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Suppression of *Aspergillus* and *Penicillium* Species by Organic Extracts of Common Soft Corals and Sponge Species Habited in Hurghada, Red Sea, Egypt

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Abstract

The ethanolic extracts of eight soft corals and five sponges were screened for antifungal activity (AU) against pathogenic fungi infected corals. The data revealed that the AUs, recorded by different crude extracts (ethanol and ethyl acetate) of soft corals, ranged between 0.0 and 49.0, which was achieved by the extract of *S. gracile* against *A. fumigatus*. In addition, the values of AUs, recorded by different crude extracts (ethanol and ethyl acetate) of sponge species, revealed that the AU ranged between 0.0 and 36.0, which was achieved by extract of *Suberea mollis Row* against *P. auratiogriseum.* Cleary, our findings proved that both ethanolic and ethyl acetate extracts of soft corals (*S. polydactyla, S. gracile, S. glaucum, S. trocheliophorum, S. ehrenbergi,* and *X. macrospiculata*) were the most effective extracts that exhibited the most potent AUs. Moreover, the most effective extracts were tested to determine the MICs against the most affected fungi and the lowest effective MIC was 10 mg/mL against several pathogenic fungi. On the other hand, the GC/MS profiles of the most effective extracts were: fatty acids and their derivatives, terpenoid (Nootkaton-11,12-epoxide, caryophyllene, geranyl-a-terpinene, etc.), steroid (corticosterone, cis-calamenene, etc.), and others. An additional trail proved the efficacy of palmitic acid (as a potent example of bioactive constituents found in crude extracts) when was compared to Treflucan.

Keywords: Antifungal Activity, Organic Extract, Soft Corals, Sponge, Red Sea.

1. Introduction

Marine invertebrates have been considered as potent resources for bioactive metabolites that are new in structure and biologically active. Potential bioactivities of numerous compounds extracted from marine species include: anti-fungal, antibacterial, antiviral, antimalarial, anti-cancer, antihelmintic, anti-inflammatory, anticoagulant, and other bioactivities (Somnath and Ghosh, 2010; Datta *et al.*, 2015). Soft corals, besides sponges, are among common marine invertebrates which have been concerned for new bioactive substances (Ibrahim *et al*., 2012; Liu *et al*., 2019).

In particular, soft corals are known to produce a wide array of secondary metabolites, particularly diterpenoids and steroids, and often characterized by uncommon structural features and potent bioactivities (Liu *et al*., 2019). The remarkable abundance and diversity of bioactive small molecule which have been isolated from soft corals have made these organisms an important source of new drug candidates for human diseases, particularly for their anti-inflammatory activity (Putra and Murniasih, 2016). For instance, Kelman *et al*. (1998) studied different developmental and reproductive features of the Red Sea soft coral; *Parerythropodium fulvum,* which showed antimicrobial activity towards many co-occurring and potentially pathogenic marine bacteria. Cheng *et al*. (2009) obtained antimicrobial activities from the soft coral; *Nephthea erecta* and *Nephthea chabroli*. Furthermore, Ibrahim *et al.* (2012) examined the extracts of ten soft corals from Egyptian Red Sea, which exhibited clear antibacterial activities against tested pathogens.

In addition, sponges are considered promising resources to supply future treatments for sever diseases such as cancer, a number of viral diseases, malaria and inflammation (Ibrahim *et al.*, 2018). Sponges produce a different kind of chemical substances with numerous carbon skeletons (such as fatty acids, terpenoids, alkaloids, polyketides, polyacetylenes, sterols, peptides, etc.), which have been found to be the main component interfering with human pathogenesis at different sites. The fact that different diseases have the capability to fight at different sites inside the body can increase the chances to produce targeted medicines (Anjum *et al*., 2016). Many of such compounds possess antibacterial, antiviral, antifungal, antimalarial, antitumor, immunosuppressive, and cardiovascular activity (Anjum *et al*., 2016), besides antifouling properties (Qian, *et al.*, 2006).

Thus, the current work investigated the suppression of *Aspergillus* and *Penicillium* growth *in vitro* by marine bioactive products extracted from soft corals and sponges

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species in Hurghada, Red Sea, Egypt. In addition, this study extended to determine the general nature of the most potent compounds via gas chromatography/mass spectrometry (GC/MS) technique.

