ANTIMICROBIAL ACTIVITY OF MARINE SAMPLES COLLECTED FROM THE DIFFERENT COASTSOF TURKEY

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Abstract

During our search for bioactive compounds from Turkish marine sources, we have detected antimicrobial activity of some of the extracts that have been prepared from sponges, and tunicate and soft corals from different coasts of Turkey. All sampleswere extracted with methanol. Antimicrobial activity test was conducted against 5 microbial pathogens; (two gram positive bacteria, one yeast and two gram negative bacteria) methicillin-resistant *Staphylococcus aureus*(ATCC 33591), vancomycin-resistant *Enterococcusfaecium* (EF379), *Candida albicans*(ATCC 14035), *Pseudomonas aeruginosa*(ATCC 14210), *Proteus vulgaris*(ATCC 12454), respectively. While crude extracts and filtrated *Axinellaverrucosa* extract showed strong antimicrobial activity, some of the sponge species showed strong or modest activity depending on their localities and concentrations.

Keywords: Marine sponge, tunicate, soft coral, secondary metabolite, antimicrobial activity

Türkiye'nin Farklı Kıyılarından Toplanan Denizel Örneklerin Antimikrobiyal Aktiviteleri

Türkiye denizlerinde bulunan süngerlerdeki bioaktif maddelerle ilgili devam eden çalışmalarımız kapsamında, bu çalışmada değişik kıyılardan toplanan sünger, tunikat ve yumuşak mercan örneklerinin metanollü ekstrelerinin antimikrobiyal etkisi incelenmiştir. Antimikrobiyal çalışma için 5 mikrobiyal patojen iki Gram (+) bakteri,iki Gram (-) bakteri ve bir mantar metisillin dirençli-*Staphylococcusaureus* (ATCC 33591), vankomycin- dirençli *Enterococcus faecium* (EF379), *Candida albicans* (ATCC 14035), *Pseudomonas aeruginosa* (ATCC 14210) ve *Proteusvulgaris* (ATCC 12454) kullanılmıştır. Konsantrasyon ve lokaliteye bağlı olarak sünger örneklerinin kuvvetli veya orta duyarlı antimikrobiyal etki gösterdiği tespit edilmiştir. İncelenen türler içinde *Axinellaverrucosa*'nın hem ham ekstresinde hem de süzülmüş ekstresinde güçlü antimikrobiyal aktivite gösterdiği bulunmuştur.

Anahtarkelimeler: Deniz süngeri, tunikat, yumuşak mercan, *sekonder metabolitler*, antimikrobiyal etki

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Introduction

The ocean which covers almost70 % of the planet's surface provide a huge biodiversity with potential as immeasurable source of natural products. Untilnow more than 17,000 marine secondary metabolites have been described of which sponges are responsible for more than 5300 different products (1). The chemical diversity of sponge metabolites is notable, in addition to the unusual nucleosides, terpenes, sterols, peptides, alkaloids, fatty acids and etc. (2). Several marine natural products have a significant biological activity and many of them are currently, in different phases of clinical trials as drug candidates. In the United States there are three FDA approved marine derived drugs, namely cytarabine, vidarabine and ziconotide. Currently, trabectedin has been approved by the European Agency for Evaluation of medicinal products (EMEA) and is completing key Phase III studies in US for approval (3). Marine sponges are among the richest sources of pharmacologically active compounds from marine organisms. Manyinteresting secondary metabolites have been isolated from various species of sponges including powerful antiviral, antimalarial, antitumor and anti-inflammatory agents (4,5). Screening of marine sponge extracts for antibacterial activity led to the isolation and characterization of a wide range of active compounds, (alkylpiperidine, bromopyrrole and pyrroloiminoquinone alkaloids, sesquiterpene-quinones /- hydroquinones, terpenoids, phenolic compounds, peptidesand proteins, polyketide sand polysaccharides) including some promising therapeutic leads (6,7). The extracts of different sponge species were also found to be active against a wide spectrum of bacterial strains isolated from hospitalized human patients (8). Extensive research has been done to unveil the antimicrobial compounds of spongesincluding different genera such as, *Axinella, Ircinia, Agelas*, *Dysidea*and results are amazingly diverse and productive (9.10,11,12,13).

Screening of organic extracts from marine sponges is a common approach to identify biomedically important compounds. The objective of our study was screening of methanolic extracts of 33 marine samples collected from different coasts of Turkey for antimicrobial activities with the aim of identifying novelcompounds with interesting and potentially useful therapeutic activities.

