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Comparison of chemical compounds associated with sclerites from healthy and diseased sea fan corals (*Gorgonia ventalina*)

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Background: The roles of gorgonian sclerites as structural components and predator deterrents have been widely studied. Yet, their role as barriers against microbes has only recently been investigated, and even less is known about the diversity and roles of the chemical compounds and mineral composition associated with the sclerites. **Methods:** Here, we examine the volatile organic compound fraction (VOCs) associated with sclerites from healthy and diseased *Gorgonia ventalina* sea fan corals to understand their possible role as a stress response or in defense of infection. We identified those compounds that are present in sclerites, measured the oxidative potential of these compounds, and analyzed the mineral composition sclerites from diseased and healthy *G. ventalina* colonies. **Results:** The results showed that sclerites harbor a great diversity of VOCs. Overall, 70 compounds were identified, the majority of which are novel with unknown biological roles. The majority of VOCs identified exhibit multiple immune-related roles including antimicrobial and radical scavenging functions. The free radical activity assays further confirmed the anti-oxidative potential of some these compounds. The anti-oxidative activity was, nonetheless, similar across sclerites regardless of the health condition of the colony, although sclerites from diseased sea fans display slightly higher anti-oxidative activity than the healthy ones. No differences in the mineral composition were detected regardless of the health state of the corals **Discussion:** Sclerites harbor great VOCs diversity, the majority of which are novel to sea fans or any other corals. Yet roles of the compounds vary from anti-oxidant to antimicrobial compounds. On the other hand, the fact that the mineral composition did not different across type of sclerites suggesting that the health state of the corals have intangible effects on the mineralization of the sclerites.

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ABSTRACT

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INTRODUTION

Sea fans corals (*Gorgonia* sp.) are one of the top competitors of the Caribbean coral reefs ecosystems. Similar to their closer-relatives, the scleractinian corals, sea fans have suffered several environmental disturbances such as aspergillosis disease outbreaks that seriously

compromised the viability of many populations across the Caribbean (Kim et al., 2004). Yet, in contrast to the scleractinans, sea fans have overcome these disturbances and continue to thrive. In fact, gorgonians are the dominant corals in many reefs formerly dominated by scleractinian corals. Their relative success is provided in part, by their strong immune defenses, which have allowed gorgonians to maintain their internal homoeostasis and health (Sabat & Toledo-Hernández 2015).

Gorgonians have a rather diverse repertory of defensive mechanisms to cope and respond to disturbances, both abiotic and biotic (Toledo-Hernández & Ruiz-Diaz 2014). For instance, to respond to pathogen infection, gorgonians are equipped with chemical pathways such as the melanin cascade that prevent or reduce the pathogen dissemination throughout the host tissue (Petes et al., 2003; Mydlarz et al., 2008). Concomitantly with this response, gorgonians have the ability to activate the production of peroxidase enzymes, which induce the production of reactive oxygen species with cytotoxic roles and cellular signaling (Mydlarz & Harvell 2007). Furthermore, gorgonians are also characterized for their diverse production of secondary metabolites, many of which are thought to be immune-related compounds due to their anti predation and microbial properties (Harvell, Fenica & Green 1988; Kim 1994; Jense et al., 1996; Smith et al., 1996; Geiser et al., 1998; Kim et al., 2000a, 2000b, Alker et al., 2004).

Gorgonians also have sclerites, which are skeletal elements mainly composed of calcium carbonate surrounding an organic matrix made up primarily of glycoproteins, spread throughout the gorgonians' epidermis, mesoglea, and axial skeleton (Kingsely & Atatbe 1982, 1984; Harvell & Suchanek 1987, Van Alstyne & Paul 1992). These sclerites are thought to have multiple defensive roles. For instance, sclerites are provide structural support to gorgonians by reducing the elasticity and stiffness of the axial skeleton against water motion (Koehl 1982; Lewis & Willis, 1991). In addition, sclerites play a role in deterring predation from gastropods and reef fishes (Harvell, Fenical & Greene 1988; Van Alstyne & Paul 1992; Koh et al., 2000). Sclerites have also been suggested to confer protection against pathogens through a process known as tissue purpling (i.e., darkening sclerites through the accumulation of pigments; Leverette et al., 2008). In fact, a recent study showed that sclerites act as a physical and chemical barrier against microbial infections (Toledo-Hernández et al., 2016). Overall, these findings raise questions