2. Materials and methods

2.1. The study area

Marine living samples were collected away from 5 km a way of Hurghada city centre, Egyptian Red Sea. This area is adjacent to the National Institute of Oceanography and Fisheries, Red Sea branch, at latitudes of 27˚ 17' 13" N and longitudes of 33˚ 46' 43" E. It extends to about 150 m seawards and ends with a lagoon of 5 m depth. This area forms a lagoon, which has a sandy bottom and is covered with algal and sea grass mats. The reef following it is ribbon-like and composed of many hard and soft coral species.

2.2. Fungal culture and reference fungi

Potato dextrose agar (PDA) medium was applied to isolate and culture fungi. It was also used in the suppression trails. Pathogenic fungi isolated from infected hard corals were identified by the same authors and then used as reference pathogenic strains. However, they were: *Aspergillus niger, A. flavus, A. terreus, A. parasiticus, A. fumigatus, Penicillium chrysogemnum, P. oxalicum, P. crustosum, P. aurantiogriseum,* and *P. echinulatum* (El-Morsy *et al*., 2017).

2.3. Sampling and identification marine organisms

SCUBA and snorkeling diving were followed to collect eight soft coral and five sponge samples. Soft corals species were identified according to Reinicke (1995) and Fabricious and Aldersdale (2001), while sponges' species were identified according to Collin *et al.* (2005)., including; morphological characteristics such as the color, size, shape, and form of the sampled specimens were examined and compared. Underwater images are especially important because soft corals emit different colors in the water. Moreover, certain organs in the specimen were observed under light microscope (Olympus CX41 with 100× and 400× magnification).

2.4. Preparation of different organic extracts

With 200 mL of ethanol and ethyl acetate solvent, 80 g of each marine sample (sponges and soft coral) were macerated well. They were filtered by filter paper after soaking for two weeks. In order to obtain crude extracts, solvents were evaporated using a rotary evaporator **(**Ballantine, 1987). Following the Backus and Green Process, bioactive substances were extracted from the sponge with some modifications. A weight of 5 g of the sponge was placed in 100 mL methanol. The quantity of ethanol and ethyl acetate used was 20 times as much as the sponge weight (5%, w/v). After the solution filtration, the residue was dried in the air in the room and then stored in a 4°C refrigerator until the next level (Hutagalung *et al*., 2014).

2.5. Antifungal activity of different extracts against reference fungi

The suppression of different extracts from selected sponges and soft corals against the isolated fungi was assessed by the well-cut diffusion method. Three hundred milliliters of PDA medium were prepared and then inoculated with 1.5 mL of fungal spore suspension (10^6 spores/mL). Fifty millimetres of PDA were poured into all plates. After solidifying, 5 mm-wells were punched out and 100 µL of extracts were moved into each well. At 28^{o}C, all plates were incubated for 5 days. A good result was shown by measuring the radius of inhibition zone (Y) around the each well (X) linearly in mm after the incubation period. The activity was expressed as absolute unit (AU) was calculated by dividing Y^2 per X^2 (El-Masry *et al*., 2002).

2.6. Minimal inhibitory concentrations (MICs)

The MICs were also determined by well-cut diffusion method through preparing five concentrations in mg/mL (10, 30, 50, 100, and 200) for each extract against tested fungi (*A. niger, A. terreus, A. fumigatus, P. chrysogenum, P. oxalicum,* and *P. aurantiogriseum*). All extracts were previously prepared as stock solutions (500 mg/mL) and then were sterilized by 0.45 µL filter and stored at 4°C. After preparing and autoclaving PDA medium, the tested fungi were amended in ratio of 5 mL spore suspension (10^6 spores/mL) per a litre of medium. The tested extracts were added in the five concentrations as mentioned and then all plates were incubated for 5 days at 28°C. It should be taken in consideration that the MIC of certain extract is determined as "the lowest concentration of extract, which inhibits visible growth of the microbe around the well or disc" (Devi *et al*., 2013).

2.7. Composition analysis of potent extracts using GC/MS spectroscopy

The crudes extracted from collected soft coral and sponge species, either in ethanol and ethyl acetate, were prepared for GC/MS analysis. Extracts prepared as mentioned before were concentrated until complete dryness and finally re-suspend in appropriate volume of solvent. However, the filtrate was subjected to GC/MS analysis (Perkin Elmer, Waltham, MA, USA) according to Muller *et al*. (2002) and Thakur and Pandey (2016) procedures.

