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Original Article

Evaluation of Cytotoxic and Antioxidant Activity and Total Phenolic Content of Some Soft Corals from the Persian Gulf

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Abstract

Marine soft corals contain a variety of secondary metabolites with diverse, biological activities, including cytotoxic and anti-oxidant. The aim of this study was to evaluate the total phenolic content, antioxidant and cytotoxic activities of some soft corals of Persian Gulf including: *Junceella juncea*, *Cavernularia* sp., white *Menella* sp., brown *Menella* sp., *Virgularia* sp., *Sinularia compressa*, *Sinularia variablis* and *Sinularia polydactyla* were collected from Persian Gulf and extracted by maceration with methanol-ethyl acetate (1:1) solvent. The extract was evaporated and the total phenolic content was evaluated by using the Folin-ciocalteu reagent. The antioxidant activity of corals was tested by using DPPH photometric assay and cytotoxic activity of them against MCF-7 and OVCAR-3 cancer cell lines were performed. The cell survivals of MCF-7 and OVCAR-3 cell lines were decreased by increasing the concentration of the extracts. The brown *Menella* sp. showed the highest cytotoxic activity against MCF-7 with IC_{50} values of 325.45±2.57 µg/ml and *S. polydactyla* showed the highest cytotoxic activity against OVCAR-3 cell line with IC_{50} values of 260.99±7.93 µg/ml. White *Menella* sp. showed the highest antioxidant activity with IC_{50} values 0.056 µg/ml. Finally *Cavernularia* sp. had the most polyphenol content with 186.33 mg/L. Key words: soft corals, antioxidant DPPH photometric assay, total phenolic content, Folin–Ciocalteu method, cytotoxic activity, Persian Gulf.

1. Introduction

The surface of Earth consists of 70% oceans and more than 300,000 described species of plants and animals that leads to biodiversity of the planet. Macroscopic plants and animals have adapted to all regions of the oceans, including polar, temperate, and tropical areas. The diversity in species is extraordinarily rich on coral reefs, where there are around 1,000 species per m^2 in some areas, and the Indo-Pacific Ocean has the world's greatest tropical marine biodiversity. Marine environments are an incomparable source for natural-product discovery, with structural

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characteristic not typically discover in terrestrial plant secondary metabolites [1]. Marine bioactive compounds isolated and identified from marine organisms have protecting effect against predators, communicating and breeding [2]. In the last decade, wide range of new structures have been isolated from marine environments, pointing that marine environments render a great number of novel bioactive structures [3, 4]. According to the study of Munro and et al, marine animals can be introduced as a source of bioactive compounds with pharmaceutical potential activity in comparison with microorganisms, marine plants, terrestrial plants and terrestrial animals [1]. This is distinctly point out that marine invertebrates are a prosperous source by virtue of the fact of higher occurrence of significant cytotoxic activity [5].

The alcyonaceans, often called also soft corals, are really recognized only by microscopic examination of the calcareous particles (sclerites). Soft corals are colonies of small animals known as polypoid cnidarians (shortened to polyps) [1]. These animals have diverse pharmacological activities including cytotoxic, anti-proliferative, anti-nociceptive, gastro-protective, hepatoprotective, anti-fungal, anti-bacterial, anti-viral, anti-malarial, anti-inflammatory, anxyolitic, neuro-protective, and immunomodulator activities [6-17]. The genus *Junceella* (phylum Cnidaria, class Anthozoa, order Gorgonacea, family Ellisellidae) [18] has shown pharmacological activities such as anti-fouling [19], cytotoxic anti-inflammatory effects and inhibitory activity against the HBeAg express of hepatitis B virus [20]. The genus *Cavernularia* belongs to the marine cnidarian (class *Anthozoa*) in the family *Vertillidae* [21]. *Virgularia* sp. is genus of invertebrate belonging to the order *Pennatulacea* (class *Anthozoa*) and has exhibited cytotoxic effects against P-388 cancer cells [22-23]. Different species of the genus *Menella* sp. (family Plexauridae) that has shown pharmacologic activities such as: inhibitory effect on the release of elastase by human neutrophils [24], inhibitory effects on the generation of superoxide anion by human neutrophils, Tumor cell growth inhibitory activity and cytotoxic activities [25-27]. The genus *Sinularia* belongs to the Coelenterata, Alcyonacea [28] (Anthozoa: Octocorallia) [29] has various pharmacological activities such as anti-inflammatory, antituberculosis, CNS depressant hypotensive, cytotoxic, antimicrobial effects and cardiac vasorelaxant [30].