about the immunological role of chemical compounds associated with sclerites: are immune-related compounds such as antimicrobials and antioxidants associated with sclerites and, do they increase in response to infection? Gorgonians react to a pathogen infection by increasing the synthesis of immune-related compounds (Alker, et al., 2004). However, it is unknown if these compounds are associated with the sclerites, or if the mineral fraction of the sclerites is impacted by the infection or the immune response of the sea fan (i.e., the role in immunity is detrimental to the structural role of sclerites, or vice versa). Here we examine the volatile organic compound fraction (hereafter VOCs) associated with sclerites from diseased and healthy *G. ventalina* colonies to identify compounds that may have an immunological role. In addition, we also perform radical scavenging activity assays to examine the oxidative potential of chemicals associated with sclerites from diseased and healthy colonies. Finally, we measured the mineral composition of sclerites from diseased and healthy sea fans to better understand the implications of sclerites having multiple functional roles that may be antagonistic when exposed to stress, such as a microbial infection.

METHODS

Tissue collection and sclerites isolation

Sclerites used for this study were isolated from ten healthy and ten diseased *Gorgonia ventalina* colonies located at a depth of 1.5-2.0 m from El Escambron beach, San Juan, Puerto Rico (18º28'00''N, 66º05'12''W). The tissue samples were collected under permit 2012-IC-086 issued to Claudia P. Ruiz Diaz, University of Puerto Rico (UPR) Rio Piedras campus, given by the Puerto Rico Department of Natural Resources, Commonwealth of Puerto Rico. Healthy colonies showed neither lesion nor tissue purpling, whereas diseased colonies showed at least one lesion usually overgrown by algae and surrounded by a ring of purple tissue (Nagelkerken et al., 1997). One tissue fragment approximately 4 cm^2 was cut from the edge of each healthy fan (hereafter HH). In diseased fans, 2 cm^2 samples were cut from both healthy tissue at the edge of the fan (healthy-diseased or HD) as well as from the diseased area (diseased-diseased or DD). Fragments were placed individually in 50 mL centrifuge tubes and brought to the laboratory on ice. Once in the laboratory, each fragment was further cut into smaller fragments of 2-3 cm^2 in size and placed in 20.0 mL-sterilized leveled vials filled with 16.0 mL of distilled water. Then, to disassociate sclerites from the soft tissue matrix, vials were vortexed at room temperature,

sonicated for 5 minutes, and then allowed to settle for approximately 4 hours. This procedure was repeated over a period of 5-7 days to minimize impacts on the structure of the sclerites. After disassociated, sclerites were rinsed several times with distilled water, individually transferred to clean 55 mm Pyrex petri dishes, and dried at 35 ^{o}C for 72 hrs (Figure 1).

Elemental components of sclerites

The relative proportion of element components of sclerites from and HH, HD and DD was determined by energy dispersive X-ray fluorescence (EDXRF) spectroscopy. To accomplish this, a randomly selected sub-set from each sclerite sample was coated in gold for 15 seconds. Then, each sample was irradiated with an electron beam of 20 kv on a JEOL 6480LV scanning electron microscope (SEM) under vacuum conditions.

Gas chromatography-mass spectrometer analysis

From each dried sclerite sample, 0.10 g were ground in a porcelain mortar and the homogenized powder was transferred to a 1.5 mL micro-test tube with 1 mL of acetone. Each tube was vortexed and centrifuged at 6,000 rpm for 5 min. The resulting extract for each sample was filtered and transferred to 1.5 mL vials (32 x12 mm) for splitless injection into an Agilent 7890A gas chromatograph (GC) coupled to an Agilient 5975 C mass-selective detector. The injection temperature was 250 °C. Separation of samples was performed using a fused silica capillary column (SLB®-5 ms 30 m × 0.32 mm × 0.25 µm film thickness; Supelco, Bellefonte, PA). The initial column temperature was held at 70 °C for 1 min, then increased by 8 °C min^{-1} until reaching 270 °C, where it was held for 3 minutes, for a total run time of 29 minutes per sample. Ultrapure helium at constant-flow of 1.2 mL min^{1} was used as the carrier gas. Analysis of the generated chromatograms was performed using the AMDIS version 2.7/NIST version 2.0 computer program and the criteria for selection in the analysis for the screening compounds were: (1) R- match >800; (2) S/N > 15; and (3) area >1000. Compound identification was confirmed through the use of appropriated standards and retention time index (http://www.nist.gov/srd/nist1a.cfm). To determine the potential biological roles of each of the detected compounds, a scientific literature review using the IUPAC names of each compound was conducted.