2.8. Effect of common constituents in extracts and antifungal drug (Treflucan)

From the GC/MS profile of ethanolic and ethyl acetate extracts, the presence of many potent compounds [such as; 6-octen-1-ol, 3,7-dimethyl-, (R)- (citronellol); 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z); pentadecanoic acid, 14-methyl-, methyl ester (palmitic acid); Trans-13-octadecenoic acid, methyl ester (Stearic acid); tridecanoic acid, methyl ester] was revealed as the common constituents in both extracts. The commercial palmitic acid was selected to be examined against the most affected fungi during the screening tests in comparison to commercial antifungal drug (Treflucan). However, the fungal discs of strains *A. niger* and *P. auratiogriseum* were inoculated individually on the top of the sterilized PDA plates according to Amer and Ibrahim (2019). The punched wells were filled with different concentrations of palmitic acid and Treflucan. Later, all plates were incubated at 28°C for 5 days. The results were obtained by measuring the diameter of inhibition zone around each well in millimeter.

2.9. Statistical treatment

Data were expressed as mean of three readings ± SD and was treated with a ANOVA test using SPSS software version. A P-value of < 0.05 is considered as significant value.

3. Results and discussion

3.1. Identification of marine organisms

Eight soft coral species collected from Red Sea were identified as: *Nephthya pacifica, Sarcophyton ehrenbergi, S. glaucum, S. trocheliophorum, S. gracile, Sinularia polydactyla, Xenia macrospiculata*, and *Dendronephthya klunzingeri*. Also, five selected sponge species collected from Red Sea were identified as: *Hyrtios erectus, Calliospongia* sp., *Amphimedon* sp., *Suberea mollis*, and *Ircinia* sp. However, macrographs of these species are shown in Figure 1.

