Cytotoxic Effect of Crude Extract of Sea Pen *Virgularia gustaviana* on HeLa and MDA-MB-231 Cancer Cell Lines

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**Abstract**—Marine organisms such as soft coral, sponge, ascidians, and tunicate containing rich source of natural compound have been studied in last decades because of their special chemical compounds with anticancer properties. The aim of this study was to investigate anti-cancer property of ethyl acetate extracted from marine sea pen *Virgularia gustaviana* found from Persian Gulf coastal (Bandar Abbas). The extraction processes were carried out with ethyl acetate for five days. Thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were used for qualitative identification of crude extract. The viability of HeLa and MDA-Mb-231 cancer cells was investigated using MTT assay at the concentration of 25, 50, and a 100 μl/ml of ethyl acetate is extracted. The crude extract of *Virgularia gustaviana* demonstrated ten fractions with different Retention factor (Rf) by TLC and Retention time (Rt) evaluated by HPLC. The crude extract dose-dependently decreased cancer cell viability compared to control group. According to the results, the ethyl acetate extracted from *Virgularia gustaviana* inhibits the growth of cancer cells, an effect which needs to be further investigated in the future studies.

**Keywords**—*Virgularia gustaviana*, Cembrane Diterpene, anti-cancer, HeLa cancer Cell, MDA-Md-231 Cancer cell.

I. INTRODUCTION

Cancer is one of the major causes of death in the world despite the increasing advances in prevention and treatment [1]. Since the current treatments for cancer like chemotherapy and radio-therapy are not always effective, and there is a possibility of recurrence due to side effects of current anti-cancer agent, the scientists have focused on natural products as anti-cancer drugs, and among the natural compounds which are extracted from marine organisms [2]. Use of medicinal marine resources in recent years has attracted the attention of many scientists [3]. Ability of these compounds to kill cancer cells and their mechanisms of action have been reported in successive articles [4]. Marine environment has a wide range of condition such as the Arctic cold water to warm water, high pressure, lack of light and high salinity. Hence, the above mentioned conditions led to the creation of marine compound structures and metabolic pathways with high biotechnological potential [5]. Marine invertebrates have an ability to produce toxic compounds to defend themselves from other marine organisms. These animals released chemical compound into the water. The biodiversity of the marine invertebrate with different chemical products is most likely suitable for research use as clinical target [6]. Marine soft coral is the most general source of cembrane diterpene. Many cembrane-type compounds have been shown to exhibit cytotoxicity against a variety of tumor cells [7].

In this study, we are focused on marine sea pen *Virgularia gustaviana*. Sea pens are the feather-like colony of polyps, this animal's body contains two main parts: the primary part is anchored to soft bed. Rachis is the other part which contains polyps. The aim of this study is the assessment of cell viability of crude extract of sea pen *Virgularia gustaviana* on cervical and breast cancer cells.

II. MATERIALS AND METHODS

A. General Experiments

TLC was carried out on percolated Kieselgel 60 F254 (0.25 mm, Merck), and spots were visualized on UV light. HPTLC was carried out on plate (Silica gel 60 F 254 glass 20*10 cm, Merck, Germany). HPLC was performed using a system comprised of a Cecil pump, a Cecil photodiode array detector, and a Rhodyne injection port. A normal phase column (Hibar 250 × 10 mm, Merck, silica gel 60, 5 μm) was used for HPLC.

B. Animals

Marine sea pen *Virgularia gustaviana* was collected with patrolling the intertidal zone of the estuary Seura in Bandar Abbas. It was frozen immediately after the collection and was moved to the Laboratory of Tissue Engineering Research Center for Nanotechnology of Beheshti University of Medical Sciences, and in -20 °C until the trial was held.

C. Extraction

In order to obtain organic extract, the frozen sea pen was chopped into small pieces (1 kg, wet weight), and all samples were freeze dried and extracted with Ethyl Acetate at room temperature (2L×3). The combined ethyl acetate extracts were filtered by Whatman filter paper (number 1), and the filtered...
solution was extracted using distilled water. Finally, the solvent was removed by evaporation using rotary evaporator to afford 80 g dark orange gummy residue [8].

D. HPLC

Ethyl acetate crude extracts of Virgularia gustaviana were studied qualitatively by HPLC. Elution conditions followed by Acetonitrile-Deionized water (pH 3.5 adjusted with phosphoric acid) with 60:40 v/v ratio as mobile phase, and flow rate of 1.5 ml min⁻¹. The highest absorbance was in 220 nm from 70:30 [9].