Materials and methods

Marine Samples

Sponge species (*Ircinia sp., Petrociaficiformis, Dysideaavara, Agelasoroides, Axinellaverrucosa, Aplysinaaerophoba, Chondrillanucula, Agelasoroides, Axinellapolypoides, Axinelladamicornis, Sarcotragusspinulosa, Cicalyptacarballoi, Irciniafasciculata, Chondrosiareniformis*), soft coral (*Eucinellacingularis*) and tunicate (*Aplidiumelegans*) were collected by scuba divers in different coasts of Turkey (Fethiye, Ayvalık, Danaadası, Güvercinlik, İbrice, Kaş, Kemer, Sinekli, Turgutreis) in March 2012, and were identified by Dr. Bülent Gözcelioğlu (one of the authors). Sponge samples, soft coral, and tunicate were deposited at Ankara University, Faculty of Pharmacy, Ankara, Turkey.

Methods

Preparation of the extracts

Fresh sponge samples were chopped intosmall pieces andextracted individually with methanol (3 x 50 mL) for several times at room temperature. The extracts were filtered and evaporated in vacuo until dryness. Methanolic extracts from 33 marine samples were evaluated for their antimicrobial properties. In order to obtain LC/MS data on these samples, an aliquot of each extract was filtrated on C18 Sep-Pack cartridge using MeOH as eluent. Microbroth dilution assays were carried at a concentration of 250 μg/ml using both original crude extracts and C18 fractions against 5 microbial pathogens: two gram positive bacteria methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE), one yeast *Candida albicans* fermented in non-shaking (CA) and shaking conditions (CA_S) and, two Gram negative bacteria *Pseudomonas aeruginosa* (PA) and *Proteus vulgaris* (PV) (14).

Results and Discussion

Strong antimicrobial activities were observed against gram positive bacteria for a few number of crude extracts at a concentration of 250 ug/mL. Moreover, MeOH filtration on C18 Sep–Pack improved the amount of secondary metabolites in the fractions by removing salts and lipid-like molecules and resulted in an increase of the hits rate. Sample species, their localities and % inhibition values of crude extracts and filtrated extracts are shown in Tables 1, 2, 3 by the concentrations of 250 ug/ml and50ug/ml, respectively.

Table 1 Species, localities and % inhibition values of extracts 5X (250ug/ml)
[Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcusfaecium* (VRE),*Candidaalbicans*(CA), *Pseudomonas aeruginosa* (PA), *Proteus vulgaris* (PV)]

	Taxonomy	**Location**	MRSA	VRE	CA	CA_S	PA	PV
1	*Ircinia oros*	Fethiye 1	0	0	7	0	7	0
2	*Axinella verrucosa*	Fethiye 5	**101**	**100**	27	23	26	3
3	*Petrocia ficiformis*	Ayvalık 1	0	0	0	0	0	13
4	*Ircinia oros*	Ayvalık 2	0	0	0	0	0	16
5	*Dysidea avara*	Ayvalık 3	0	0	6	0	6	1
6	*Agelas oroides*	Ayvalık 4	0	0	0	11	0	4
7	*Axinella verrucosa*	Ayvalık 5	0	0	0	15	0	10
8	*Aplysina aerophoba*	Ayvalık 6	0	0	1	0	0	0
9	*Chondrilla nucula*	Ayvalık 10	0	0	3	0	2	0
10	*Agelas oroides*	Ayvalık 13	0	0	10	0	10	10
11	*Axinella polypoides*	Danaadası	0	0	13	4	13	0
12	*Petrocia ficiformis*	Danaadası	0	0	15	30	15	15
13	*Ircinia oros*	Fethiye 1	0	0	13	17	13	14
14	*Petrocia ficiformis*	Fethiye 2	19	0	0	30	1	19
15	*Agelas oroides*	Fethiye 3	0	0	18	6	17	8
16	*Axinella damicornis*	Fethiye 4	0	3	13	32	12	9
17	*Axinella verrucosa*	Fethiye 5	13	0	14	0	13	0
18	*Dysidea avara*	Fethiye 7	10	12	15	0	14	1
19	*Sarcotragus spinulosa*	Fethiye 10	3	0	0	0	0	0
20	*Axinella polypoides*	Fethiye 11	**100**	**96**	**101**	0	**99**	**100**
21	*Ircinia variabilis*	Fethiye 13	0	0	9	0	9	8
22	*Petrocia ficiformis*	Fethiye 14	0	0	12	0	11	9
23	*Petrocia ficiformis*	Fethiye 14	0	0	0	0	0	17
24	*Dictyonella incisa*	Fethiye 15	**81**	0	15	1	15	12
25	*Chondrilla nucula*	Güvercinlik	0	0	19	23	18	0
26	*Dysidea avara*	İbrice 4	0	41	21	23	20	0
27	*Agelas oroides*	Kaş 2	0	0	17	0	17	0
28	*Cicalypta carballoi*	Kaş 5	0	**54**	8	0	7	8
29	*Ircinia fasciculata*	Kemer 3	0	8	0	0	0	0
30	*Cliona viridis*	Sinekli 8	0	0	0	0	0	14
31	*Ircinia variabilis*	Sinekli 9	3	0	9	4	9	15
32	*Agelas oroides*	Turgutreis 17	0	0	16	15	16	10
33	*Chondrosia reniformis*	Turgutreis 23	0	0	17	10	16	41

