ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 7:2 (2013) 96-104

records of natural products

Radical Scavenging Effect of Different Marine Sponges from Mediterranean Coasts

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(Received June 5, 2012; Revised January 17, 2013; Accepted February 11, 2013)

Abstract: To find new products reducing free radical damage is very important research area in recent pharmaceutical investigations. Considering this information, different marine sponges distributed in Mediterranean coasts of Turkey were screened for their antioxidant capacity. Methanolic extracts of eleven species from six different localities were investigated for their 2,2-diphenyl-1-picrylhydrazil (DPPH), nitric oxide (NO) and superoxide (SO) radical scavenging activities. Dose dependent radical scavenging activity was observed and the results were found to be comparable to that of known antioxidative compounds, ascorbic acid, quercetin and BHA. The most significant scavenging activity was determined for the methanolic extracts of *Dysidea avara*, *Axinella cannabina*, *Axinella damicornis*, *Agelas oroides* and *Ircinia fasciculata.* In addition, localities of the sponges were found to be effective for the potency of their activities.

Keywords: Sponges; *Axinella*; *Agelas*; *Ircinia*; *Dysidea;* Radical scavenging effect.

1. Introduction

Different types of oxygen species such as superoxide anion, singlet oxygen and hydroxyl radicals along with peroxides and transition metals have degenerative effects to living cells and DNA in human body. The role of free radicals and reactive oxygen species are becoming increasingly important in the pathogenesis of diabetes, arteriosclerosis, cardiovascular diseases, cancer and several neurodegenerative disorders. Resent investigations have indicated that effective antioxidants are getting increasingly important in disease prevention and therapy [1-3]. Marine organisms are an important source of novel molecules for new drug discovery and drug development researches. Long evolutionary history of marine organisms makes them very diverse in secondary metabolites production. A great number of secondary metabolites from marine organisms have been extensively investigated for their bioactive properties and demonstrated interesting antiinflammatory, cytotoxic, immunomodulating, antimicrobial, antiviral, neurosuppresive and analgesic activities [4-6]. Potential antioxidative activity of different marine organisms were also studied and found to be interesting for

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 3/15/2013 EISSN:1307-6167

research in detail [7,8]. Sponges (phylum *Porifera*) are primitives of the animals that have existed for 700–800 million years. They are the most widespread species with the approximately 15,000 species in marine environments [9]. Sponges are also one of the most interesting taxa of marine organisms because of the large number of the bioactive secondary metabolites, some of which are currently under preclinical trials and many others have been reported to display various types of biological activities *in vitro* [5,10]. Particularly, with regard to sponge species from Mediterranean coasts, a lot of cytotoxic and antioxidant compounds have been isolated. However, bioactivity screening studies on Mediterranean marine invertebrates are very limited and need to be investigated [7,8]. In the present work, methanolic extracts of different sponges from the Mediterranean coast of Turkey have been screened for their radical scavenging activity against DPPH, nitric oxide and superoxide radicals to understand their antioxidative potential.

2. Materials and Methods

2.1. Plant Material

Tested species and their localities are listed in Table 1 and shown in the map 1.

Table 1. Tested species and their localities

Species	Location	Species	Location
Agelas oroides	Ayvalık	*Agelas oroides*	Kemer
Dysidea avara	Ayvalık	*Dysidea avara*	Kemer
Axinella damicornis	Ayvalık	*Ircinia fasciculata*	Kemer
Axinella cannabina	Ayvalık	*Axinella damicornis*	Kemer
Axinella cannabina	Hatay	*Ircinia spinulosa*	Kemer
Axinella damicornis	Hatay	*Ciocalypta carballoi*	Kaş
Ircinia spinulosa	Hatay	*Petrocia ficiformis*	Kaş
Agelas oroides	Hatay	*Sarcotragus sp.*	Turgutreis
Unknown sample	Hatay	*Axinella verrucosa*	Turgutreis
Ircinia fasciculata	Hatay	*Chondrilla nucula*	Güvercinlik

Map 1. Localities of the tested species in Mediterranean coasts

2.2 General

2,2-diphenyl-1-picrylhydrazil (DPPH), nitro blue tetrazolium (NBT), sodium nitroprusside, gallic acid, ascorbic acid were obtained from Sigma-Aldrich Chem Co (St. Louis, MO). 3-*t*-butyl-4-

hydroxyanizole (BHA) was purchased from Nacalai Tesque Co. (Kyoto, Japan). Sulfanilamide and napthylethylenediamine dihydrochloride were obtained from Merck Co. (Darmstadt, Germany).

