In vitro Evaluation of Bioactivity of Dictyonella incisa from Turkey

Hajar HEYDARI 1  Bülent GÖZCELIOĞLU 2  Belma KONUKLUGİL 1 

1 Ankara University, Pharmacy Faculty, Pharmacognosy Department, TR-06100 Tandoğan, Ankara - TURKEY
2 Scientific and Technological Research Council of Turkey (TÜBİTAK), TR-06420 Bakanlıklar, Ankara - TURKEY

Article Code: KVFD-2018-19309    Received: 03.01.2018    Accepted: 14.03.2018   Published Online: 14.03.2018

Abstract

Marine species are known to produce structurally unique pharmaceutically potent secondary metabolites. During the course of our studies on Turkish marine sponges, we have collected Dictyonella incisa from Turkey’s coast and methanolic extract was investigated for antioxidant, cytotoxic and antimicrobial activity. Antioxidant activity was evaluated by the superoxide radical scavenging assay, cytotoxic activity was determined against HCT-116 and HEp-2 cell lines by MTT assay. Antimicrobial activity was tested against some Gram positive, Gram negative bacteria and yeasts. Also cholesterol and cholestan isolated and the structure were identified by NMR methods. This is the first work on the bioactivity and secondary metabolites of D. incisa collected from Turkish coasts.

Keywords: Antimicrobial, Antioxidant, Cytotoxic activity, Dictyonella incisa

INTRODUCTION

Marine species are rich sources of bioactive compounds used in food and pharmaceutical industries [1]. In the last decades, many bioactive compounds have been isolated from various marine species like sponges, tunicates corals and etc. Sponges are spineless animals belonging to phylum [2]. They are great sources of secondary metabolites which have pharmacological properties. Therefore, sponges have shown widely diverse activities like anti-HIV, antibacterial, cytotoxicity, anti-fouling properties [3-5]. Sponges appear to be one of the richest phylum in toxigenic species; there are approximately 15,000 sponge species which 150 of them are living in freshwaters [6]. In search of novel pharmaceutically active substances from sponges, we have engaged in bioactivity screening of sponges. In this regard, Dictyonella incisa was selected for screening of antioxidant, antimicrobial and cytotoxicity activities. Dictyonella incisa is a sponge species which belongs to Dictyonellidae family and Dictyonella genus (World Register of Marine Species). Turkey is a country of peninsula surrounded by the Black Sea at the north, the Aegean Sea at the west, and the Mediterranean Sea at the south. Despite its long coastal line, which is 8300 km in total, there have been limited works on its marine prosperity from the view point of isolation of secondary metabolites and bioactivities. Three records were found according to the literature search. One of these is related to anti-inflammatory activity and isolation of fatty acids [7]. The others are about isolation of ketosteroid and sterol [8,9].
In our continuing search for secondary metabolites and bioactivity of Turkish marine organisms, we have reported the isolation of cholesterol and cholestane, also antioxidant, antimicrobial and cytotoxic activities of *D. incisa* from Turkish Sea.

**MATERIAL and METHODS**

**General**

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-terazolium-bromide (MTT) and nitroblue tetrazolium (NBT) were purchased from Sigma Aldrich, sabouraud 4% dextrose agar, muller hinton broth, sabouraud dextrose broth and ethyl acetate was purchased from Merck. McCoy’s 5A medium, fetal bovine serum (FBS), streptomycin and glutamine were from PAA (PAsching, Austria), HCT 116 colon cancer cells and Hep-2 human larynx epidermoid carcinoma cell lines were kindly provided by Bert Vogelstein and Refik Saydam Hygiene Center, Ankara Turkey respectively. Molecular devices Spectra MAX 190 Microplate Reader helps to get absorbance.

**Preparation of Extract**

*Dictyonella incisa* was collected from Seferihisar, Turkey in 2014, by a scuba diver and identified by Dr. Gözcelioğlu. A voucher specimen was deposited at the Pharmacognosy Department of Faculty of Pharmacy, Ankara University. The sample (204.4 g) was cutted to small pieces and then extracted by methanol (500 mL). The extract was dried under vacuum and kept at 4°C until uses.