Free radical scavenging assay

Free radical scavenging activity of sclerite extracts was estimated using 1,1,-diphenyl-2-picrylhydrazyl (DPPH). Briefly, 0.10 g of homogenized powder from each of the sclerite samples was placed into labeled and clean 50 mL centrifuge tubes filled with 5.0 mL methanol. Then, each tube was vortexed for 10 sec, placed in a water bath at 40 °C for 40 min, and sonicated afterward for 30 min. The samples were then centrifuged for 10 min (6,000 rpm) at 25 °C. Afterwards, 1.0 mL of the supernatant was transferred to a clean 50 mL centrifugal tube containing 5.0 mL methanol. Then, 39 µL of DPPH (0.09 mM) and 1 µL of each sclerite extract were added to a 96-well microplate and the absorbance of the each sample was recorded at 0, 1, 4 and 5 minutes and then every 5 minutes for a one-hour period at 560 nm in a Thermo Scientific Multiscan FC spectrophotometer. Scavenging activity in this study was expressed as IC_{50}, which represents the concentration of extracts (mg/mL) needed to inhibit 50% of the DPPH radicals. To calculate IC_{50} for each sclerite sample, the percent inhibition of radical production (%I) was estimated using the following equation (Mishra et al., 2012):

$$\%I = (A_i - A_f) \cdot 100 / A_i \,,$$

where A_i and A_f represent the initial and final absorbance, respectively. A linear regression analysis was performed with extract concentration as the independent variable and %I as the dependent variable. Finally, the resulting linear regression equations with a correlation coefficient (R^2) greater than 80% were used to estimate the IC_{50}.

Statistical analyses

A one-way ANOVA analysis was conducted to detect significant differences between elements, including the Ca/Mg ratio, and sclerite type (HH, HD, and DD). In this analysis, the relative proportion of elements (in percent weight) was assigned as the dependent variable, while sclerite type was assigned as the independent variable. For the analysis of free radical scavenging, a one-way ANOVA analysis was performed to detect for statistical differences between the IC_{50} index and the sclerite types, with the IC_{50} index assigned as the dependent variable andsclerite type assigned as the independent variable In all analyses, P-value was fixed at 0.05. Statistical analyses were performed using R version 3.2 (R Core Team 2015).

RESULTS

Elemental components of sclerites Ten different elements, namely O_2, Ca, C, Mg, S, Al, N, Si, and Cl were detected in the surveyed sclerites, yet their detection was variable. For instance, C, O_2, Mg, Ca and S were detected in over 95% of the sclerites surveyed while Al and Na were detected at least once per sclerite type but with less frequency (i.e., 20-80%) than the aforementioned elements. Si and Cl were the least represented as each one was detected only once, both in a single HH sample.

The elements were also represented in different proportions. For instance, the predominant elements (% weight > 1) across all sclerite samples were O_2, Ca, Mg and Mg. By contrast, S, Al, Na, N, Si and Cl were represented in trace amounts (% weight < 1) across all sclerites samples (Table 1). Yet, the one-way ANOVA analyses independently conducted for C, O, Na, Mg, S and Ca failed to detect significant differences among the sclerite type (Table 2). No analysis where conducted for the remaining elements, as these exhibited less than five detection per sclerites sample.

Volatile organic compounds

A total of 70 VOCs were ... the sampled sclerites; 37% of these were identified from DD sclerites, while 35% and 29% were identified from DH and HH sclerites, respectively. In general, three major groups were identified: alcohols, alkanes and alkenes. When data was organized based on sclerite type, alcohols and secondarily alkanes were the most frequently identified VOCs in all samples, although the number of identified alkenes was higher in HD sclerites (Figure 2). Furthermore, DD sclerites exhibited the highest number of exclusive compounds with a total of 17 unique VOCs, followed by HH and HD sclerites with 15 and 8 unique VOCs, respectively (Table S1; Figure 3). The remaining VOCs were either shared across all sclerite types or between two distinct combinations of sclerites (Figure 3).