2.3. Preparation of the extracts

Sponges were collected using scuba between 5 and 50 m depth in the coasts of Hatay, Ayvalık, Kaş, Kemer and Bodrum (Guvercinlik and Turgut Reis) in Mediterranean Region of Turkey (Map 1). Collected materials were preserved in 70% ethanol. After storing, materials were extracted with methanol (3 x 50 mL) at room temperature and extracts were concentrated under reduced pressure. Methanolic extracts were used for the bioactivity experiments.

2.3. DPPH radical scavenging effect

DPPH radical scavenging activity of MeOH extracts was assessed by the decoloration of MeOH solution of DPPH spectroscopically; butyl-4-hydroxyanisole (BHA) and ascorbic acid were used as reference compounds. MeOH solutions (200 μL) of the samples at various concentrations were added to 1 mM DPPH/MeOH solution (50 μL). The reaction mixture was shaken vigorously and the absorbance of remaining DPPH was measured at 520 nm after 30 min. The radical scavenging activity was determined by comparing the absorbance with that of blank (100%) containing only DPPH and solvent. All the analyses were done in 3 replicates [11,12].

2.4. SO radical scavenging effect by alkaline DMSO method

The method of Elizabeth and Rao was used for the detection of superoxide radical scavenging activity of the extract with slight modification. Briefly, a superoxide radical was generated in a nonenzymatic system. The reaction mixture containing 10 μL of NBT (1 mg/mL solution in DMSO) and 30 μL of the extract or reference compounds were dissolved in DMSO. 100 μL of alkaline DMSO (1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) was added to give a final volume of 140 μL and the absorbance was measured at 560 nm using microplate reader [12,13].

2.5. NO scavenging activity

In order to determine the NO radical scavenging activity of extracts, 60 μL of a serial diluted sample were added into a 96-well flat-bottomed plate. Following this, 60 μL of 10 mM sodium nitroprusside, dissolved in phosphate buffered saline (PBS), were added to each well and the plate was incubated under light at room temperature for 150 min. Finally, an equal volume of the Griess reagent (1% sulfanilamide, 0.1% napthylethylenediamine dihydrochloride, 2.5% H_3PO_4) was added to each well in order to measure the nitrite content. After chromophore was formed at room temperature in 10 minutes, absorbance at 577 nm was measured in a microplate reader [12,14,15].

3. Results and Discussion

Free radical mediated cell damage in many different diseases and importance of marine organisms as a source of different secondary metabolites, led us to determine radical scavenging activity of different marine sponges from different localities [16]. The samples were collected from the coasts of Hatay, Ayvalık, Kaş, Kemer and Bodrum (Guvercinlik and Turgut Reis) in Mediterranean Region of Turkey. Radical scavenging effects of eleven different marine sponges from six localities (totally 20 samples) were screened against three different radicals: 2,2-diphenyl-1-picrylhydrazil (DPPH), nitric oxide (NO) and superoxide (SO) radicals. The studied extracts exhibited dose dependent scavenging activity in various strength. Methanolic extracts of *Axinella cannabina* (788 μg/mL) from Ayvalık, *Agelas oroides* (553.7; 545.1 μg/mL) from Kemer and Ayvalık, *Axinella damicornis* (519.5; 738.5 μg/mL) from Kemer and Ayvalık, *Axinella verrucosa* (432.32 μg/mL) from Turgut Reis showed radical scavenging activity against DPPH radical and the strongest activity was

observed for the Kemer sample of *Dysidea avara* with 92.8 μg/mL IC_{50} value (Table 2). Ascorbic acid, quercetin and BHA showed very high radical scavenging activity in lower concentrations (< 50 μg/mL).

