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Antibacterial and DPPH Free Radical-Scavenging Activities of Methanolic Extracts of *Aaptos* sp. (Marine Sponges)

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ABSTRACT

This study reports on the evaluation of the antioxidant and antibacterial activities of twelve methanolic extracts (A-L) of *Aaptos* sp., collected from various locations of Terengganu Islands, namely Pulau Bidong, Pulau Kapas, Pulau Perhentian and Pulau Redang. The antioxidant activity of the twelve specimens was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The antibacterial bioassay against 5 bacteria, i.e. *Bacillus subtilis* (*gram-positive*), *Escherichia coli* (*gram-negative*), *Bacillus proteus, Streptococus agalatea* and *Streptococus fecalis,* was carried out using the disc-diffusion method. In the DPPH method, all extracts exhibited moderate and strong radical scavenging activity when compared to the standards used, i.e. quercetin and butylated hydroxyanisole (BHA) with the inhibition percentage in the range of 55–89%. In particular, specimen H exhibited the strongest radical scavenging activity with IC_{50} value of 0.1mg/ml. On the contrary, all the specimens showed antibacterial activity at least against one test organism. Interestingly, specimens C, G and L, which were collected from Perhentian, Bidong and Kapas Islands respectively, exhibited weak to strong activity against all bacterial strains. Beside that, specimen F (collected off Redang Island) was weakly bactericidal only against *Bacillus proteus*. Meanwhile, specimen G (collected off Bidong island) was primarily selected for further isolation to yield cholestan-3β-ol and aaptamine.

Keywords: DPPH free radical scavenging, antibacterial, *Aaptos* sp., cholestan-3β-ol, aaptamine

INTRODUCTION

The ocean provides a huge resource bank to the discovery of novel compounds. Marine sponges, as one of the most interesting phyla with respect to pharmacological active marine compounds, were investigated widely in the last decade (Blunt *et al.*, 2005). More than 5000 different compounds have been isolated from about 500 species of sponges (Rifai *et al.*, 2005). An extensive study has also been done on the isolation of bioactive compounds from marine sponges worldwide. However, only a few studies have reported on the isolation of chemical compounds from the Malaysian sponges, and these include *Pseudaxinyssa* sp (Fernandez *et al.*, 1992) and *Leucoploeus fenestrata* (Siraj *et al.*, 1988). A few reports have also revealed cytotoxicity and liver metabolizing enzyme activity of the Malaysian sponge extracts (Abas *et al.*, 1999; Habsah *et al.*, 2005a, 2005b). Recently, the isolation of bioactive compound, from marine sponge-derived fungi, has gained a great attention, which resulted in

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the isolation of brefeldin A. mycophenolic acid and cladosporin (Nor Ainy *et al.*, 2005).

Aaptos sp., a marine sponge from the family Suberitidae, has been found as a rich source of 1*H*-benzo[*d*,*e*][1,6]-naphthyridine alkaloid, aaptamine which comprises of α-adrenoceptor blocking activity (Nakamura *et al.*, 1982) and other pharmacological activities including anti-tumour, anti-viral, anti-microbial, and PKC or GFAT enzyme inhibitor (Bobzin *et al.*, 2000; Coutinho *et al.*, 2002). To date, a few aptaminoid analogues, including aaptamine, 9-demethylaaptamine, bisdemethylaaptamine, bisdemethylaaptamine-9-*O*-sulfate, isoaaptamine, aaptosamine, aaptosine, 9-demethyloxyaaptamine and 4-methyloxyaaptamine, have been isolated from this species (Nakamura *et al.*, 1982; Rudi and Kashman, 1993; Herlt *et al.*, 2004). Besides, isoagelaxanthin A, 3-[(13-methylhexadecyl)oxyl-1,2-propanediol and 3-[(15-methyloctadecyl)oxyl-1,2-propanediol were also successfully isolated from this species (DNP on CD-ROM, 1982-2001). Considering the importance of *Aaptos* sp, the researchers also made an attempt to isolate antibacterial compounds.

