Egyptian Journal of Biology, 2000, Vol. 2, pp 28-33
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Antimicrobial activity of some sponges from the Gulf of Aqaba

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ABSTRACT

The antibacterial and anticandidal activities of sponges collected from the southern part of the Gulf of Aqaba were investigated. Methanol extracts of ten sponge species were tested a gainst six test microorganisms: Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus;* the Gram-negative bacteria *Escherichia coli* and *Proteus vulgaris* and the yeasts *Candida albicans* and *C. tropicalis.* Three species only named *Acanthella carteri, Ircinia felix* and *Ircinia strobilinia* had broad spectrum antimicrobial activity. On the other hand, growth promotion was stimulated by *Ircinia felix* towards *Escherichia coli.* Investigating different bio-activity of sponges may open new avenues for introducing novel marine compounds into pharmaceutical industry. Also, screening the inhibitory or promoting activities of sponge extracts may reflect the ecological mechanisms of fouling organisms settled on the sponge substratum.

KEYWORDS: Antimicrobial activity, sponges, Gulf of Aqaba, Sinai, Egypt

INTRODUCTION

Sponges, which constitute phylum Porifera, are the most primitive of the multicellular animals having no true organs. Except for hundreds of freshwaters species, 5000 species of sponges live in all seas (Green 1977). Rocks, shells, submerged timbers, corals or even soft sand and mud bottoms provide suitable substratum for sponges (Barnes 1982). They feed on microorganisms such as small plankton and bacteria through pumping mechanism and bear large amounts of symbionts such as bacteria and blue green algae (Wilkinson 1987).

An array of different mechanisms have been selected by sponges as antifouling defenses. This includes special surface structures (e.g. protruding spicules), continuous surface renewal, mucous production, shedding of the epidermis or production of biological active or toxic compounds (Mebs *et al.* 1985; Dyrynda 1986; Davis & wright 1989; Wahl 1989; Bakus *et al.* 1990; Wahl & Banaigs 1991; Turton 1992; Ilan *et al.* 1996). These substances exhibit species-specific toxicity action against a wide range of organisms including microorganisms, invertebrates, vertebrates and algal propagules (Jackson & Buss 1975; Green 1977; Amade *et al.* 1982; Ayling 1983). Also, experimental and circumstantial evidences point to an allelochemical action in substrate competition (Muricy *et al.* 1993), as well as antipredation and antifouling effects (Bakus *et al.* 1986).

Sponges have been considered as valuable and interesting marine source of secondary metabolites. Many biologically active substances have been successively isolated from sponges (Schmitz *et al.* 1984; Fusetani 1987; Kitagawa 1987) especially from demosponges (Bergquist & Bedford 1978). Some antibiotics and anticancer products were extracted from various sponge species (Rotem *et al.* 1983).

The purpose of this study is to investigate the antimicrobial activity of crude extracts of sponges collected from the Gulf of Aqaba against Gram-positive and Gram-negative

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bacteria and yeast. This investigation was done for primarily chemical and pharmacological evaluation of sponges from the Gulf of Aqaba.

MATERIALS AND METHODS

Sampling: Sponges were collected by SCUBA diving using hand and knife for cutting hard-textured tissues from five sites along the southern part of the Gulf of Aqaba (Table 1) during 1998. Sponge specimens were gathered, cleaned from associated biota, placed in plastic, then frozen at −10°C before extraction. Frozen specimens were transported to the laboratory for further investigations.

Extraction: Crude extracts were prepared from ten species belonging to nine genera of coral reef sponges (Table 1). Frozen sponges were macerated by blender and soaked overnight in methanol (20 ml/5g wet weight), then filtered. The filtrate was used for investigating the antimicrobial activity against the test organisms. Filter paper discs (6 mm) were impregnated with 20 µl of the extract and the methanol was allowed to evaporate at room temperature.

Table 1. Test microorganisms used for screening the antimicrobial and anticandidal activities of sponge crude extracts. (NRRL = Northern Regional Research Laboratory).

Microorganism	NRRL strain	Classification
Bacillus subtilis	NRS-744	Gram-positive
Staphylococcus aureus	B-767	Gram-positive
Escherichia coli	B-3704	Gram-negative
Proteus vulgaris	B-123	Gram-negative
Candida albicans	Y-477	Yeast
Candida tropicals		Yeast

Antimicrobial bioassay: Four bacterial strains and two yeasts were used for assaying the antimicrobial activity of sponges The microorganisms listed in Table (2) were obtained from the United States Department of Agriculture, Northern Regional Research Laboratory (NRRL), Peoria, Illinois, USA. An inoculum of each bacterial strain was suspended in 5 ml of Nutrient Broth and incubated overnight at 37°C. Yeast cultures were suspended in 5 ml Sabauraud's Dextrose Broth and incubated for 48-72 hours at 30°C. The cultures were diluted with Broth before use (1:10, volume:volume).