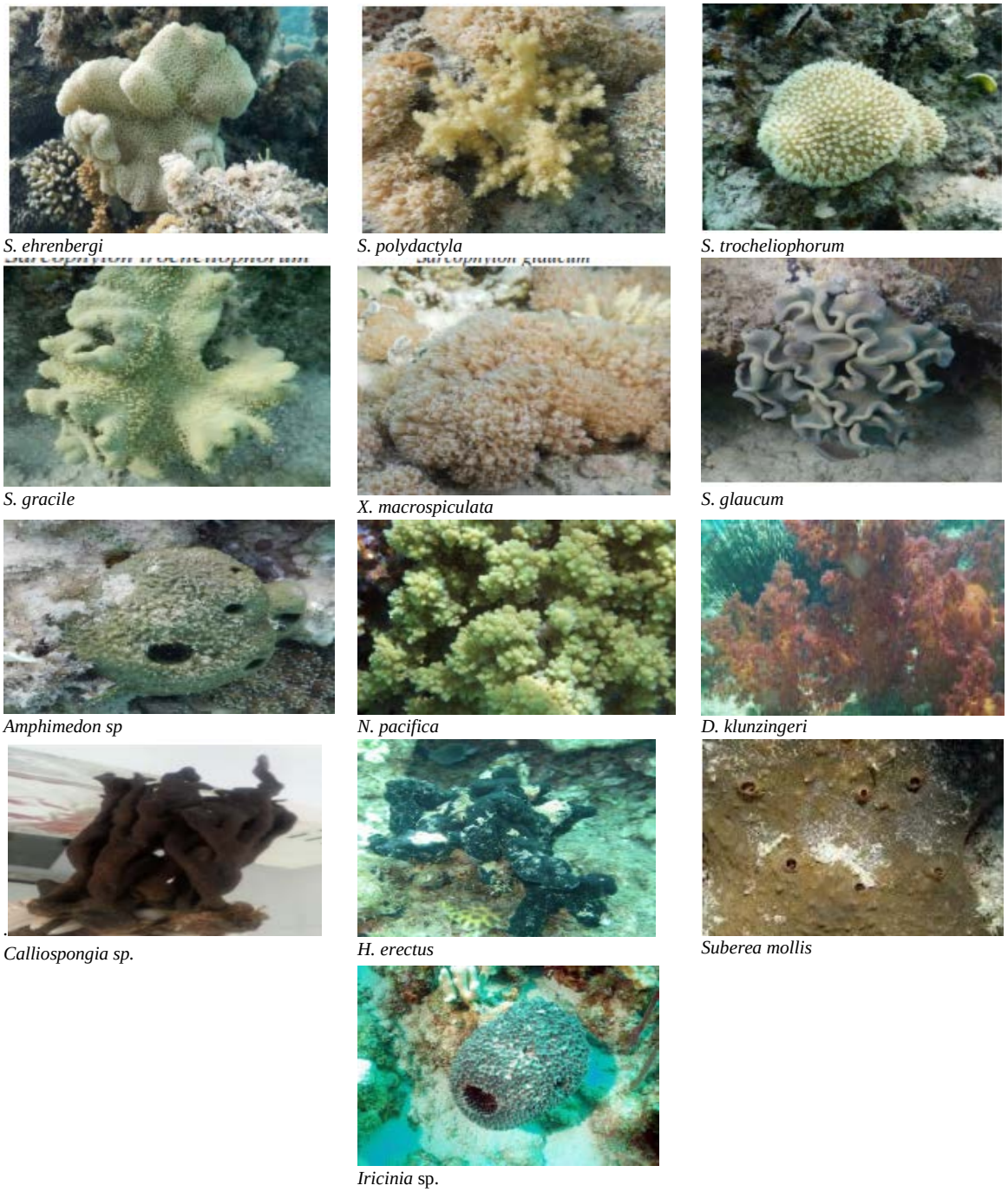


Figure 1. Selected soft coral and sponge species used in screening the antifungal activity, collected from Egyptian Red Sea, at Hurghada city.

Mycopathogens of aquatic animals have become the focus of considerable attention because of the high occurrences of fungal diseases in wild populations and aquaculture. Most marine fungal infections, once established in an individual, are often lethal and difficult to treat. This suggests that these fungi will continue to be troublesome pathogens of marine organisms (Noga, 1990). In comparison, marine species, such as sponges, soft corals, sea hares, bryozoans, sea slugs, and marine microorganisms, etc., are rich sources of novel bioactive metabolites (Blunt *et al*., 2005). Recently, many studies have confirmed the antibacterial, antifungal and antiviral activities of bioactive compounds extracted from marine organisms (Somnath and Ghosh, 2010; Ibrahim *et al*., 2018; 2020a; 2020b).

3.2. Screening of antifungal activity of coral extracts

Preliminarily, the present study investigated the inhibition of the fungal growth *in vitro* through applying selected marine bioactive extracts came from common Red Sea soft corals and sponges, Egypt.

In general, ethanolic extracts of eight soft coral and five sponge species were then screened for antifungal activity expressed as AU using well-cut diffusion technique. Result shown in Table 1 presents the AU recorded by different extracts (ethanol and ethyl acetate) of soft coral species against tested fungi. However, data revealed that the AU ranged between 0.0 and 49.0, which was achieved by the extract of *S. gracile* against *A. fumigatus*. This AU value was followed by 42.3 for extracts of both *S. glaucum* and *S. trocheliophorum* against *P. auratiogriseum* and *A. fumigatus*, respectively. Relatively, there were many other high records. The lowest value, rather than negative records, was 1.4 against *P. echinulatum*, which was given by extract of *X. macrospiculata*.

In addition, result shown in Table 2 presents the AU recorded by different extracts (ethanol and ethyl acetate) of sponge species against tested fungi. However, data revealed that the AU ranged between 0.0 and 36.0, which was achieved by extract of *Suberea mollis* against *P. auratiogriseum.* This value was followed by *Amphimedon* sp. extract against both *P. oxalicum* (AU = 31.6) and *P. crustosum* (AU = 30.0). However, there were many other relatively high records. Also, the lowest value, rather than negative records, was 2.0 against *P. echinulatum* which was occurred by extract of *Calliospongia sp.*

Table 1: Screening of antifungal activity for soft coral species extracts by well-cut diffusion technique (AU) against both *Aspergillus* and *Penicillium* species.

