Fascaplysinopsis sp*.- extensively studied for it biological activities prevents proliferation of Human colon carcinoma and osteogenic sarcoma cell lines and normal fibroblasts through Cyclin-dependent kinase 4 Inhibition [29].

Prostate cancer has become the most common cancer in humans for two decades [26], thus, we found it useful to evaluate the cytotoxic power, in vitro, on the PC3 prostate cancer line, commonly used as models of drug susceptibility [30]. Our results show that the dichloromethane extract inhibits the proliferation of PC3 cells in a dose-dependent manner. Gordaliza and al. 2010 attribute the anticancer properties of marine sponge extracts to their secondary metabolites [31]. Indeed, previous reported studies have demonstrated that *Cliona* genera contains a lot of secondary metabolites with biological activity which can be an great source of original molecules [32], [33].

Cliona species have usually yielded modified cytotoxic peptides and alkaloids, such as clionamides [34]. Yet, it is still unclear whether these metabolites are produced only by the sponges themselves or synthesized by the microorganisms living in symbiosis with them. Usually, Marine sponges contain varied and abundant microbial communities such as fungi, bacteria and microalgae. In some cases, these microbial associates comprise as much as 40% of the sponge volume and can contribute significantly to the host metabolism via e.g. photosynthesis or nitrogen fixation [35] and production of antibiotic substance to a competitive role for space and nutrient [18].

IV. CONCLUSION

Our study highlights the cytotoxic effect of *C. viridis* suggesting its potential application in the treatment of cancer. These species could be considered as potential sources of anticancer compounds. However, further investigations are essential for the chemical characterization of the active principles responsible for the cytotoxic effect.

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