Table 2 Species, localitiesand % inhibitionvaluesoffiltratedextracts 5X (250ug/ml)

ID	Taxonomy	Location	MRSA	VRE	CA	CA_S	PA*	PV*
1	*Ircinia oros*	Fethiye 1	3	0	0	-	3	7
2	*Axinella verrucosa*	Fethiye 5	6	2	0	-	0	20
3	*Petrocia ficiformis*	Ayvalık 1	0	0	0	-	0	9
4	*Ircinia oros*	Ayvalık 2	11	1	0	-	0	15
5	*Dysidea avara*	Ayvalık 3	0	**70**	0	-	1	11
6	*Agelas oroides*	Ayvalık 4	13	0	0	-	17	13
7	*Axinella verrucosa*	Ayvalık 5	0	0	0	-	9	0
8	*Aplysinaaerophoba*	Ayvalık 6	2	0	0	-	17	0
9	*Chondrilla nucula*	Ayvalık 10	16	0	0	-	0	0
10	*Aplidium elegans*	Ayvalık 11	0	0	0	-	0	0
11	*Eucinella singularis*	Ayvalık 12	0	0	0	-	5	0
12	*Agelas oroides*	Ayvalık 13	0	0	0	-	0	20
13	*Axinella polypoides*	Danaadası (32m)	10	0	0	-	10	0
14	*Petrocia ficiformis*	Danaadası	0	0	0	-	16	0
15	*Ircinia oros*	Fethiye 1	43	0	0	-	9	0
16	*Petrocia ficiformis*	Fethiye 2	0	0	0	-	7	0
17	*Agelas oroides*	Fethiye 3	**98**	**96**	0	-	12	**95**
18	*Axinella damicornis*	Fethiye 4	0	0	0	-	5	0
19	*Axinella verrucosa*	Fethiye 5	**100**	**100**	0	-	5	0
20	*Dysidea avara*	Fethiye 7	**94**	**87**	0	-	28	0
21	*Sarcotragus spinulosa*	Fethiye 10	**61**	0	0	-	0	0
22	*Axinella polypoides*	Fethiye 11	18	0	0	-	22	47
23	*Ircinia variabilis*	Fethiye 13	10	4	0	-	0	4
24	*Petrocia ficiformis*	Fethiye 14	14	10	0	-	19	9
25	*Petrocia ficiformis*	Fethiye 14	0	0	0	-	0	8
26	*Dictyonella incisa*	Fethiye 15	**100**	**95**	0	-	7	0
27	*Chondrilla nucula*	Güvercinlik	3	0	0	-	15	0
28	*Dysidea avara*	İbrice 4	**82**	62	0	-	3	0
29	*Agelas oroides*	Kaş 2	**66**	**91**	0	-	0	0
30	*Ciocalyptacarbolloi*	Kaş 5	41	**75**	0	-	30	0
31	*Ircinia fasciculata*	Kemer 3	**77**	26	0	-	0	0
32	*Agelas oroides*	Turgutreis 17	17	0	0	-	20	0
33	*Chondrosia reniformis*	Turgutreis 23	0	0	0	-	0	**51**

Table 3 Species, localitiesand % inhibitionvaluesoffiltratedextracts 1X (50ug/ml)