1, *A. flavus*; 2, *A. niger*; 3, *A. fumigatus*, 4, *A. parasiticus*; 5, *A. terreus*; 6, *P. auratiogriseum*; 7, *P. chrysogenum*; 8, *P. oxalicum*; 9, *P. echinulatum*; 10, *P. crustosum.*

Species/extract	Antifungal activity (AU)									
	1	2	3	4	5	6	7	8	9	10
Ethanol crude extract:										
D. klunzingeri	0.0±0.0	9.6±0.17	4.4±0.06	3.6±0.10	0.0±0.0	0.0±0.0	4.4±0.0	0.0±0.0	1.2±0.06	6.8±0.06
N. molle	6.3±0.06	9.0±0.10	23.0±0.17	8.4±0.15	1.4±0.0	0.0±0.0	4.8±0.12	0.0±0.0	1.4±0.12	4.0±0.21
S. ehrenbergi	7.8±0.06	7.3±0.15	13.7±0.20	6.3±0.25	12.3±0.21	4.4±0.20	4.0±0.0	4.0±0.15	9.0±0.26	4.0±0.21
S. glaucum	6.8±0.21	4.8±0.15	5.8±0.10	4.8±0.06	0.0±0.0	0.0±0.0	12.30.15	12.3±0.15	13.7±0.25	12.3±0.15
S. trocheliophorum	4.0±0.12	5.8±0.21	5.8±0.12	0.0±0.0	4.0±0.20	0.0±0.0	4.0±0.12	12.3±0.15	16.0±0.12	7.80.10
S. gracile	5.3±0.15	4.0±0.15	6.8±0.26	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	9.0±0.25	0.8±0.0	0.0±0.0
S. polydactyla	17.6±0.31	17.6±0.12	23.0±0.21	10.9±0.17	21.2±0.15	24.0±0.15	16.0±0.12	25.0±0.15	16.0±0.21	7.8±0.15
X. macrospiculata	4.8±0.06	5.3±0.21	10.9±0.12	8.4±0.21	5.3±0.12	4.8±0.12	9.0±0.06	8.4±0.06	12.3±0.12	12.3±0.06
Ethyl acetate crude extract:										
D. klunzingeri	0.0±0.0	6.8±0.06	4.0±0.18	0.0±0.0	0.0±0.0	0.0±0.0	2.6±0.06	0.0±0.0	5.3±0.12	4.0±0.15
N. molle	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
S. ehrenbergi	5.8±0.10	10.9±0.15	5.7±0.23	9.0±0.10	20.3±0.17	2.6±±0.0	16.0±0.21	5.3±0.12	10.9±0.15	4.0±0.12
S. glaucum	2.6±0.0	22.1±0.23	36.0±0.29	16.0±0.15	20.3±0.20	3.2±±0.06	36.0±0.30	4.0±0.20	20.3±0.25	6.3±0.21
S. trocheliophorum	2.6±0.0	4.0±0.0	42.3±0.87	3.6±0.15	4.4±0.0	0.0±0.0	0.0±0.0	4.0±0.15	0.0±0.0	6.3±0.15
S. gracile	4.8±0.15	6.5±0.20	49.0±1.04	7.3±0.10	25.0±0.36	0.0±0.0	30.3±1.01	4.0±0.20	32.5±1.51	0.0±0.0
S. polydactyla	5.8±0.10	9.0±0.31	10.9±0.89	4.4±0.06	6.3±0.26	4.0±0.15	12.3±0.15	5.3±0.20	16.0±0.36	4.0±0.0
X. macrospiculata	4.0±0.15	9.0±0.31	10.2±0.12	6.3±0.42	7.3±0.12	3.2±±0.0	4.8±0.26	0.0±0.0	1.4±0.0	4.0±0.10

Actually, soft corals have been investigated by scientists globally for their diversity of chemical constituents and biological activities (Liang and Fang, 2006). Bowden *et al*. (1984) discovered that soft coral;

Lobophytum crasscopiculatum yielded cembranolides compound, which have antimicrobial activities. Kelman *et al*. (1998) studied the Red Sea soft coral; *Parerythropodium fulvum*, which exhibited antimicrobial activity against several co-occurring and potentially pathogenic marine bacteria. Geffen and Rosenberg (2005) showed antibacterial from the coral; *Pocillopora damicornis*. Cheng *et al*. (2009) obtained antimicrobial activities from *N. erecta* and *N. chabroli*. Ibarhim *et al.* (2012) previously investigated number of these corals from Egyptian Red Sea (particularly; *S. acutum, S. spongosium, S. gracile, S. glaucum, Sinularia gardineiri, S. leptoclados, Lopophytum pauciliforum, Dendronephthea sp., N. pacifica,* and *X. macrospiculata*). However, their extracts showed obvious antibacterial activities against some common pathogens.