E. Cell Culture

HeLa and MDA-MB-231 cell line are used to evaluate the cytotoxic effect of the tested extracts. Cells were routinely cultured in RPMI 1640 (Chemi-Con International, Temecula, CA, USA). Media were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin [10]. Cells were maintained at 37 °C in humidified air containing 5% CO₂. For serum (FBS) and 1% penicillin/streptomycin [10]. Cells were has been cultured with the Fractions, was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay [12]. The HeLa and MDA-MB-231 at density of 5 × 10⁴ per well were seeded in 24-well plate and incubated in incubator with 5% CO₂ at 37 °C, for one day. Then, cells were treated with Ethyl acetate extract with various concentrations 100, 50, 25 µl and incubated at 37 °C for 24h. The cultures without treatment were used as the control group. After 24h, the vitality of the cancer cells, which had been cultured with the Fractions, was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [12]. The HeLa and MDA-MB-231 with decrease of dose-dependently, which is shown on Fig. 2 and Table I.

Table I. PERCENTAGE OF HE-La AND MDA-MB-231 CELLS ADHESION BY DIFFERENT CONCENTRATIONS OF THE ETHYL ACETATE EXTRACT OF GENUS VIRGULARIA GUSTAVIANA

<table>
<thead>
<tr>
<th>Doses</th>
<th>Viability (%)</th>
<th>HeLa</th>
<th>MDA-Mb-231</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>90.00±1.00</td>
<td>18.00±0.57</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>36.00±1.00</td>
<td>49.00±0.57</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>73.00±2.08</td>
<td>82.67±1.45</td>
<td></td>
</tr>
</tbody>
</table>

IV. DISSECTION

The main objective of this study was characterization result of HPLC and TLC analyzed and assessment of capability of organic ethyl acetate extract of Virgularia gustaviana collected from Bandar Abbas costal to inhibit growth of cancer cell. Characterization of ethyl acetate extract by HPLC showed that 13 main compounds were eluted by acetonitrile: deionized water as a mobile phase.

Marine environment is important area of natural compound with potential effect as therapeutic agents [14]. Many cytotoxic compounds were extracted from marine invertebrate such as soft corals, sponge, bryozoan, and etc. [15]. In the past decade, scientists have been focused on anti-cancer properties of marine invertebrate bioactive compounds, for instance pachymatins from Pachymatiasma johnstonii genus of marine sponge [16], bryostatins from Bugula neritina the marine bryozoan [17], didemnin B from Trididemnum solidum a marine tunicate [18]. Marine soft coral which contain coelenterata, octocorallia, alcyonacea families have been approved as a potential foundation of steroids and diterpenoids. The prostanoids from C. viridis have been proposed as anti-tumor agent in some types of human tumors [19]. Australins A-D from ethanoic extract of C. australis showed high cytotoxic effect on MCS-7, MDA-MB-231 and HepG2 cancer cell lines [8]. The ethyl acetate extract of P. viridis showed cytotoxic effects towards MCF 7 than for HCF 116 [20]. Treatment of extracts of the genus Simularia on SCC25 and HaCaT cells indicated cytotoxic effects [21]. Methanolic extract of the soft coral Sarcophyton pauciplicatum showed cytotoxic activity against a panel of eight human cancer cell lines [22]. In last study identification
of fatty acid extracted of sea pen *Virgularia gustaviana* and their anti-bacterial and anti-fungal activity were measured with MIC and MBC methods and the results shown that arachidonic acids of chloroform extract has best activity against *Staphylococcus aureus* on 125 µg/ml dose in MIC method [23]. Also, anti-inflammatory effect of chloroform and hexane extracts of mentioned genus showed strong activity in mice ears even at low doses, which is probably due to 54% arachidonic acid [24]. In this study, the ethyl acetate extract of marine sea pen *Virgularia gustaviana* presented high cytotoxic effects on HeLa and MDA-MB-231 cancer cell lines. From these hopeful results, ethyl acetates extracted from sea pen show strong cytotoxic effects on human cervical cancer cell line HeLa and Breast cancer cell line MDA-Mb-231. In that purification and identification of any of 18 compounds, assessment of cytotoxic effect of each compound needs more studies.

Fig. 1 HPLC chromatograms of 20 µl injection of Ethyl acetate extract of *Virgularia gustaviana*
In conclusion, characterization and biological experiments presented that there are 13 different compounds with different Rf on Ethyl acetate extracted of sea pen *Virgularia gustaviana* which showed high cytotoxic effect on HeLa and MDA_Mb-231 cancer cell lines.

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REFERENCES