Evidence concerning the possible biological roles of the VOCs identified in sclerites was obtained for 44% the compounds. Most were associated with immune related roles such as anti-microbials and anti-oxidants. Less represented roles were related to cytotoxic and apoptotic compounds, cell membrane components such as steroids and oxidized steroids derivatives, and intermediate signaling molecules.

Free radical scavenging assay

Thirteen out of the 30 sclerites sampled had R^2 >80% and thus were used for the free radical scavenging assays. Of these, six were HH sclerites, whereas four and three were DD and HD sclerites, respectively. The free radical scavenging IC_{50} ranged from 0.21 mg dry extract/mL to 0.01 mg dry extract/mL DPPH. On average, HH sclerites exhibited the highest IC_{50} followed by HD sclerites and lastly DD sclerites (Figure 4). These differences were not statistically different.

DISCUSSION

Gorgonian corals are renowned for their rich production of natural compounds. In fact, before the advent of aspergillosis disease of gorgonians during the early 90s during which the numbers of gorgonian-related studies greatly increased, gorgonian studies focused on their capacity to synthesize a wide range of secondary metabolites (Coll 1992; Almeida et al., 2014). Such studies have disclosed not just a great diversity of chemical compounds associated with these corals (i.e., sterols and lipid derived compounds), but also diverse biological roles, from feeding deterrence to antimicrobial compounds.

In contrast, literature centered on the chemical composition of sclerites is scarce. Previous studies have showed that sclerites are partially made of an organic matrix, largely glycoproteins which are rich in aspartic acid (Kingsley & Watabe 1982, 1984). This organic matrix could be divided into water soluble and insoluble fractions, the former displaying collagen-like characteristics (Kingsley et al., 1990). Yet, the role of sclerites as chemical barriers against microbes has only recently been investigated. Here we present the first screen of chemical compounds in sclerites from *G. ventalina* colonies under contrasting health conditions to better understand their potential biological roles. Over 60 VOCs associated with sclerites were identified. The majority of these compounds have not been reported previously in corals and little is known of their biological roles (Table 1S). However, some of the VOCs identified could be derivatives of other compounds such as alcohols, which have undergone esterification reactions to form esters that are import fractions of lipids and steroids of living cells! Furthermore, several of these VOCs were common to all sclerites types, whereas others were found in two or exclusively in one of the three types of sclerites examined here (Table 1S; Figure 3). Some of the compounds identified in this study have been characterized as free radical scavengers (Table 1S). The oxidative analysis assays performed in this study, which revealed

247 that extracts from the three sclerites types exhibit antioxidant activity, further confirm this fact.
248 Furthermore, in this analysis, extracts from DD sclerites showed slightly more oxidative activity
249 than extracts from HH and HD sclerites, suggesting that the diseased state may induce an
250 increase in the production of oxidative compounds, likely in response to oxidative stress
251 resulting from the immune mechanisms orchestrated by sea fans against pathogen infections.
252 The fact that diseased tissue displays dramatically lower alkanes (compounds associated with
253 chemical signaling) and higher alkenes (compounds with higher antioxidant properties) relative
254 to healthy tissue may be evidence for such a response mechanism
255
256 Several of the immune responses described for sea fans such as phagocytosis, peroxidase
257 enzymatic activity, and melanization (Olano & Bigger 2000; Midlarz & Harvell 2007), are also
258 involved in the production of reactive oxygen species (ROS) and other free radical molecules,
259 inducing oxidative stress in the coral. Consequently, to counteract this oxidative stress, sea fans
260 have developed inducible response mechanisms such as increased production of non-enzymatic
261 antioxidant scavengers, which neutralize the potential harmful impact of ROS and other free
262 radical molecules (Yost, Jones & Mitchelmore 2010; Shahbudin et al., 2011). In the present
263 study we were unable to determine if antioxidants are an inducible defense mechanism in
264 sclerites, but their presence in all three types of sclerites studied suggest they may be used as a
265 prophylactic measure.
266
267 Increased antioxidant capacity in corals has also been linked to thermal stress. Several studies
268 have reported that corals under thermal stress exhibit higher antioxidant potential than those
269 under normal temperature conditions (Downs et al., 2002; Griffin & Bhagooli 2004). In fact,
270 over-expression of genes involved in the oxidative-stress response has been reported to increase
271 in acroporids after they have undergone thermal stress (Császá, Senaca & Oppen 2009). The sea
272 fans utilized in the present study were not exposed to thermal stress prior to sampling and thus
273 temperature stress is unlikely to play a role in the results observed.
274
275 Compounds known to have antimicrobial properties such as alcohols and esters were also
276 identified in sclerites (Table 1S). Many of these compounds have been isolated from terrestrial
277 plants. Nonetheless, the fact that gorgonians produce chemical compounds with antimicrobial