Table 2: Screening of antifungal activity for sponge species extracts by well-cut diffusion technique against both *Aspergillus* and *Penicillium* species.

Species/extract	Antifungal activity (AU)									
	1	2	3	4	5	6	7	8	9	10
Ethanol crude extract:										
H. erectus	4.9±0.10	10.9±0.15	16.0±0.55	6.3±0.12	9.0±0.21	9.0±0.35	0.0±0.0	0.0±0.0	2.2±0.12	7.3±0.17
Calliospongia	2.6±0.12	23.0±0.38	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.3±0.15	0.0±0.0
Amphimedon	5.3±0.25	18.5±0.90	25.0±0.75	5.7±0.06	16.0±0.44	16.0±0.55	12.9±0.20	20.3±0.75	13.7±0.30	23.0±0.82
Suberea mollis	0.0±0.0	0.0±0.0	4.0±0.06	0.0±0.0	12.3±0.35	16.0±0.30	0.0±0.0	4.0±0.15	0.0±0.0	4.0±0.15
Iricinia sp.	0.0±0.0	4.0±0.21	16.0±0.44	4.0±0.10	0.0±0.0	9.0±0.31	0.0±0.0	5.7±0.36	9.0±0.31	9.0±0.45
Ethyl acetate crude extract:										
H. erectus	3.6±0.15	23.0±0.55	13.7±0.45	10.9±0.45	9.0±0.35	13.7±0.36	16.0±0.56	16.0±0.10	16.0±0.42	16.0±0.31
Calliospongia	0.0±0.0	17.6±0.31	16.0±0.25	5.8±0.31	0.0±0.0	13.7±0.40	15.2±0.21	0.0±0.0	2.0±0.21	0.0±0.0
Amphimedon	0.0±0.0	9.0±0.50	20.2±0.51	16.0±0.65	13.0±0.31	28.1±0.56	13.7±0.31	31.6±0.20	16.0±0.20	30.0±0.56
Suberea mollis	0.0±0.0	0.0±0.0	25.0±0.46	4.0±0.10	7.8±0.32	36.0±0.50	0.0±0.0	0.0±0.0	2.3±0.06	9.0±0.46
Iricinia sp.	0.0±0.0	16.0±0.21	7.8±0.20	12.3±0.26	0.0±0.0	9.0±0.40	0.0±0.0	0.0±0.0	4.0±0.30	0.0±0.0

1. *A. flavus*; 2, *A. niger*; 3, *A. fumigatus*, 4, *A. parasiticus*; 5, *A. terreus*; 6, *P. auratiogriseum*; 7, *P. chrysogenum*; 8, *P. oxalicum*; 9, *P. echinulatum*; 10, *P. crustosum*.

Additionally, sponge extracts exhibited antimicrobial activities. For instance, sponge aqueous extract was used as antifungal activity (Perdicaris *et al*., 2013) and antibacterial against certified strains of bacteria **(**Galeano and Martínez, 2007; Ibrahim *et al*., 2018**)**. Dhinakaran and Lipton (2012) examined the antifungal activity of the organic extracts of the sponge; *Sigmadocia pumila* against various fungal strains such as: *Trichoderma viride, Fusarium spp., A. niger, Candida albicans, P. chrysogenum,* and *A. flavis*. Hence it is assumed that the sponge exhibited high antimicrobial activities. Ibrahim *et al.* (2018) also collected five marine sponges from the Levantine Basin in the vicinity of Alexandria city, Egypt (Spongia sp., *Cinachyrella* sp., *Ciocalypta penicillus*, *Axinella verrucosa*, and *Plakortis simplex*) and their results showed that the acetone extract of *Spongia* sp. had a broad spectrum followed by extracts of *C. penicillus* and *A. verrucosa*.