Table 2. Investigated sponge species and their collection sites along the Gulf of Aqaba.

Sponge organism	Collection site	Description	Fouling organisms
Family: Theonellidae *Theonella conica* Kieschnick	Shark Observatory (27° 43' 93" N & 34° 15' 41" E).	massive, cup-shape with narrow central cavity, reddish brown	-
Family:Spirastrellidae *Acervochalina* sp.	Ras Um El Sied (27° 50' 50" N & 34° 10' 53" E).	vase shape, with wide osculum, surface has finger-like projections, brown in color	-
Family: Latrunculiidae *Negombata coraticata* Carter	Ras Um El Sied (27° 50' 50" N & 34° 10' 53" E).	massive robe shape, branched lobate flagelliform, red in color	-
Family: Axinellidae *Acanthella carteri* Schmidt	Ras Nusrani (28° 00' 47" N & 34° 26' 17" E).	fan shape, rough surface with projecting spicules	-
Axinyssa sp	Ras Um El Sied (27° 50' 50" N & 34° 10' 53" E).	orange color encrusting, with scattered oscula, flabellate lamellar growth blue in color	-
Family: Ceratoprellidae	Ras Nusrani	encrusting, with wide	

Grayella cyathophora de Laubenfels	(28° 00' 47" N & 34° 26' 17" E).	oscula, ostia area also clear & distributed creamy in color	-
Family: Biemnidae *Biemna ehrenberga* Keller	Rowaisia (28° 10' 59" N & 34° 26' 28" E).	enlarged with volcano mountains- like fistulas, burrowing into soft sediments, black in color	-
Family: Callyspongidae *Siphonochalina siphonella* Schmidt	Ras Um El Sied (27° 50' 50" N & 34° 10' 53" E).	parallel branching tubes, soft surface, pink in color	-
Family: Thorectidae *Ircinia felix* Lieberkühn	Monqateaa (28° 12' 29" N & 34° 25' 30" E).	enlarged, with clear oscula on surface, color is black	Foraminefera, shrimps, crabs, gastropoda, brittle stars, seaurchins and seaweed
Ircinia strobilina Lamark	Monqateaa (28° 12' 29" N & 34° 25' 30" E).	enlarged, with clear fibers on surface, color is black	Foraminefera, shrimps, crabs, brittle stars and seaweed

The disc diffusion method (Ericsson & Sherris 1971) was used in screening methanol extracts of sponges for antimicrobial activity. Petri dishes seeded with 20 ml of either Nutrient Agar or Sabouraud Agar and 1ml of microbial culture. Discs of the methanol extracts of sponges were been screened for antimicrobial activity. Control discs dipped in either 95% methanol or distilled water was used as controls in each assay.

The experiment was repeated three times. Petri dishes were pre-incubated one hour at 5°C to permit maximum diffusion of the extracts into the medium. Plates were incubated at 30°C for 24-72 hours and the diameter of inhibition zone (mm) was determined.

RESULTS AND DISCUSSION

The antibacterial and anticandidal activity of sponges collected from the southern part of the Gulf of Aqaba were tested against two Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*; two Gram-negative bacteria: *Escherichia coli, Proteus vulgaris* and two yeast: *Candida albicans, C. tropicals.* Five of the tested microorganisms are human pathogenic, including one sensitive Gram-positive bacteria, *Staphylococus aureus.* Thirty percent of the sponges tested showed a broad spectrum antimicrobial activity against five test microorganisms. Those active sponges were *Acanthella carteri, Ircinia strobilinia* and *Ircinia felix* (Table 3). The active species belong to the sponge families Axinellidae, and Thorectidae. Despite the two species *Acanthella carteri* and *Axinyssa* sp. belong to the family Axinellidae, only one species (the former) showed biological activity. Gram-positive bacteria and yeasts were more sensitive to methanol extracts of sponges than Gram-negative bacteria. Strong to moderate activity was observed from methanol extracts of sponge species against both Gram-positive bacteria and yeast, while weak activity was recorded against Gram-negative bacteria.

Antibacterial activity of species from the genera *Acanthella* and *Iricinia* was recorded. Moreover, active compounds produced from *Acanthella Klethra* exhibit cytotoxic and antimalarial activities. Also agglutination and toxicity were produced from the extracts of the sponge species *Acanthella Klethra, Acanthella* sp., *Ircinia campana, I. Microconulosa* and *I. Strobilina* (Burkholder & Ruetzler 1969; Amade *et al.* 1982; McCaffery & Endean 1985).

On the other hand, growth promotion of the bacterial strain *Escherichia coli* was produced from the methanol extract of the sponge *Ircinia felix* (Table 3). Similar results were obtained by Muricy *et al.* (1993) who recorded that 48% of their sponge sample promoted the growth of *E. coli.* In the present study the sponge species *Ircinia felix* is characterized by the presence of high matrix bacteria population in its mesohyl (Ilan *et al.* 1994). However, this

cannot be taken as a rule as there are some sponges, which proved to inhibit the growth of bioassay bacteria, still has mutualistic symbiotic bacteria living in their tissues.