activities is not surprising. Multiple culture-based studies using extracts from several gorgonians species have revealed the antimicrobial potential of these extracts (Kim1994, 2000; Jensen et al., 1996; Alker, Smith & Kim 2001; Alker et al., 2004; Ward et al., 2007). Nonetheless, in this study, no relationship could be established between the origin of compounds (i.e., diseased or healthy corals) and their microbial roles, as the majority of the potential roles of the compounds could not be obtained from the scientific literature. Nonethelass, many of these compounds could be precursors or intermediaries of potentially biologically relevant molecules that were trapped within the sclerites during their formation. If indeed some of the compounds isolated from sclerites are precursors of biologically active molecules, as well as environmental cues or internal stressors, sclerites could be used as archives of metabolic pathways or past climatic and biotic stress events.

This study also compared the mineral composition of sclerites isolated from gorgonians under contrasting health conditions. The results revealed no differences in the mineral composition of sclerites regardless of the health state of the corals. Furthermore, the fact that the minerals surveyed have similar compositions across all sclerites, suggests that the mineral fraction of sclerites may have a negligible or no immune-related role at all. Other studies have found that the mineral composition and consequently mineralization of gorgonian sclerites could be controlled by environmental cues. For instance, previous studies have shown that Mg/Ca ratios of sclerites from several gorgonians are positively correlated with water temperature (Chave 1954; Weinbauer & Velimirov 1995). Additional studies are needed to better understand the role of sclerites and how they are impacted by external and internal cues when exposed to both abiotic and biotic stressors.

CONCLUSIONS

Sclerites harbor great VOCs diversity, the majority of which are novel to sea fans or any other corals. This diversity is likely an underestimate and the use of other types of extraction solvents (polar and none polar solvents), surveying several positions within the same colony, or increasing the number of individuals surveyed could greatly increase the number of compounds identified. Likewise, VOCs exhibit multiple roles as identified from the scientific literature, and many other roles would be identified by utilizing bioassays. Furthermore, several of the

identified VOCs could potentially be precursors of biologically active compounds that were trapped within the sclerites during their formation. On the other hand, previous studies have linked sclerites mineralization with water temperature. If indeed some of the compounds isolated from sclerites are precursors of biologically active molecules as well as the environmental cues or internal stressors, sclerites could be used as archives of metabolic pathways or past climatic and biotic stressful events. Yet, addition research will be needed to further clarify this hypothesis.

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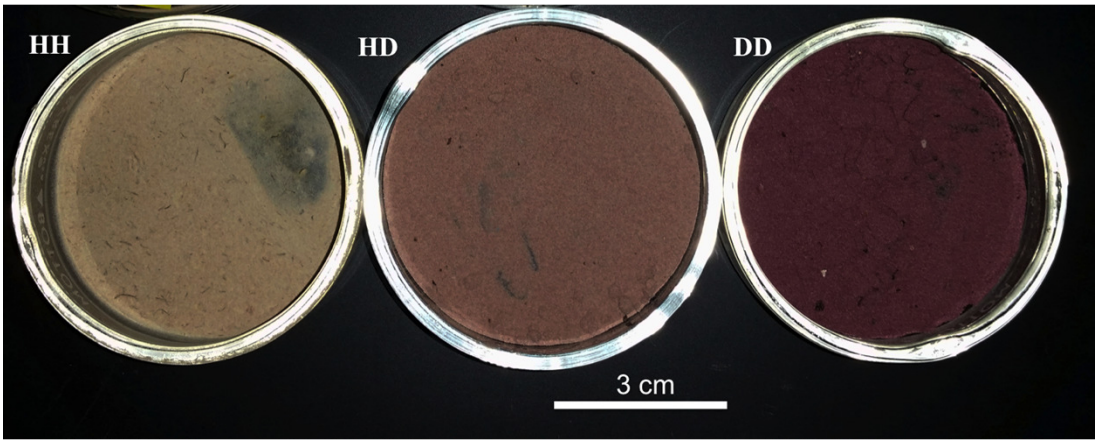