More recently, Ibrahim *et al.* (2020a) studied treating pathogenic bacteria (*Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus*) isolated from human stool and urine samples by the crudes extracted from sponge (*Negombata magnifica*, *Siphonochalina siphonella*, and *H. erectus*) and soft coral (*S. glaucum* and *X. macrospiculata*). Their data were also promising in such manner. Ibrahim et al. (2020b) evaluated the antimicrobial properties of three species of sponge collected from Red Sea, Hurghada, Egypt. They were identified as; *Cinachyrella arabica*, *Ciocalypta penicillus* and *Axinella verrucosa.* Their data revealed the positive values of antifungal and antibacterial activities in extracts, especially against *A. hydrophila, S. aureus* and *P. notatum*.

Data in both Table 1 and 2 were expressed as mean of three readings ± SD. Correlations between antifungal activities and fungal species in case of ethanol crude extracts were significant; where P-value < 0.001. Also, they were significant in case of ethyl acetate crude extracts; where P-value < 0.05. In very few cases of the latter condition, they were not significant; where P-value > 0.05 (Table 3).

Table 3: Results of the ANOVA test showing significance between type of crude extract and antifungal activity (AU) presented in Table 1 and 2.

AU/Fungal species	For data in Table 1				For data in Table 2			
	Ethanol crude extract		Ethyl acetate extract		Ethanol crude extract		Ethyl acetate extract	
	F	Significance	F	Significance	F	Significance	F	Significance
AU/1	3208.538	0.000	1946.164	0.000	1100.923	0.000	1635.571	0.000
AU/2	2227.401	0.000	1.541	0.223	1335.330	0.000	1711.002	0.000
AU/3	6310.098	0.000	1.288	0.317	1435.691	0.000	1.223	0.361
AU/4	2190.637	0.000	2747.693	0.000	5235.750	0.000	451.171	0.000
AU/5	11384.127	0.000	4.172	0.009	2271.668	0.000	1547.146	0.000
AU/6	11384.127	0.000	2940.268	0.000	1066.844	0.000	1921.672	0.000
AU/7	127.686	0.000	3593.907	0.000	12870.750	0.000	2255.653	0.000
AU/8	9916.997	0.000	902.128	0.000	1470.158	0.000	60203.400	0.000
AU/9	4928.935	0.000	1237.145	0.000	2213.636	0.000	2148.664	0.000
AU/10	2846.210	0.000	1233.613	0.000	1258.791	0.000	3847.546	0.000

1. *A. flavus*; 2, *A. niger*; 3, *A. fumigatus*, 4, *A. parasiticus*; 5, *A. terreus*; 6, *P. auratiogriseum*; 7, *P. chrysogenum*; 8, *P. oxalicum*; 9, *P. echinulatum*; 10, *P. crustosum*.

3.3. MICs of the most effective extracts

Thereafter, the MICs were determined against the most affected fungal pathogens. The MICs were detected by applying five concentrations (10, 30, 50, 100, and 200 mg/mL) for each extract obtained from soft corals (*S. polydactyla, S. gracile, S. glaucum, S. trocheliophorum, S. ehrenbergi,* and *X. macrospiculata*) against the most affected fungal pathogens *(A. niger, A. terreus, A. fumigates, P. aurantiogriseum, P. chrysogenum,* and *P. oxalicum).*

Data in Table 4 illustrates the MICs of *S. polydactyla* ethanolic extracts were 10 mg/mL against all tested *Aspergillus* species. The MICs of *S. gracile* ethanolic extracts were 30 mg/mL against *A. niger* and *A. fumigatus* and 200 mg/mL against *A. terreus.* The MICs of *S. glaucum* ethanolic extracts were 50 mg/mL against both of *A. niger* and *A. fumigatus* and 100 mg/mL against *A. terreus.* The MICs of *S. trocheliophorum* ethanolic extracts were 30 mg/mL against both of *A. niger* and 50 mg/mL against *A. fumigatus* and *A. terreus.* The MICs of *S. trocheliophorum* ethanolic extracts were 30 mg/mL against both of *A. niger* and 50 mg/mL against *A. fumigatus* and *A. terreus.* The MICs of *S. ehrenbergi* ethanolic extracts 10 mg/mL against both of *A. terreus* and *A. fumigatus* and 30 mg/mL against *A. niger* The MICs of *X. macrospiculata* ethanolic extracts 30 mg/mL against all tested *Aspergillus* species.