NBT assay was carried out to test whether the extracts scavenge superoxide anions or not. In this assay, alkaline DMSO is used as a superoxide generating system, reacts with NBT to give colored diformazan. While all the extracts showed dose dependent SO scavenging activity, their potency was found very low. Methanolic extracts of *Axinella cannabina* (1286.9 μg/mL) from Ayvalık, *Dysidea avara* (34.1 μg/mL), *Ircinia fasciculata* (1675.9 μg/mL) and *Axinella damicornis* (1579.6 μg/mL) from Kemer and *Agelas oroides* (1617.2 μg/mL) from Hatay showed SO scavenging activity (Table 3). Similar to the DPPH results, SO scavenging activity of *Dysidea avara* (34.1 μg/mL) was found comparable to that of standard compounds quercetin (39.9 μg/mL) and ascorbic acid (< 50 μg/mL).

Moreover, NO scavenging effect of the samples was determined using Griess reagent. NO is a small molecule generated in biological system by nitric oxide syntheses. It is an abundant reactive radical that acts as an important biological signaling molecule in a variety of physiological process, such as blood pressure regulation, defense mechanism against pathogens, smooth muscle relaxation and immune regulation. Increased NO concentration may lead to nitrosylation reactions that can alter the structure of protein and inhibit their normal function. When SO reacts with NO, it produces more oxidative and active molecules peroxy nitrite ion (ONOO-), which causes DNA fragmentation and lipid peroxidation [17,18]. In this study, five of the tested extracts showed moderate NO scavenging activity and their IC_{50} values were found as follows (Table 4): 1977.7 μg/mL for *Agelas oroides*, 1759.1 μg/mL for *Dysidea avara*, 1937.9 μg/mL for *Ircinia spinulosa* and 1150 μg/mL for *Axinella damicornis* from Kemer; 1668.9 μg/mL *Axinella cannabina* from Hatay. Only the methanolic extract of *Ciocalypta carbolloi* (700.7 μg/mL) from Kaş showed activity more than that of quercetin (777.88 μg/mL). NO scavenging activities of BHA and ascorbic acid were also found dose dependent but very low in the same conditions with the extracts (> 2 mg/mL).

Concerning DPPH, NO and SO radical scavenging activity of the tested extracts, DPPH radicals were found to be the most scavenged free radicals. While methanolic extract of *Axinella cannabina* from Ayvalık showed both DPPH and SO scavenging activity, *Axinella damicornis* and *Agelas oroides* from Kemer showed both DPPH and NO scavenging activity. In addition, *Ircinia fasciculata* from Kemer scavenged NO and SO radicals significantly. Methanolic extract of *Dysidea avara* from Kemer found the most effective sample for DPPH (92.8 μg/mL) and SO (34.1 μg/mL) radicals with very low IC_{50} values comparable to that of known antioxidants. In addition, samples from Kemer were also found remarkably more effective comparing the other samples. These results indicated that the localities of the sponges are important for the activity of the samples. Effect of the locality may be arisen from the endophytic microorganisms living together with the sponges or the composition of the water around the collection area [19,20]. It is well known that seasonal changes influence various abiotic factors such as temperature, pH, and salinity as well as biotic factors like morphology and epifaunal diversity ultimately responsible for the biosynthesis of sponge's secondary metabolites [21]. In addition, sponges often have associated symbiotic microbial populations. In some cases, these microorganisms and not sponge cells are the likely source of the isolated secondary metabolites. For example, the polybrominated biphenyl ether antibiotics isolated from the sponge *Dysidea herbaceaare* really produced by the endosymbiotic cyanobacterium *Oscillatoria spongeliae* [9]. Concerning our results, tested sponges samples should be investigated from the view point of endophytic microorganisms.