The aim of this study was to evaluate the anti-oxidant and cytotoxic activities of eight soft corals, from the genus *Junceella*, *Cavernularia*, *Virgularia*, *Menella*, and *Sinularia* that were collected from the Persian

Gulf to find the best cytotoxic extract for future investigations and phytochemical analyzes.

2. Material and Methods

2.1. Samples Collection

Eight soft corals *Junceella juncea*, *Cavernularia* sp., white *Menella* sp., brown *Menella* sp., *Virgularia* sp., *Sinularia compressa*, *Sinularia variablis* and *Sinularia polydactyla* were collected off the coast of Larak, Persian Gulf, Iran (Latitude: 26°53′14.05″N 56°23′33.53″E) in September 2017 at depths of 10 to 15 m and stored at −20 °C until extraction. Collected organism were identified by Arash shirvani, marine reasercher from Modern Bio-Treasures of Qeshm Research Center, Central Marine Fisheries Research Institute, Qeshm, Iran.

2.2. Preparation of Extracts

The specimens of each coral were grinded and then 27.5, 163.7, 122.8, 51.1, 48.0, 253.2, 47.2 and 70.0 grams of wet weight of *J. juncea*, *Cavernularia* sp., *Virgularia* sp., white *Menella* sp., brown *Menella* sp., *S. compressa*, *S. variablis*, and *S. polydactyla* respectively were air-dried in the shade, at room temperature (25°C). Then samples were extracted four times with methanol: ethyl acetate (1:1) (500 mL) [31]. Finally the extracts were concentrated at 30 °C with Rotary evaporator.

2.3. Cell Lines

MCF-7 cell line was obtained from cell bank, the Iranian biological resource Center (IBRC). OVCAR-3 cancer cell line was provided from Pasteur Institute of Iran (Tehran, I.R. Iran).

Cell lines were cultured in RPMI-1640 nourished with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 µg/ml of streptomycin, and were incubated at 37 °C in 5% CO2 air and 95% humid air [32].

2.4. Cell Viability Assay with MTT Reduction

Cell viability was distinct by applying the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; Sigma). MCF-7, OVCAR-3 cancer cell lines and AGO1522 normal cell line were seeded at 7×10^3 cells in each well of a 96-well plate. After overnight incubation to allow cell attachment, the RPMI 1640 in each well was replaced with media containing various concentrations (10, 50, 100 and 500µl) of soft coral extracts and incubated for 48 h. Samples were dissolved in DMSO (Dimethyl Sulfoxide) and diluted with RPMI. The final DMSO concentration in all treatments, including sample and control groups was 1% of total medium volume. Then, 20 µl of MTT (5 mg/ml in PBS) was added to each well including sample and control groups and the cells were incubated at 37 °C for 4 h. Then 100 µl DMSO was added to each well to dissolve insoluble formazan. Finally the absorbance values were read by a on a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments) at 570 nm to determine the number of viable cells [31]. The IC50 value which is defined as the concentration of test compound that is required to kill fifty

present of cancer cells, was determined after MTT test [33].

2.5. Antioxidant Activity

2.5.1. DPPH Photometric Assay

Sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 100, 50 and 25 µg/mL, in ethanol. One mL of a 0.3 mM DPPH ethanol solution was added to 2.5 mL of sample solutions of different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity (AA) using the following formula:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\}$$

Ethanol (1.0 mL) plus plant extract solution (2.5 mL) was used as a blank. DPPH solution (1.0 mL; 0.3 mM) plus ethanol (2.5 mL) was used as a negative control. The positive controls were those using the standard solutions. The EC50 values were calculated by linear regression of plots where the abscissa represented the concentration of tested soft corals extracts and the ordinate the average percent of antioxidant activity from three separate tests [34].