Table 3: The antimicrobial activity of sponge methanol extracts from collected specimens along the Gulf of Aqaba. (-) no zone of inhibition, (+) 1-10 mm zone of inhibition, (++) 11-20 mm zone of inhibition, (+++) >20 mm zone of inhibition, (P) growth promotion.

Sponge organism	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Candida albicans*	*Candida tropicalis*
Family: Theonellidae	-	-	-	-	-	-
Theonella conica						
Family: Spirastrellidae						
Acervochalina sp.	-	-	-	-	-	-
Family: Latrunculiidae	-	-	-	-	-	-
Negombata coraticata						
Family: Axinellidae	++	+++	+	++	++	+++
Acanthella carteri	(12.7± 3.5)	(23.0 ± 2.0)	(9.0 ± 1.0)	(18.0 ± 0.3)	(11.8 ± 6.0)	(23.3 ± 4.0)
Axinyssa sp	-	-	-	-	-	-
Family: Ceratoprellidae	-	-	-	-	-	-
Grayella cyathophora						
Family: Biemnidae	-	-	-	-	-	-
Biemna ehrenberga						
Family: Callyspongidae	-	-	-	-	-	-
Siphonochalina siphonella						
Family:Thorectidae	+++	++	P	++	+++	+++
Ircinia felix	(21.3 ± 2.5)	(18.3 ± 0.7)		(12.5 ± 0.5)	(23.3 ± 4.0)	(20.8 ± 3.3)
Ircinia strobilina	++	++	-	+	++	++
	(11.3 ± 0.5)	(13.8 ± 1.0)		(7.4 ± 0.3)	(15.0 ± 1.5)	(11.0 ± 1.0)
Control						
Methanole	-	-	-	-	-	-
Water	-	-	-	-	-	-

It could be presumed that *in vitro* antibacterial activities from sponge species *Ircinia felix, Ircinia strobilina* and *Acanthella carteri* did not have antifouling effect against some algal species which existed on the studied sponge species. The explanation would be that substances obtained by an unavoidably artificial extraction procedure may never come into contact with epibionts in the field and actually play other roles in the sponge's metabolism (Bergquist & Bedford 1978; McCaffrey & Endean 1985; Bakus *et al.* 1990; Davis *et al.* 1991; Clare *et al.* 1992; Uriz *et al.* 1992).

Kong *et al.* (1994) pointed out that the value of exploring marine natural products as potential sources of pharmaceuticals is strong. Investigating the antimicrobial, antimalarial or cytotoxicity activity of sponges may open new avenues for introducing novel structures and/or biological activity for further pharmacological investigation (McCaffrey & Endean 1985; Kong *et al.* 1994). On the other hand, screening the inhibitory or promoting activity of sponge extracts may support the ecological mechanisms of fouling and antifouling organisms settled or prevented on the sponge substratum.

In conclusion, the broad spectrum activity of the sponge species *Ircinia felix, I. Strobilina* and *Acanthella carteri* from the Gulf of Aqaba may be exploited on pharmaceutical scale. It could be also useful for interpretation of fouling mechanisms of these sponge species.

Acknowledgments

The authors very appreciated for Dr. Ishrak Khafagi, Associate Professor of Biotechnology, Botany Department, Faculty of Sciences, Suez Canal University for her help during this biological assay.

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الملخص العربى

النشاط الضد حيوى لبعض الاسفنجيات في خليج العقبة

على عبد الفتاح جاب الله[١]، رؤوف قلادة[١] ، إسماعيل محرز شلبى[٢] ، تامر حلمى[١]

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تم دراسة النشاط المضاد للبكتيريا والخمائر لمستخلصات الإسفنج المنتشر فى جنوب خليج العقبة حيث درست مستخلصات الكحول المثيلى لعشرة أنواع من الإسفنج ضد سلالتين من البكتريا الموجبة الجرام *باسيلس ستلس*، *ستافيلوكوكس أوريس*، وسلالتين من البكتيريا السالبة الجرام *أيشريشيا كولاى*، *بروتس فولجاريس*، هذا بالإضافة إلى نوعين من الخمائر *كانديدا ألبكانس*، *كانديدا تروبيكالس*.

وقد وجد أن ٣٠% من مستخلصات الإسفنج لها تأثير ضد حيوي واسع المجال ضد البكتيريا والخمائر. وهي لأنواع *أكنثلا كارتيري* و*أريثنيا فيليكس* و*أريثنيا ستروبيلينا*. كما وجد أن نوع *أريثنيا فيليكس* ينتج محفزات نمو لبكتيريا القولون. ويمهد هذا البحث الطريق لمزيد من الدراسات الكيميائية علي هذه الأنواع من الاسفنجيات.