Table 2. DPPH radical scavenging effect of the sponge extracts (inhibition%±S.E.M[a])

µg /mL	Kemer					Ayvalık				Guvercinlik	Turgut Reis	
	Agelas oroides	*Dysidea avara*	*Ircinia fasciculata*	*Axinella damicormis*	*Ircinia spinulosa*	*Agelas oroides*	*Dysidea avara*	*Axinella damicormis*	*Axinella cannabina*	*Chondrilla nucula*	*Sarcotragus sp.*	*Axinella verrucosa*
50	6.7±1.6	38.6±9.1	2.1±0.7	6.9±1.2	-[b]	6.9±2.5	3.3±3.0	4.1±2.5	2.7±1.2	-	-	9.0±3.5
100	8.7±1.7	73.2±1.1	2.3±0.9	11.2±0.6	-	10.1±0.5	3.9±0.9	7.2±0.9	7.7±1.1	-	-	13.6±4.4
200	20.3±0.8	86.2±1.1	6.5±1.7	22.5±0.8	-	23.5±0.8	6.2±0.2	15.5±0.5	17.2±2.3	4.3±0.4	6.6±1.0	32.4±1.2
400	42.6±0.7	89.8±2.0	12.0±0.7	47.3±5.4	5.2±0.4	40.4±1.2	12.1±1.1	30.1±2.2	27.6±2.5	5.6±3.2	10.6±0.7	50.9±0.9
800	73.8±1.8	91.8±0.5	21.8±1.9	70.9±0.4	9.8±0.7	69.9±1.7	20.3±0.2	52.9±1.7	46.9±1.9	12.5±1.5	21.7±0.7	83.9±0.9
IC_{50}[c]	**553.7**	**92.8**	>1000	**519.5**	>1000	**545.1**	>1000	**738.5**	**788**	>1000	>1000	**432.32**

µg /mL	Hatay					Kaş			AA	Quercetin	BHA
	Axinella cannabina	*Axinella damiconis*	*Ircinia spinulosa*	*Agelas oroides*	*Unknown sample*	*Ircinia fasciculata*	*Petrocia ficiformis*	*Ciocallypta carbolloi*			
50	-	5.0±1.2	-	1.5±0.4	2.2±3.1	-	-	-	93.9±0.3	92.8±0.2	86.7±0.3
100	1.3±1.7	6.6±1.2	-	2.3±0.3	3.5±0.6	2.1±1.4	-	-	94.0±0.5	93.2±0.1	91.9±0.8
200	8.2±1.3	9.1±1.3	2.6±0.2	2.6±1.2	4.4±3.7	2.4±0.3	-	-	93.9±0.1	93.3±0.2	92.6±0.6
400	10.9±1.9	18.5±1.3	10.4±6.7	5.0±1.2	10.2±1.7	5.3±2.5	-	1.3±0.7	93.2±0.1	93.2±0.2	94.2±0.2
800	25.1±1.4	32.2±1.8	14.2±1.3	9.3±1.4	16.9±0.4	9.9±1.2	-	3.8±0.4	93.9±0.1	93.3±0.1	94.8±0.2
IC_{50}	>1000	>1000	>1000	>1000	>1000	>1000	-	>1000	<50	<50	<50

[a]n=3, [b]No Activity, [c]µg/mL

Table 3. SO radical scavenging effect of sponge extracts (inhibition%±S.E.M[a])

µg /mL	Kemer					Ayvalık				Turgut Reis		Güvercinlik
	Agelas oroides	*Dysidea avara*	*Ircinia fasciculata*	*Axinella damiconis*	*Ircinia spinulosa*	*Agelas oroides*	*Dysidea avara*	*Axinella damicornis*	*Axinella cannabina*	*Sargotragus sp.*	*Axinella verrucosa*	*Chondrilla nucula*
50	8.9±2.6	52.8±1.7	14.5±1.2	13.1±2.2	-[b]	13.6±3.9	6.8±1.7	8.2±1.1	10.3±1.1	-	-	-
250	15.0±1.7	76.3±3.4	16.1±3.4	14.3±3.1	-	24.7±3.9	9.2±3.4	22.1±1.7	29.3±4.7	-	-	-
500	19.6±2.9	81.0±2.6	19.6±3.1	18.6±5.2	-	27.8±5.4	11.1±2.6	29.6±2.8	37.8±1.2	-	-	-
1000	31.2±1.9	83.1±1.2	31.2±2.3	31.6±4.3	13.2±5.1	30.6±3.9	15.7±5.2	32.4±3.7	46.2±1.6	-	-	-
1400	44.3±1.1	89.1±1.7	44.4±3.8	47.3±5.0	24.5±4.2	32.4±2.8	26.7±1.7	33.5±2.2	47.5±1.1	-	-	-
IC_{50}[c]	>1700	**34.1**	**1675.9**	**1579,6**	>1700	>1700	>1700	>1700	**1286.9**	-	-	-