MATERIALS AND METHODS

Specimen Preparation

The marine sponges, *Aaptos* sp., were collected via SCUBA at a depth of 8 to 15 meters from Bidong, Kapas, Redang and Perhentian Island, Terengganu. Voucher specimens were deposited at the Museum Biodiversity, Institute of Oceanography, Universiti Malaysia Terengganu. Sponges were cleaned, chopped and dried in air-grafted oven (45°C), prior to extraction with methanol. The extracts were filtered and dried under reduced pressure using a rotary evaporator. The dried extracts were de-salted prior to the analysis. The Methanol extract of 12 specimens were subjected to thin layer chromatography (*Fig. 1*).

Thin Layer Chromatography (TLC)

TLC was perform using TLC sheets (Merck 1.05735.0001), which were pre-coated with silica gel GF_{254} of 0.25 mm thickness, with a mobile phase of chloroform-methanol (8:2). Silica gel plates were visualized under UV 365 nm and UV 254 nm without treatment.

Bacteria

For the purpose of antibacterial evaluation, five bacterial strains, i.e. *Bacillus subtilis* (Gram-positive), *Bacillus proteus*, *Escherichia coli* (Gram-negative), *Streptococus agalatea* and *Streptococus fecalis* were cultured in appropriate broths at 30°C for overnight, and their concentrations adjusted to 10^5-10^6 colony forming units (CFU) per ml, using a spectrophotometer (λ 600nm).

Antibacterial Disc Diffusion Method

The agar cultures of the tested micro-organisms were prepared as described by Mackeen *et al.* (1997). 10 mg of extract was loaded onto each Whatman No. 1 filter paper disc (ϕ 6 mm) and placed on inoculated agar for initial screening. The plates were inverted and incubated for 24 h at 30°C. The presence of antimicrobial activity was confirmed by the occurrence of clear inhibition zones around the disc. The assay was carried out in triplicates. The strength of the activity was classified as 'strong' for the inhibition zone having diameters of ≥15.0 mm, 'moderate' (good) for the diameters ranging from 10.0 to 14.5 mm, and weak for the one with diameters < 10 mm.

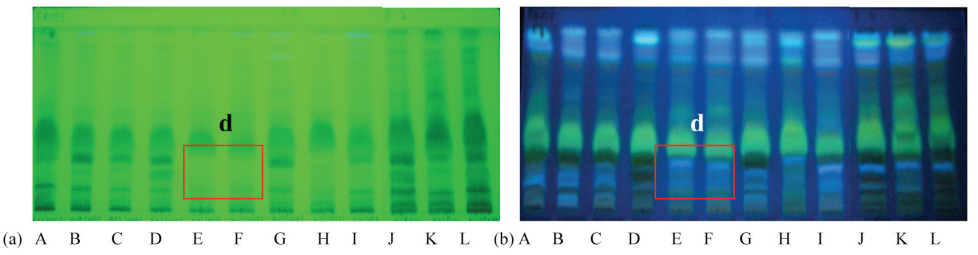

Fig. 1: TLC chromatogram of 12 specimens (band A-L) under (a) 254 nm and (b) UV 365 nm, respectively

DPPH Free Radical Scavenging Assay

The stock solutions of the specimens were prepared at 10 mg/ml in DMSO. The reaction mixture of 50 µl sample was added to 1.95 ml 0.1 mM DPPH solution in a disposable cuvette (Plastibrand® Kartell, 1940). After that, the reaction mixture was shaken and incubated for 30 min at room temperature and the absorbance was read at 517 nm against a blank. The standards used in this assay were butylated hydroxyanisole (BHA) and quercetin. The specimens showing strong activity (D, G, H, J and K) were subjected for further identification to evaluate the IC_{50} values of the different concentrations, using the 96-well micro plate method proposed by Lee *et al.* (1998) with a slight modification. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of methanol. The stock solutions of the specimens (1 mg/ml) were diluted (two fold dilution) in 96-well micro plates to varying concentrations, topping from 500 ug/ml down to the lowest of 7.81 µg/ml. Then, 5 µl of methanolic DPPH solution was added. Each well was shaken before incubation in a dark place at room temperature. After 30 minutes, the absorbance was read at 517 nm. The assay was carried out in triplicates and calculated using the following formula:

$$\text{Scavenging effect (\%)} = [A_{\text{Blank (517nm)}} - A_{\text{Sample (517nm)}} / A_{\text{Blank(517nm)}}] \times 100$$

A=Absorbance

*Isolation of **1** and **2***

Methanolic extract of B01/010/04 was primarily selected for further isolation because of its significant radical scavenging and antibacterial activities. The extract (150 g) was fractionated by silica gel 60 (0.063-0.200 mm) (70-230 mesh ASTM Merck) gravity chromatography, employing a gradient (0-100% of hexane in chloroform and then from 0-100% chloroform in methanol). Based on their TLC profiles, the fractions were combined to yield 14 fractions. The active fraction 3 (0.5 g) was further purified, using silica gel 230-400 mesh ASTM Merck (0 to 100% hexane in chloroform), to yield **1** (12.9 mg). Fraction 11 (5 g) which was then further purified by silica gel 230-400 mesh ASTM Merck (0 to 100% chloroform in methanol) and to give **2** (250 mg).