2.6. Determination of Polyphenol Content

2.6.1. Folin–Ciocalteu Method

The amount of total phenolics was determined using the Folin–Ciocalteu method [35]. A calibration curve of gallic acid (ranging from 0 to 100 mg/l) was prepared and the results, determined from regression equation of the calibration curve ($y = 0.0071x - 0.0324$, $R^2 = 0.9898$), were expressed as mg gallic acid equivalents per gramme of the sample. In this method, 1 ml of soft coral extract was diluted 10–75 times with deionized water (to obtain absorbance in the range of the prepared calibration curve) was mixed with 1 ml 3-fold-diluted Folin–Ciocalteu phenol reagent. Then 2ml, 35% sodium carbonate solution was added to the mixture. After thoroughly shaking, by adding 2 ml deionized water, the final concentration was reached to 6ml. diluted to 6 ml by adding 2 ml of water. After 30 min, blue color formed was measured at 700 nm [36].

2.7. Statistical Analysis

The cytotoxic experiment was done in triplicate. The results are given as mean standard deviation (SD). Student's t-test was used for comparison between two means and a one-way analysis of variance (ANOVA) was used for comparison of more than two means. A difference was considered statistically significant when $p \leq 0.05$.

3. Results and Discussion

3.1. Cytotoxicity Assay Results

According to the standards of the National Cancer Institute (NCI), a crude extract can be considered as active for an IC50 ≤ 20 µg/ml (37). None of the extracts (Table 1) collected from eight soft corals, *J. juncea* , *Cavernularia* sp., *Virgularia* sp., white *Menella* sp., brown *Menella* sp., *S. compressa, S. variablis, and S. polydactyla* were active against MCF-7 and OVCAR-3 cancer cell lines. The results revealed that the most active soft corals

Table 1. The IC_{50} (µg/ml) $^{\Delta}$ (Mean ± SD) values of soft corals extracts on human cancer cell lines.

Soft corals	**MCF-7**	**OVCAR-3**
	IC_{50} (µg/ml)	**IC_{50} (µg/ml)**
J. juncea	607.13±109.53	394.79±84.79
Cavernularia sp.	550.154±52.23	315.42±4.49
Virgularia sp.	364.79±7.30	322.88±13.10
White *Menella* sp.	607.13±109.53	394.79±84.79
Brown *Menella* sp.	325.45±2.57	270.30±5.017
S. polydactyla	417.21±43.61	260.99±7.93
S. variablis	361.09±36.41	265.98±6.26
S. compressa	413.53±14.65	317.79±6.84

against OVCAR-3 cancer cell line were considered: *S. polydactyla* (IC_{50}= 260.99±7.93 µg/ml), *S. variablis* (IC_{50}= 265.98±6.26 µg/ml) and brown *Menella* sp. (IC_{50}= 270.30±5.017 µg/ml). The results showed that the most active extract against MCF-7 cell lines were: brown *Menella* sp. (IC_{50}= 325.45±2.57µg/ml), *S. variablis* (IC_{50}= 361.09±36.41 µg/ml) and *Virgularia* sp. (IC_{50}= 364.79±7.30 µg/ml). The extracts vitro was more active against OVCAR-3 cell line. The results were shown in Table 1.

3.2. Antioxidant Activity Results

The antioxidant activity was measured by DPPH photometric assay. DPPH. radical scavenging activity was quantified in terms of percentage inhibition of a pre-formed free radical by antioxidants in each sample. There was a significant variation in the percentage inhibition of the DPPH$^{\cdot}$ radical by the extracts (3.5523– 90.0532 % inhibition) (Table 2).The White *Menella* sp., brown *Menella* sp., extracts exhibited the highest antioxidant capacity (90.053 % inhibition). Concurrently, *S. polydactyla* extract exhibited the lowest antioxidant capacity (3.552 % inhibition). The results were shown in Table 2.

3.3. Determination of Polyphenol Content Results

The total polyphenol content of each soft coral was quantified using the Folin–Ciocalteu reagent and compared with standard curve of gallic acid (y= 0.0071x+0.324, R2=0.9898). All eight extracts were a significant source of polyphenols; however the total amount varied significantly between extracts (0.647–186.338 mg GAE/L). The *Cavernularia* sp. extracts contained the highest amount of

Table 2. DPPH inhibition of the tested extracts of eight soft corals.