µg /mL	Hatay					Kaş			Quercetin	BHA	AA
	Axinella cannabina	*Axinella damicornis*	*Ircinia spinulosa*	*Agelas oroides*	*Unknown sample*	*Ircinia fasciculata*	*Ciocalypta carballoi*	*Petrocia ficiformis*			
50	7.3±1.7	8.6±3.6	2.3±3.1	4.5±0.9	1.6±4.5	1.1±4.5	-	1.2±3.7	57.6±1.7	-	-
250	9.2±1.9	7.9±1.2	7.1±4.7	7.2±1.9	6.6±2.1	1.8±1.8	-	1.4±2.8	82.2±2.4	-	73.8±2.5
500	11.0±3.9	16.5±2.0	16.9±1.1	13.7±1.3	18.0±3.5	11.6±3.7	-	3.7±3.9	87.8±1.4	9.5±5.2	86.2±2.1
1000	11.6±1.0	24.7±2.1	17.2±5.8	17.4±1.0	23.3±5.5	12.9±4.2	-	3.9±1.1	90.4±1.0	38.2±4.2	86.5±1.8
1400	20.4±1.0	32.1±.7	18.9±8.5	20.4±6.0	24.8±1.8	16.3±9.2	-	4.9±1.1	90.9±1.1	48.2±2.3	92.1±
IC_{50}	>1700	>1700	>1700	**1617.2**	>1700	>1700	-	>1700	**39.9**	**1420**	<50

[a]n=3, [b]No Activity, [c]µg/m

Table 4. NO radical scavenging effect of sponge extracts (inhibition%±S.E.M[a])

µg /mL	Kemer					Ayvalık				Güvercinlik	Turgut Reis	
	Agelas oroides	*Dysidea avara*	*Ircinia spinulosa*	*Axinella damicornis*	*Ircinia spinulosa*	*Agelas oroides*	*Dysidea avara*	*Axinella damicornis*	*Axinella cannabina*	*Chondrilla nucula*	*Sarcotragus sp.*	*Axinella verrucosa*
50	22.7±1.1	-[b]	23.1±1.5	22.1±1.7	-	3.1±1.4	-	8.3±0.8	8.5±0.9	5.3±0.9	2.9±1.4	5.1±0.9
250	29.0±2.1	8.5±1.9	29.9±2.3	31.5±0.2	-	7.5±0.9	11.7±5.0	17.4±2.7	20.1±2.2	10.7±2.1	10.7±2.4	19.4±1.0
500	31.7±2.1	20.9±1.2	32.4±0.8	38.3±0.4	-	16.3±1.7	14.1±3.8	25.1±0.4	27.2±0.5	21.1±2.3	10.9±1.2	30.2±3.1
1000	37.7±3.8	28.2±0.5	39.6±1.5	48.1±2.3	3.9±3.0	31.5±2.5	32.1±2.5	34.3±0.5	36.9±0.7	32.1±2.0	12.4±2.4	36.1±2.0
1400	41.0±0.5	39.1±2.7	41.3±2.4	52.5±0.8	8.8±3.4	36.5±0.5	35.0±2.2	36.3±0.2	39.0±2.0	35.8±2.0	12.7±0.2	38.2±0.9
IC_{50}[c]	**1977.7**	**1759.1**	**1937.9**	**1150**	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000