*Cholestan-3β-ol (**1**)*

White powder ($CHCl_3$), 12.9 mg: m.p. 128-129°C; $C_{27}H_{48}O$; EIMS (+) ion mode *m/z*: 388 $[M]^+$, 373 $[M + CH_3]^+$, 264, 233, 215, 201, 147; IR (KBr) V_{max}: 3400, 2930, 2850, 1657, 1467, 1375, 1331, 1170, 1137, 1078, 1039 cm^{-1} (for ^1H and ^{13}C NMR data, *see* Table 1) (Gauvin *et al.*, 1998; Dzeha *et al.*, 2002; Santalova *et al.*, 2004).

Aaptamine (***2***)

Greenish yellow crystal ($CHCl_3$), 250 mg: m.p. 111-112°C; $C_{13}H_{12}N_2O_2$; EIMS (+) ion mode *m/z*: 228 $[M]^+$, 213, 183, 170, 142; IR (KBr) *v*max: 3450, 1633, 1325, 1248, 1111, 1026, 777 cm^{-1} (for ^1H and ^{13}C NMR data, *see* Table 1) (Nakamura *et al.*, 1982; Herlt *et al.*, 2004).

RESULTS AND DISCUSSIONS

It is a known fact that sponges contain bioactive compounds which are of potential medical importance (Thakur and Muller, 2004). In this research, the results of the preliminary studies on *Aaptos* sp. were reported for the presence of antibacterial and DPPH free radical scavenging activities. *In vitro,* the antibacterial screening of twelve methanolic extracts of *Aaptos* sp.(A-L) demonstrated activity against one or more bacteria, tested with less activity than standards (gentamycin, streptomycin and penicillin), as shown in Table 2. Specimens J, K and L showed an equally strong activity against *Streptococus fecalis,* whereas specimens H and B exhibited a strong activity against *Bacillus subtilis* and *Streptococus agalatea,* respectively. Considerable antibacterial activity was also shown by few samples against certain bacteria; specimens A, B, D and L against *Bacillus subtilis*; specimens B, C, D, E, H and I against *Streptococus fecalis*; A, D, E, I, K and L against *Streptococus agalatea.* Specimen C displayed a strong activity against *Bacillus proteus*, while other extracts showed only weak activity. Meanwhile, specimens C, G and L showed a weak activity whereas all the remaining extracts were inactive against *Escherichia coli.* In conclusion, specimens C, G and L (collected off Perhentian, Bidong and Kapas islands) showed mostly strong activity against all bacteria and in contrary to this, specimen F (collected off Redang island) was weakly bactericidal against only one bacterium, i.e. *Bacillus proteus.*

TABLE 1
^{1}H and ^{13}C NMR assignments for cholestan-3β-ol (**1**) and aaptamine (**2**)

Position		A1			A2	
C#	δH (J_{HH})	δC	HMBC	δH (J_{HH})	δC	HMBC
1		37.22	C1/H19			
				7.15 *d*	142.49	H2/C4,C12,C13
2		35.7	C2/H19	(6.0)		
				6.27 *d*	99.08	H3/C2,C13
3	3.59 m	71.6		(6.0)		
4		45.07			151.52	
5		54.57	C5/H19			
				7.69 *d*	130.21	H6/C3,C4,C8
6		21.47		(6.0)		
				6.77 *d*	102.25	H7/C6,C13
7		24.43		(6.0)		
8		31.75			135.10	
9		56.49		6.92 *s*	117.90	H9/C10,C11,C13,
10		32.3			158.77	
10’				3.97 *s*	57.06	H10’/C10,C11’
11		19.82			133.27	
11’				3.85 *s*	61.22	H11’/C11,C10’
			C12/H18		134.16	
12		40.26	C17,C14			
13		36.38			114.37	C13/H7,H9
14		42.81	C14/C12,C17,H18			
15		24.05				
16		28.47				
17		56.71	C17/H21			
18	0.65 *s*	12.29	H18/C12,C14,C17			
19	0.81 *s*	12.54	H19/C1,C5,C9			
20		38.44				
	0.90 *d*					
21	(6.6)	18.88				
22		36.01	C22/H21			
23		28.23	C23/H26			
24		39.73	C24/H26			
25		28.95	C25/H26			
	0.87 *d*					
26	(6.6)	22.78	H26/C27			
	0.86 *d*					
27	(6.6)	23.04	H27/C26			