Figure 1: Image of Isolated sclerites placed on Petri dishes from healthy sea fan colonies (HH), from healthy tissue from diseased sea fans (HD), and diseased tissue from diseased sea fans (DD).

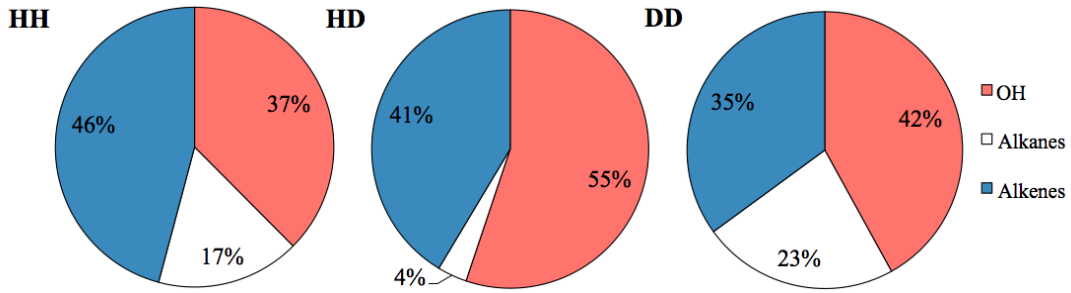

Figure 2. Percentages of the main functional chemicals in sclerites from healthy sea fans (HH), from healthy tissue from diseased sea fans (HD), and diseased tissue from diseased sea fans (DD).

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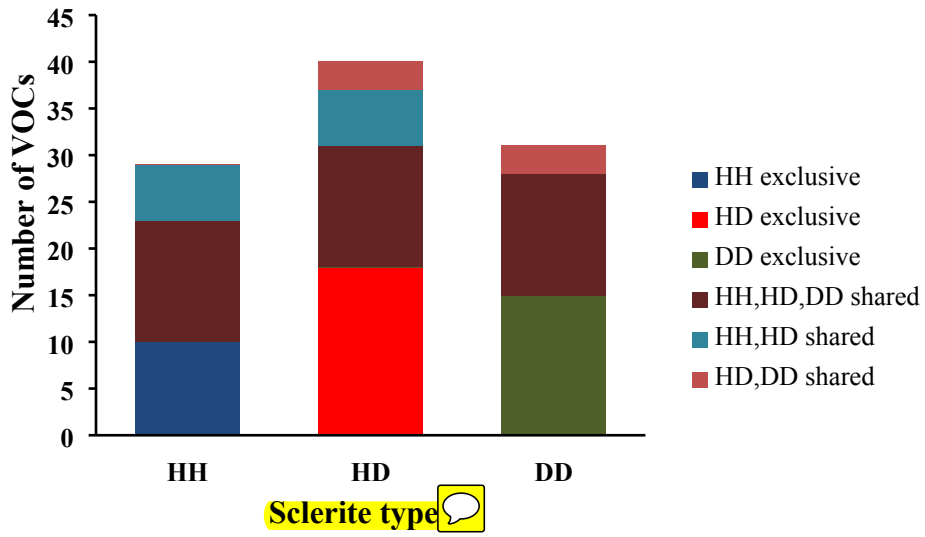

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460 **Figure 3.** Number of volatile organic compounds (VOCs) in sclerites from healthy sea fans
461 (HH), healthy tissue from diseased sea fans (HD), and diseased tissue from diseased sea fans
462 (DD).