ID	Taxonomy	Location	MRSA	VRE	CA	CA_S	PA*	PV*
1	*Ircinia oros*	Fethiye 1	6	17				
2	*Dictyonella incisa*	Fethiye 15	1	11				
3	*Petrocia ficiformis*	Ayvalık 1	7	18				
4	*Ircinia oros*	Ayvalık 2	5	2				
5	*Dysidea avara*	Ayvalık 3	9	2				
6	*Agelas oroides*	Ayvalık 4	0	7				
7	*Axinella verrucosa*	Ayvalık 5	8	7				
8	*Aplysinaaerophoba*	Ayvalık 6	3	0				
9	*Chondrilla nucula*	Ayvalık 10	3	0				
10	*Aplidium elegans*	Ayvalık 11	0	0				
11	*Eucinella singularis*	Ayvalık 12	9	35				
12	*Agelas oroides*	Ayvalık 13	12	12				
13	*Axinella polypoides*	Danaadası (32m)	35	22				
14	*Petrocia ficiformis*	Danaadası	0	19				
15	*Petrocia ficiformis*	Fethiye 2	4	21				
16	*Agelas oroides*	Fethiye 3	4	0				
17	*Axinella damicornis*	Fethiye 4	0	0				
18	*Axinella verrucosa*	Fethiye 5	0	13				
19	*Dysidea avara*	Fethiye 7	4	**96**				
20	*Sarcotragus spinulosa*	Fethiye 10	15	0				
21	*Axinella polypoides*	Fethiye 11	15	36				
22	*Ircinia variabilis*	Fethiye 13	23	23				
23	*Petrocia ficiformis*	Fethiye 14	21	33				
24	*Petrocia ficiformis*	Fethiye 14	9	33				
25	*Dictyonella incisa*	Fethiye 15	0	13				
26	*Chondrilla nucula*	Güvercinlik	10	0				
27	*Dysidea avara*	İbrice 4	0	39				
28	*Agelas oroides*	Kaş 2	1	37				
29	*Ciocalypta carbolloi*	Kaş 5	35	32				
30	*Ircinia fasciculata*	Kemer 3	**59**	5				
31	*Cliona viridis*	Sinekli 8	40	24				
32	*Agelas oroides*	Turgutreis 17	0	30				
33	*Chondrosia reniformis*	Turgutreis 23	27	11				

Tables show that crude extract of *Axinellapolypoides* has strong antimicrobial activity against MRSA (101%), VRE(96%), CA(101%), PA (99 %) and PV (100%). *Axinellaverrucosa* and *Dictyonellaincisa* collected from Fethiye, showed strong antimicrobial activity against MRSA (101% *Axinellaverrucosa*, 81% *Dictyonellaincisa*); *Axinellaverrucosa*showed strong activity against VRE (100%), as well. On the other hand, *Ciocalyptacarbolloi* collected from Kaş showed moderate activity against VRE (54%).

Filtrated extracts (250ug/ml) of *Dysideaavara*fromAyvalık had modest activity on VRE (70%), however same species collected from Fethiye had strong activity both against MRSA (94%) and VRE (87%). While *Agelasoroides* from Fethiye had strong activity against MRSA (98%), VRE

(96%), PV (95%), another *Agelasoroides*speciescollected from Kaş shows strong activity against VRE (91%) and modest activity against MRSA (66%). *Axinellaverrucosa*showed strong activity against MRSA (100%) and VRE (100%). Some other spongespecies,*Sarcotragusspinulosa* and *Dictyonellaincisa*had modest activity against MRSA (61% and 82%, respectively). *Ircinia fasciculate and Chondrosiareniformis*collected from Kemer and Turgutreis demonstrated moderate activity against MRSA (77%) and PV (51%), respectively.

Antimicrobial activity is observed only with 50ug/ml filteredextracts of twospongespecies. *Dysideaavara* from Fethiye hadstrongactivityagainst VRE (96%)and *Irciniafasciculata* from Kemer hadmodestactivity (59%). A number of studies on secondarymetabolites of the genera *Axinella, Dysidea* and *Ircinia* have been reported in the literature (14-21). According to the studies that have been conducted on these sponges, *Axinella* species contain terpenes, alkoloids and cyclopeptides, *Ircinia* genus possesses major metabolites such as linearfuranoterpenes. All these compounds may contribute to antimicrobial activity. Avorol isolated from *Dysideaavara* has antimicrobial properties. Considering secondary metabolites of *Dictyonellaincisa*, only two studiesdescribing the compound has been published (22, 23).

Results indicate that antimicrobial activity varies among sponge species. One of the reasonsfor this variety is the collection localities of the sponges. Same sponge species can show strong, modest or weak activity depending on their localities. Besides, theamounts of extracts play an important role as well. Activity results depend on the concentration of the extracts.

Conclusion

Upto date there have been many papers on chemistry of the genera*Axinella* and *Ircinia* whichconsista part of this study, in contrast to *Dictyonela incisa* that have not been intensively studied yet.Thereforefurther investigation needs to be performed for compound(s) purified from this sponge species in order to elucidate its (their) chemical structure(s) for antimicrobial activity. In conclusion, it is believed that a rich source of antimicrobial drug candidates could be obtained from the secondary metabolites of marine sponges.

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