All spectra in $CDCl_3$, ^{1}H at 400 MHz, ^{13}C at 400 MHz; assignments by ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, and HMBC experiments acquired on a Varian-Unity INOVA spectrometer.

TABLE 2
Antibacterial activity of methanol extracts of *Aaptos* sp. collected from various locations

Code	Specimens	Bacteria species*				
		B.sub	B.Pro	S.fea	S.aga	E.coli
P01/011/04	A	+	+	-	+	-
P02/010/04	B	+	+	+	++	-
P02/009/04	C	++	++	+	++	+
P03/015/04	D	+	+	+	+	-
R01/010/04	E	-	+	+	+	-
R03/007/04	F	-	+	-	-	-
B01/010/04	G	++	+	++	++	+
K01/025/04	H	++	+	+	-	-
K01/028/04	I	-	+	+	+	-
K01/010/05	J	-	+	++	++	-
K02/011/05	K	-	+	++	+	-
K03/010/05	L	+	+	++	+	+
	Gentamycin	39	15	19	21	19
Control	Penicillin	-	-	18	-	-
	Streptomycin	20	20	20	19	20

Note: B - Bidong Island; R - Redang Island; P - Perhentian Island; K - Kapas Island
* B.sub: *Bacillus subtilis*; B.pro: *Bacillus proteus*; S.aga: *Streptococus agalatea*; S.fea: *Streptococus* sp.; E.coli: *Escherichia coli* * (–) No activity, (+) weak activity (7–10-mm halo), (++) good activity (10–15-mm halo)

Twelve crude extracts of *Aaptos* sp. (A-L), from different localities, were assayed for antioxidant activity using DPPH free radical scavenging assay (Table 3). Five specimens (D, G, H, J and K) exhibited a strong free radical scavenging although they were less active as compared to butylated hydroxyanisole (BHA) and quercetin with the inhibition percentages in the range of 79-89%. The remaining extracts showed only moderate to weak activity, with the inhibition percentages in the range between 55-78%, with the weakest activity detected for the specimens collected from Redang Island; E and F (inhibition percentage of 55.37% and 58.89%, respectively). The five specimens, which showed strong activity, were further analyzed to determine the concentration values for their 50% inhibition of DPPH free radical scavenging activity (IC_{50}), using different concentrations (2-fold dilution) topping from 7.81 to 500 µg/ml. The IC_{50} value of specimens D, G, H, J and K ranged from 0.1 to 0.12 mg/ml. *In vitro* screening of antioxidant and antibacterial activity of 12 methanolic extract of *Aaptos* sp. showed that the specimen collected off Bidong Island (G) displayed potential significant activity. Unlike other specimens, which somehow exhibited moderate activity in both assays, specimens E and F had weak activities. According to the TLC profiling of all 12 specimens (*Fig. 1*), the bands in area d were absent in the TLC profile of E and F. These could justify the low DPPH free radical scavenging activity of specimens E and F.

Cholestan-3β-ol (**1**) was purified from the hexane fraction and its structure was confirmed by comparing the spectral data with the literature values. The EIMS spectrum showed the molecular ion peak at m/z 388.2, indicating the molecular formula as $C_{27}H_{48}O$ (Gauvin *et al.*, 1998). The other fragment ions were at *m/z* 373 [M-CH_3] (28), 233 ($C_{16}H_{25}O^+$) (63), 215 ($C_{15}H_{19}O^+$) (100), 147($C_{11}H_{15}^{\bullet}$) (30) and ($C_5H_7^+$) (32). The interpretation of the ^1H and ^{13}C NMR (Table 1) is in agreement with the data in the literature (Dzeha *et al.*, 2002; Santalova *et al.*, 2004).