µg /mL	Hatay					Kaş			Quercetin	BHA	AA
	Axinella cannabina	*Axinella damicornis*	*Ircinia spinulosa*	*Agelas oroides*	*Unknown sample*	*Ircinia fasciculata*	*Petrocia ficiformis*	*Ciocalypta carbolloi*			
50	23.5±1.1	-	-	5.0±0.9	-	-	5.6±0.7	17.4±2.1	7.2±3.1	-	-
250	28.8±1.1	1.1±0.4	2.0±0.3	12.5±1.9	2.4±1.8	8.6±3.8	9.1±1.2	33.6±0.7	12.8±2.1	13.1±2.5	1.2±1.9
500	34.9±2.4	13.4±4.3	16.2±1.4	19.8±1.6	14.5±3.2	20.3±0.9	12.6±1.4	41.5±0.2	29.8±1.9	22.1±2.7	8.5±2.2
1000	40.5±1.6	33.3±1.2	32.4±0.7	29.7±1.0	31.2±3.3	36.2±2.2	12.7±0.6	71.0±0.6	64.2±0.9	25.9±3.7	24.9±1.8
1400	44.7±1.1	35.5±2.5	36.6±0.5	32.4±0.3	40.5±1.3	38.0±1.9	17.3±1.8	73.5±0.2	91.6±0.5	32.7±3.9	37.5±0.9
IC_{50}	**1668**.9	>2000	>2000	>2000	>2000	>2000	>2000	**700.7**	**777.88**	>2000	>2000

[a]n=3, [b]No Activity, [c]µg/

Turkey has 1542 km coast of Mediterranean Sea. Although biodiversity of Mediterranean Sea is well known, biological and phytochemical studies on species of Mediterranean Sea are very limited. In this study, eleven different marine sponges from six localities (20 samples) were tested for their radical scavenging activity and five samples, *Dysidea avara, Axinella cannabina, Axinella damicornis, Agelas oroides* and *Ircinia fasciculata* were found promising for further researches. This work reveals that Mediterranean Fauna is an interesting source of new antioxidative sponge extracts. In another bioactivity screening study of Mediterranean sponges, extracts of *Agelas oroides* and *Axinella damicornis* from coastal water of Monastir,Tunisia, appeared to be quite promising due to their capacity to inhibit the growth of *Pseudomonas aeruginosa* and gentamycin resistant strains *Listeria monocytogenes* and *Enterococcus feacalisas* [22]. Very resent screening study on marine sponges, showed that *Irsinia spinulosa* and *I. fasciculata* samples of Hatay collections exhibited potent antibacterial properties towards different microorganisms. They also found the methanolic extracts of *Ircinia* and *Dysidea* species displayed promising results in AChE inhibition test over 50% [23]. In addition, the prenylated hydroquinones isolated from the marine sponges *I. fasciculata* from Kemer were found to show cytotoxic and antioxidative activities and inhibit NF-kB signaling in H4IIE hepatoma cells and protein kinases [8]. *Agelas* species have been reported to have bromopyrrol type alkaloids and different type of fatty acids in major amounts. Marine natural products isolated from *Agelas oroides* collected from the island of Gokceada in Aegean Sea exhibited *in vitro* antiplasmodial activity as well as trypanocidal and leishmanicidal activities without any mammalian cells cytotoxicity [24]. In our study, *Dysidea avara* was found the most promising extract for the tested experiments. Previously, secondary metabolites, avarol and avarone, marine sesquiterpen hydroquinones were isolated from *Dysidea avara* with different pharmacological properties including antiinflammatory and antipsoriatic effects. Antipsoriatic properties of avarol attributed to in part due to the down-regulation of several inflammatory molecules, such as TNF-α and NF-κB in psoriatic skin [25-27]. Supporting to the pervious results, further investigations need to clarify mode of action of the active extracts and future studies will focus on the chemical composition of *Dysidea avara*.

Acknowledgments

This work was partially assisted by Ankara University Research Foundation (No: 09B3336005)

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