**Antimicrobial Activity**

The stock solutions of extract were prepared in dimethyl sulphoxide (DMSO) at a final concentration of 512 µg/mL and sterilized by using 0.22 µm Millipore Membrane Filter (MA 01730, USA). Antimicrobial activity of sample was determined against *Staphylococcus aureus* (ATCC 43300), *Staphylococcus epidermidis* (ATCC 12228) and *Bacillus subtilis* (ATCC 6633) as Gram positive strains; *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (RSKK 574) as Gram negative strains, *Candida albicans* (10231) and *Candida parapsilosis* (ATCC 22019) as yeast strains. Antimicrobial activity was screened according to the EUCAST recommendations with broth microdilution method. Antimicrobial activity was performed by a modified microdilution method as described in CLSI M07-A9 standard for bacteria and CLSI M27-A3 standard for yeasts (CLSI 2008; 2012) The tested two fold serial dilutions of the extract were between 256 and 0.5 µg/mL. The sealed microplates were placed in a humid chamber and incubated at 35°C for 24 and 48 h for bacteria and yeasts, respectively. The lowest concentration of the extract that completely inhibited macroscopic growth of the microorganism was accepted as minimum inhibitory concentration (MIC).

**In-Vitro Cytotoxic Activity Assay (MTT Test)**

HEp-2 human cells (human larynx epidermoid carcinoma) and HCT 116 colon cancer cells were grown in DMEM (Dulbecco’s modified Eagle’s medium) supplemented with 10% fetal bovine serum, glutamine (2 mM) and 1% streptomycin in a humidified atmosphere of 5% CO₂, 95% air at 37°C. Cells were plated in a 96-well-plate with 1 × 10⁴ cells/well of concentration. After 48 h incubation methanolic extract (25-200 mg/mL DMSO) of *D. incisa* was added to cell in different concentrations (1000, 500, 250, 125, 62.5, 32.2, 16,1 µg/mL). Subsequently, MTT reagent (0.5 mg/mL in sterile phosphate buffer) was added directly to the wells and incubated for 4 h. The absorbance was measured at 570 nm. The percentage growth inhibition was calculated using following formula, 200 µL of cells (Hep-2) were added without extract as control group. Results was calculated after 24, 48 and 72 h[10].

\[
%\text{Cell Inhibition} = \left[ \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right] \times 100
\]

**Superoxide Radical Scavenging Activity by Alkaline DMSO Method**

Sodium hydroxide was added to air saturated DMSO for generating the superoxide radical. Alkaline DMSO was prepared by 5 mM NaOH in 0.1 mL water to 1 mL DMSO. Ten µL NBT (1 mg/mL) was added to 100 µL alkaline DMSO and 30 µL of extract at different concentrations, to give final volume of 1.4 mL. The absorbance was measured at 560 nm. The experiment was performed in triplicate. Quercetin and Ascorbic acid was used as standards.[11]

\[
\%\text{Inhibition} = \left[ \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right] \times 100
\]

**Cholesterol and Cholestane Isolation**

The methanol extract of *D. incisa* (8.9 g) was partitioned between distilled water (50 mL) and n-hexane (200 mL). The polar residue was partitioned by chloroforme (250 mL). n-hexane and chloroforme fraction was evaporated under the vacuum. The cholesterol was obtained from the n-hexane fraction (2.5 g), loaded to the Silica column chromatography and eluted by ethyl acetate and hexane gradiently. The cholestane was obtained from the chloroform fraction, loaded to the Silica column chromatography and eluted by chloroforme and methanol gradiently. Obtained compounds (white amorphous) were washed with methanol several times then dissolved in chloroforme and sent to Nuclear Magnetic Resonance (NMR). The structures of the compounds were identified by ‘H NMR.

**RESULTS**

*Dictyonella incisa’s* extract was tested against Gram positive, Gram negative strains, and yeast. According to the results, extract was shown to have antimicrobial activity against *S. aureus*, *S. epidermidis* (MIC: 62.5 µg/µL) and against *C. albicans* and *C. parapsilosis* (MIC: 1.56
and 0.78 respectively). The results were shown in Table 1. Cytotoxicity activity of the extract was established by MTT assay against HCT-116 (colon cancer cells lines) and HEp-2 (human larynx epidermoid carcinoma), according to the results D. incisa’s extract was shown to have higher cytotoxicity against HCT-116 than Hep-2 cell lines. The results were shown in Table 2.

Antioxidant activity of D. incisa’s methanolic extract was determined by SO (Superoxide Radical Scavenging Activity) assay, according to the results it was show dose dependent antioxidant activity (24.62% inhibition in 800 µg/µL) where quercetin and ascorbic acid were used as standard. The results were shown in Fig. 1.

Cholesterol and cholestane were isolated from D. incisa, the structure of these compounds were identified by ‘H NMR and compared with literature. Cholesterol and cholestane structure was shown in Fig. 2.[12,13].