Soft coral	**DPPH (% inhibition)**	**IC_{50} (µg/ml)**
J. juncea	78.153	0.123
Cavernularia sp.	57.904	0.237
Virgularia sp.	69.272	0.173
White *Menella* sp.	90.053	0.056
Brown *Menella* sp.	80.107	0.112
S. polydactyla	3.5524	0.543
S. variablis	34.991	0.366
S. compressa	61.634	0.216

Table 3. Total Polyphenol measurement of the five genus of soft corals by Folin-Ciocalteu assay.

Soft coral	**Phenolic content (mg/l)**
J. juncea	0.64
Cavernularia sp.	186.33
Virgularia sp.	34.30
White *Menella* sp.	109.94
brown *Menella* sp.	3.60
S. polydactyla	47.60
S. variablis	63.32
S. compressa	62.05

polyphenols (186.3380 mg GAE/L) and *J. juncea* extracts contained the lowest amount of polyphenols (0.6478mg GAE/L) (Table 3).

3.4. Disscussion

This research showed that some corals collected from the Persian Gulf were active as antioxidant and contained phenolic compounds. In the cytotoxic assay *S. polydactyla* showed the highest activity against OVCAR-3 cancer cell line with IC_{50}= 260.99±7.93 µg/ml and brown *Menella* sp. exhibited the highest activity against MCF-7 with IC_{50} value of 325.45 ± 2.57µg/ml. This results maybe because of cytotoxic diterpenes like new cembranolide diterpenes that were isolated from hybrid soft coral *Sinularia maxima* × *Sinularia polydactyla* by Haidy Nasr Kamel and coworkers. Cembranolide diterpen has shown strong cytotoxic activity

against the SK-BR3 (breast cancer) cell line and HeLa and HeLa-Apl (cervix cancer) cell lines with GI50 values of 0.039, 0.48, and 0.56 μM, respectively [38]. Menecubebane B a sesquiterpene isolated from *Menella* sp.by Qi Peng and coworkers revealed moderate cytotoxicity against the Eca9706 and HeLa cell lines involved with IC_{50} values 20.8 and 30.6 μM, respectively [39]. The phytochemical constituents of the genus *juncealla* sp. [40], *Cavernularia* sp. [41], *Virgularia* sp. [42], *Menella* sp. [43] and *Sinularia* sp. [44] were evaluated in previous literatures. Although it was the first time that the cytotoxic and antioxidant activity and total phenolic content of soft corals of the Persian Gulf were evaluated. The antioxidant activity was measured by DPPH photometric assay. The use of DPPH* provides an easy and rapid method to evaluate the antiradical antioxidant activity, and Based on the mechanism of reduction of the DPPH molecule extensively described in the literature [45] that is correlated with the presence of hydroxyl groups on the antioxidant molecule, it is estimated that high activity of polar extracts is probably due to the presence of substances with an available hydroxyl group. In this study, *Menella* sp. extract showed the highest antioxidant activity with 90.05 % inhibition and IC_{50}=0.056 μg/ml, indicating that antioxidant or active compounds of different polarity could be present in *Menella* sp. Analyses of the Folin-Ciocalteu type are convenient, simple, require only common equipment, and have produced a large body of comparable data. The assay in fact measures all compounds readily oxidizable under the reaction conditions and its very inclusiveness allows certain substances to also react that are either not phenols or seldom thought of as phenols (e.g., proteins). Finally in this experiment the *Cavernularia* sp. extract contained the highest amount of polyphenols (186.3380 mg GAE/L) and *Menella* sp. showed the highest anti-oxidant activity with IC_{50} value of 0.056μg/ml. There was not any correlation between antioxidant activity and total phenolic content of the soft corals extracts. Totally, extracting solvent remarkably affected total polyphenol content and antioxidant activity of extracts [36].

4. Conclusion

Marine soft corals are a rich source of potent compounds with various pharmacological activity and pharmaceutical potentials. This research showed that soft corals were active as an antioxidant and contained phenolic compounds with low cytotoxic activity. So it can be recommended using solvent with different polarity for extraction and partitioning, and evaluating the active constituents of this species, may provide useful comparative information in the future.

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