TABLE 3
Free radical scavenging activity (%) of methanol extract of *Aaptos* sp. collected from various locations

Code	Specimen	Free radical scavenging activity (%)	IC_{50} (mg/ml)
P01/011/04	A	73.84 ± 1.0	NT
P02/010/04	B	76.32 ± 0.3	NT
P02/009/04	C	78.69 ± 1.9	NT
P03/015/04	D	80.51 ± 0.5	0.13
R01/010/04	E	55.37 ± 0.1	NT
R03/007/04	F	58.89 ± 1.3	NT
B01/010/04	G	78.80 ± 0.5	0.12
K01/025/04	H	89.28 ± 0.7	0.11
K01/028/04	I	78.57 ± 0.8	NT
K01/010/05	J	81.57 ± 0.7	0.26
K02/011/05	K	81.05 ± 2.3	0.12
K03/010/05	L	72.21 ± 0.9	NT
Standard	BHA	94.38 ± 0.6	0.04
Standard	Quercetin	94.15 ± 0.6	0.04

NT-not tested

The structure of aaptamine (**2**) was determined by interpreting the data of 1D and 2D-NMR, and it was also in agreement with the literature value (Nakamura *et al.*, 1982; Herlt, 2004). The EIMS spectrum of **2** showed that the molecular formula as $C_{13}H_{12}N_2O_2$ with molecular weight 228 $[M]^+$ (46). Other fragments ion are 213 (100), 183 (23), 170 (36), 142 (13). The ^{1}HNMR spectrum showed the presence of two methoxy group at δ 3.97 (3H, s, 8-OCH_3) and 3.85 (3H, s, 9-OCH_3). In the aromatic region δ 6 – 8 ppm, 5 peaks integrated a proton each was observed, which were at δ 6.27 (J = 6.81 *Hz*, H-3) and δ 7.68 (J = 6.87 Hz, H-2), δ 7.15 (J = 7.56 Hz, H-5) and 6.77 (J = 6.78 Hz, H-6). The assignment of carbons and protons of **2** and the HMBC correlation is given in Table 1. These biological activities, in all the samples, might be contributed by aaptamine and demethyloxyaaptamine (DNP on CD-ROM, 1982-2001).

CONCLUSIONS

In conclusion, to the best of the researchers' knowledge, Bidong Island is a suitable location for the collection of sample in search of bioactive constituent from *Aaptos sp.* From the results gathered from the *in vitro* screening, specimen G was found to yield a cholestanol compound known as cholestan-3β-ol (**1**) and an alkaloid called aaptamine (**2**). The isolation of the other bioactive compounds is in progress.

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REFERENCES

Abas, H.H., Zulfigar, Y. and Chan, K.L. (1999). Cytotoxicity and drug metabolism screening of several marine sponges from Pulau Pasir, Kedah and Pulau Aur, Johor. *Asean Review of Biodiversity and Environmental Conservation, (ARBEC)*, 1-5.

Blunt, J., Copp, B.R., Munro, M.H.G., Northcote, P. T. and Prinsep, M.R. (2005). Marine natural product. *Natural Product Report, 22*, 15-61.

Bobzin, S.C., Yang, S. and Kasten, P. (2000). Application of liquid chromatography-nuclear magnetic resonance spectroscopy to the identification of natural products. *Journal of Chromatography B*, 748, 259-267.

Coutinho, A.F., Chanas, B., E. Souza, T.M.L., Frugrulhetti, I.C.P.P. and Epifanio, R.D.A. (2002). Anti HSV-1 alkaloids from a feeding deterrent marine sponge of the genus *Aaptos. Heterocycles, 57*, 1265-1272.

Dictionary of Natural Product (DNP) on CD-ROM, Version 10:1. (1982-2001). Chapman& Hall/ CRC.

Dzeha, T., Jaspars, M. and Tabadravu, J. (2003). Chionasterol, a triterpenoid from the Kenyan marine green macroalga *Halimeda macroloba. Western Indian Ocean J. Mar. Sci., 2*, 157-161.

Fernandez, R., Siraj, O., Feliz, M., Quinoa, E. and Riguera, R. (1992). Malaysiatin, the first cyclic heptapeptide from a marine sponge. *Tetrahedron Letters, 33*, 6017-6020.