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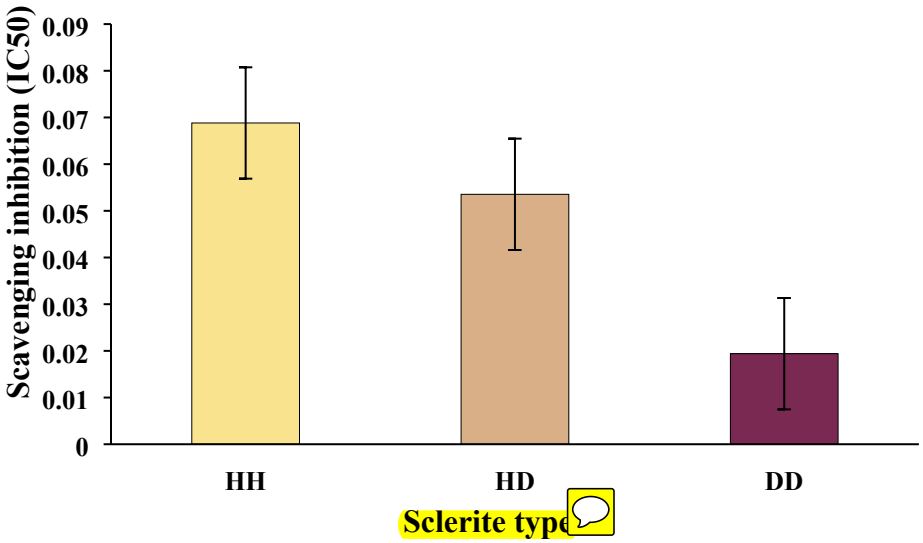

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475 **Figure 4.** Average of free radical scavenging analysis expressed as IC_{50} of sclerites from healthy
476 sea fans (HH), healthy tissue from diseased fans (HD) and diseased tissue from diseased fans
477 (DD). Bars denote standard errors.

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Table 1. Average of the most dominant elements (% weight > 1) of sclerites isolated from; healthy sea fans (HH); healthy tissue from diseased sea fans (HD); and diseased tissue of diseased sea fans (DD). Numbers in parenthesis represent the standard error.

Sclerite type	Mineral composition (in %weight)			
	C	O	Mg	Ca
HH	23.74 (1.35)	38.52 (0.85)	3.06 (0.03)	34.04 (1.90)
HD	29.00 (2.63)	35.86 (1.50)	2.84 (0.02)	31.23 (1.72)
DD	28.73 (1.35)	37.27 (1.35)	2.95 (1.35)	29.75 (1.35)

Table 2. One-way ANOVA results comparing the % weight of elements as the dependent variable versus sclerite types as the independent variable.

Elements	One-Way ANOVA analyses				
	df	**SS**	**Mean S**	**F-statistic**	***p-value***
C	2	172.64	86.321	2.166	0.135
O	2	33.54	16.771	1.277	0.297
Na	2	0.044	0.022	1.08	0.354
Mg	2	0.228	0.114	0.51	0.636
S	2	0.005	0.003	0.134	0.875
Ca	2	94.17	47.086	1.357	0.275

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540 **Table S1:** List of volatile organic compounds (VOCs) isolated from sclerites from: healthy fans (HH), from healthy
541 tissue from diseased fans (HD) and diseased tissue (DD) and their respective biological roles.