**Cholesterol** (CDCl₃, 400MHz): H₁: δ1.61 (ddd, J =13.7, 3.1, 2.5 Hz), H₂:1.76 (ddddd, J =13.2, 10.2, 2.5, 2.4 Hz), H₃:2.34 (dd, J =14.6, 2.3Hz), H₄:5.31 (dd, J =9.1, 5.2 Hz), H₅: 2.1(ddd, J =13.1,10.2,9.1 Hz), H₆:1.46(qd, J =10.2,3.3 Hz), H₇:1.47 (ddd, J =10.2,10.2, 3.3 Hz), H₈:1.49 (dddd, J =13.1,10.3,10.2, 3.2 Hz), H₉:1.58 (dd, J =13.07,3.17, 2.55 Hz), H₁₀:1.6(ddddd, J =13.7,8.0,4.6 Hz), H₁₁:1.57(dddd, J =13.17,13.19,0.93, 2.51 Hz), H₁₂:1.6(ddddd, J =13.7,8.0,4.6 Hz), H₁₃:1.63(dddd, J =13.7,8.0,4.6 Hz), H₁₄:1.49(dddd, J =7.70,6.79,4.58 Hz), H₁₅:0.87(s,3H), H₁₆:1.22 (s, 3H), H₁₇:0.47 (dddd, J =13.16,3.19, 3.09,2.5 Hz), H₁₈:1.04 (d, J =6.8 Hz), H₁₉:0.79(dt, J =7.3,2.7 Hz), H₂₀:1.23 (quint, J =7.18 Hz), H₂₁:0.66 (q, J =7.2 Hz), H₂₂:1.47(dddd, J =13.2,3.2,3.1, 2.5 Hz), H₂₃:0.73-0.84 (d, J =6.6 Hz),OH: 2.63(s, 1H).

**Cholestane** (CDCl₃, 400MHz): H₁, H₄: δ1.53 (ddd, J =13.92,6.95, 4.82, 1.73 Hz), H₂, H₃: 1.6 (dddd, J =13.92,8.02, 3.28, 1.53 Hz), H₅:1.25 (m, J =10.26 Hz), H₆: 1.75 (dq, J =13.05, 2.78 Hz), H₇: 1.53 (dddd, J =13.87,8.0, 4.48,1.65 Hz), H₈: 1.36 (tdd, J =10.25,10.20, 2.79 Hz), H₉: 1.24 (dd, J =10.25, 10.18, 3.28 Hz), H₁₀: 1.5 (dddd, J =13.07,10.24, 10.18, 3.18 Hz), H₁₁: 1.67 (dd, J =13.08,3.18, 2.53 Hz), H₁₂: 1.55 (dd, J =10.29,7.5, 4.82 Hz), H₁₃: 1.5 (dddd, J =13.92,8.24, 4.18, 1.18 Hz), H₁₄: 1.65 (dd, J =13.75,13.79, 4.65, 1.73 Hz), H₁₅: 1.66 (dd, J =8.25, 4.63, 4.3 Hz), H₁₆: 0.75(s,3H), H₁₇:1.2 (s, 3H), H₁₈: 1.45 (m, J =7.28 Hz), H₁₉: 1.04 (m, J =6.8 Hz), H₂₀:0.72 (dt, J =7.3,7.2 Hz).

### Table 1. Antimicrobial activity of D. incisa’s extract (MIC value)

<table>
<thead>
<tr>
<th>Extract and Standards</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus ATCC 43300</td>
</tr>
<tr>
<td>D. incisa</td>
<td>62.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.625</td>
</tr>
<tr>
<td>Miconazole</td>
<td>No determined</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Among the marine organisms; marine sponges (Phylum Porifera) are the oldest and simplest multicellular animals on earth. A huge number of bioactive compounds have been
isolated from marine sponges. The majority productive marine producers of novel and unique compounds are sponges, with more than 250 new secondary metabolites reported every year. This great potential has revived applications of secondary metabolites as therapeutics and at present, a number of promising compounds are in clinical and preclinical trials such as Discodermolide, Monanchocidin, Renieramycin M, Hetronemin (anticaner), Tsitsikammamine C, Psammaphysin H (antimalarial) Norbatzelladine L (antiviral), (−)-ageloxime D (antifungal), Manolide, Spongiones A-D (antiinflammatory) and Eryloside F (cardiovascular agent)

REFERENCES


**Acknowledgment**

This work was supported by Projects of Scientific Investigation of Ankara University (BAP), Project No: 14LO237001.