Gauvin, A., Smadja, J., Aknin, M. and Gaydou, E. M. (1998). Cylopropane-containing sterols in the marine sponge *Petrosia spheroida. Comparative Biochemistry and Physiology, Part B 121,* 451-456.

Habsah, M., Zalilawati, M.R., Nurhana, F., Khamsah S.M., Jasnizat, S., Ali, A.M., Lajis M.N., Faizah S-Harisson and Mohamad H. Z. (2005a). Cytotoxic activity of extracts of *Aaptos* spp. (marine sponges) against HL60 and MCF-7 cell lines (pp. 313-316). In Ibrahim Jantan, Jamia Azdina Jamal, Juriyati Jalil, Nor Fadillah Rajab, Hidayatul Fathi Othman & Jacintha Santhanam (Eds.), 21st *Annual Seminar of Malaysian Natural Product Society.*

Habsah, M., Wan Ainur, N.W.A.J., Khamsah S.M., Nurhana, F., Ali, A.M., Lajis M.N. and Mohamad H.Z. (2005b). Cytotoxic activity of the Methanol extracts of some marine sponges (*Halichondria* sp.) (p.289-294). In Ibrahim Jantan, Jamia Azdina Jamal, Juriyati Jalil, Nor Fadillah Rajab, Hidayatul Fathi Othman and Jacintha Santhanam (Eds.), 21st *Annual Seminar of Malaysian Natural Product Society.*

Herlt, A., Mander, L., Rombang, W., Rumampuk, R., Soemitro, S., Steglich, W., Tarigan, P. and Nussbaum, F.V. (2004). Alkaloids from marine organisms. Part 8: Isolation of bisdemethylaaptamine and bisdemethylaaptamine-9-O-sulfate from an Indonesian *Aaptos* sp. marine sponge. *Tetrahedron, 60*, 6101–6104.

Lee, S.K., Mbwambo, Z.H., Chung, H.S., Luyengi, L., Gamez, E.J.C., Mehta, R.G., Kinghorn, A.D. and Pezzuto J.M. (1998). Evaluation of the antioxidant potential of natural products. *Combinatorial Chemistry & High Throughput Screening, 1,* 35-46.

Mackeen, M.M., Ali, A.M., El-Sharkawy, S.H., Salleh, K.M., Lajis, N.H. and Kawazu, K., (1997). Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables. *International Journal of Pharmacognosy, 35*, 174–178.

Nakamura, H., Kobayashi, J. and Ohizumi, Y. (1982). Isolation and structure of aaptamine a novel heteroaromatic substance possessing α-blocking activity from the sea sponge *Aaptos aaptos. Tetrahedron Letters, 23*, 5555-5558.

Nor Ainy, M., Cole, A., Munro, M., Blunt, J., Cummings, N., Lang, G. and Mitova, M. (2005). Marine-invertebrate-derived fungi and their bioactive compounds (pp.35-47). In Ibrahim Jantan, Jamia Azdina Jamal, Juriyati Jalil, Nor Fadillah Rajab, Hidayatul Fathi Othman and Jacintha Santhanam (Eds.), 21st *Annual Seminar of Malaysian Natural Product Society.*

Santalova, E.A., Makarieva, T.N., Gorshkova, I.A., Dmitrenok, A.S., Krasokhin, V.B. and Stonik, V.A. (2004). Sterols from six marine sponges. *Biochemical Systematics and Ecology, 32*, 153-167.

Siraj, O., Tenenbaum, L.V., Manes, L. and Crews, P. (1988). Novel marine sponge derived amino acids 7. The fenestins. *Tetrahedron Letters, 29,* 5489-5492.

RUDY, A. and KASHMAN, Y. (1993). Aaptosine-a new cytotoxic 5,8-diazabenz[cd]azulene alkaloid from the Red Sea sponge *Aaptos aaptos. Tetrahedron Letters, 34*, 4683-4684.

RIFAI S.A., FASSOUANE, A., EL-ABBOUYI, A. WARDANI, A. KIJJOA and VAN SOEST, R. (2004). Screening of antimicrobial activity of marine sponges extracts. *Medical Mycology, 15*, 33-38.

THAKUR, N.L. and MULLER, W.E.G. (2004). Biotechnological potential of marine sponges. *Review Article Current Science, 86*, 1506-1512.