VOCs	Sclerites			Biological Function	Reference
	HH	HD	DD		
2-Hexadecanol	✓			Anti-microbial, anti-oxidant	Senthil et al., 2016
Dodecyl acrylate	✓			anti-oxidant	Joo et al., 2010
3,6-dione- (5,17,20) Cholestane-	✓			cytotoxic	Ktari et al., 2000
2-methyl-2-Undecanethiol	✓			Anti-microbial	Meenakshi et al., 2013
2-Tetradecene	✓			Anti-microbial	Roy et al., 2010
1-Tridecene	✓			Unknown	
Bis(2-ethylhexyl) phtalate	✓			Apoptosis inductor	Priya AM, Jayachandran S
1-Monostearoylglycerol	✓			Unknown	
5-octadene (E)	✓			Unknown	
2-ethyl-1-Decanol		✓		Anti-microbial	Kumar et al., 2013
2-methyl 1-Dodecanol		✓		Anti-microbial	Kuppuswamy et al., 2013
7-Hexadecene		✓		Anti-microbial	Mujeeb et al., 2014
1-Undecanol		✓		Unknown	
9-methyl 1-Undecene,		✓		Antibacterial	Okwu and Ighodaro 2010
2-Tridecanol		✓		Unknown	
Geranylgeranylacetate		✓		Unknown	
3-Tetradecene,		✓		Unknown	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol		✓		Unknown	
4-Nonene, 2-methyl-		✓		Unknown	
4-Tetradecene		✓		Unknown	
5-Tetradecene,		✓		Unknown	
5-Tridecene,		✓		Unknown	
5,22-dien-3-ol, (3·)- Cholesta-		✓		Unknown	
Methyl stearate		✓		Unknown	
Phytol, acetate		✓		Unknown	
Tetradecane		✓		Unknown	
15-Heptadecenal		✓		Unknown	
2-Hexyl-1-octanol			✓	anti-oxidant	Shah and Ahmed 2016
2-Monopalmitin			✓	anti-oxidant	Kumar et al., 2013
2-Propyl-1-pentanol			✓	Unknown	
Bis (7-methyloctyl) ester Phthalic acid			✓	Anticancerous, Anti-oxidant	Sivasubranian & Brindha

2-Tetradecanol			✓	Unknown	
4-Ethyl-2-octanol			✓	Unknown	
7-methyl -Undecene -,			✓	Unknown	
5-Eicosene,			✓	Unknown	
5-Cholestene-3-ol, 24-methyl-			✓	Unknown	
2-methyl Nonadecane -			✓	Unknown	
Heptadecane			✓	Unknown	
1-Decene			✓	Unknown	
4-Decene			✓	Unknown	
2-Methyl-1-undecanol			✓	Unknown	

Table S1 (continue)

VOCs	Sclerites			Biological Function	Reference
	H H	H D	D D		
1-Decanol, 2-hexyl	✓	✓	✓	Anti-microbial	Xiangwei et al 2006
17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	✓	✓	✓	Anti-microbial	Altameme et al 2015
3-Octadecene, (E)	✓	✓	✓	Anti-microbial	Lages et al 2010
Campesterol	✓	✓	✓	Antioxidant	Senthil et al., 2016
Cholesta-4,6-dien-3-ol, (3)	✓	✓	✓	Product of the oxidation of Cholesterol	Liu and Shan 2006
Cyclopropane, 1-(2-methylbutyl)-1-(1-methylpropyl)	✓	✓	✓	Anti-fungal	Sheoran N et al 2014
Dodecane, 2,6,11-trimethyl	✓	✓	✓	Oil in from the leaves of Roses	Hosni et al 2010
Ergosta-5,22-dien-3-ol, (3·,22E,24S	✓	✓	✓	Apoptsis agonist activity	Byju et al 2014
1-Dodecene	✓	✓	✓	Anti-microbial	Senthil et al., 2016
1-Tetradecene	✓	✓	✓	Unknown	
2-Butyl-1-decene	✓	✓	✓	Unknown	
Cetene	✓	✓	✓	Unknown	
Nonadecane	✓	✓	✓	Anti-fungal	Omoruyi et al 2014
4-Trifluoroacetoxytridecane	✓	✓		Unknown	
Benzoic acid, 4-ethoxy-, ethyl ester	✓	✓		Anti-microbial,	Sheela and Uthayakumari 2013
Cholesterol	✓	✓		Anti-oxidant, citotoxicity	Peng et al 1979
Ergost-5-en-3-ol, (3·)-	✓	✓		Anti-oxidant	Ponnamma and Manjunath 2012
Hexadecanoic acid, methyl ester	✓	✓		Anti-fungal	Flavier et al 1997
1-Octanol, 2-butyl	✓	✓		Unknown	
1-Undecene, 7-methy	✓		✓	Unknown	
3-Hexadecene, (Z)	✓		✓	Unknown	
Cholestane-3,6-dione, (5)	✓		✓	Oxidized cholesterol derivatives- cytotoxic	Osada et al 1999
Cholest-5-en-3-ol, 24-propylidene-, (3·)		✓	✓	Oxidized cholesterol derivatives- cytotoxic	Osada et al 1999
Undecane, 2-methyl		✓	✓	Unknown	
Glycerol 1-palmitate		✓	✓	Unknown	

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