Table 4: MICs of extracts (mg/mL) from selected soft coral against the most previously affected *Aspergillus* species [a= concentration of extract (mg/mL), while b= zone, mm].

	MICs (mg/mL)/Fungus					
	A. niger		*A. terreus*		*A. fumigatus*	
Species/extract	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate
S. polydactyla	10^a	30^a	10^a	30^a	10^a	30^a
	10^b	9^b	11^b	9^b	10^b	9^b
S. gracile	30^a	30^a	200^a	10^a	30^a	10^a
	5^b	5^b	11^b	13^b	9^b	11^b
S. glaucum	50^a	10^a	100^a	10^a	50^a	10^a
	10^b	10^b	9^b	10^b	12^b	10^b
S. trocheliophorum	30^a	50^a	50^a	30^a	50^a	10^a
	5^b	10^b	11^b	8^b	11^b	11^b
S. ehrenbergi	30^a	10^a	10^a	30^a	10^a	10^a
	8^b	7^b	12^b	11^b	8^b	$9^{b\,b}$
X. macrospiculata	30^a	30^a	30^a	30^a	30^a	30^a
	10^b	9^b	10^b	8^b	11^b	10^b

On the other side, the MICs of both of *S. polydactyla* and *X. macrospiculata* ethyl acetate extract were 30 mg/mL against all tested *Aspergillus* species. The MICs of both of *S. S. gracile* ethyl acetate extract were 10 mg/mL against both of *A. terreus* and *A. fumigatus* and 30 mg/mL against A. *niger*. The MICs of both of *S. S. glaucum* ethyl acetate extract were 10 mg/mL against all tested *Aspergillus* species. The MICs of both of *S. trocheliophorum* ethyal acetate extract were 50, 30 and 10 mg/mL against A. *niger, A. terreus* and *A. fumigatus,* respectively. The MICs of both of *S. ehrenbergi* ethyl acetate extract were 10 mg/mL A. *niger* and *A. fumigatus* and 30 mg/mL against *A. terreus.*

The results in Table 5 revealed that the MICs of *S. polydactyla* ethanolic extract were recorded as 10 mg/mL against both of *P. aurantiogriseum* and *P. chrysogenum* and 30 mg/mL against *P. oxalicum*. The MICs of *S. gracile* ethanolic extract were 50, 100 and 30 mg/mL against *P. aurantiogriseum, P. chrysogenum* and *P. oxalicum,* respectively. The MICs of *S. glaucum* ethanolic

extract were 10 mg/mL against *P. chrysogenum* and 30 mg/mL against both of *P. aurantiogriseum*, and *P. oxalicum*. The MICs of *S. trocheliophorum* ethanolic extract were 30 mg/mL against *P. oxalicum* and 50 mg/mL against both of *P. aurantiogriseum* and *P. chrysogenum.* The MICs of *S. ehrenbergi* ethanolic extract were 30 mg/mL against *P. aurantiogriseum* and 50 mg/mL against both of *P. chrysogenum* and *P. oxalicum.* The MICs of *X. macrospiculata* ethanolic extract were 10, 30 and 100 mg/mL against *P. aurantiogriseum, P. chrysogenum* and *P. oxalicum*, respectively.

On the other hand, the MICs of *S. polydactyla* ethyl acetate extract were recorded to be 10 mg/mL against *P. aurantiogriseum* and 30 mg/mL against both of *P. chrysogenum* and *P. oxalicum.* The MICs of both of *S. gracile* and *S. glaucum* ethyl acetate extract was recorded to be 10 mg/mL against *P. aurantiogriseum* and 50 mg/mL against both of *P. chrysogenum* and *P. oxalicum.* The MICs of both of *S. trocheliophorum* ethyl acetate extract was recorded to be 10, 100 and 50 mg/mL against *P. aurantiogriseum, P. chrysogenum* and *P. oxalicum*, respectively. The MICs of both of *S. ehrenbergi* ethyl acetate extract was recorded to be 30 mg/mL against *P. aurantiogriseum* and 50 mg/mL against both of *P. chrysogenum* and *P. oxalicum*, The MICs of both of *S. ehrenbergi* ethyl acetate extract was recorded to be 30 mg/mL against all tested *Penicillium* species.

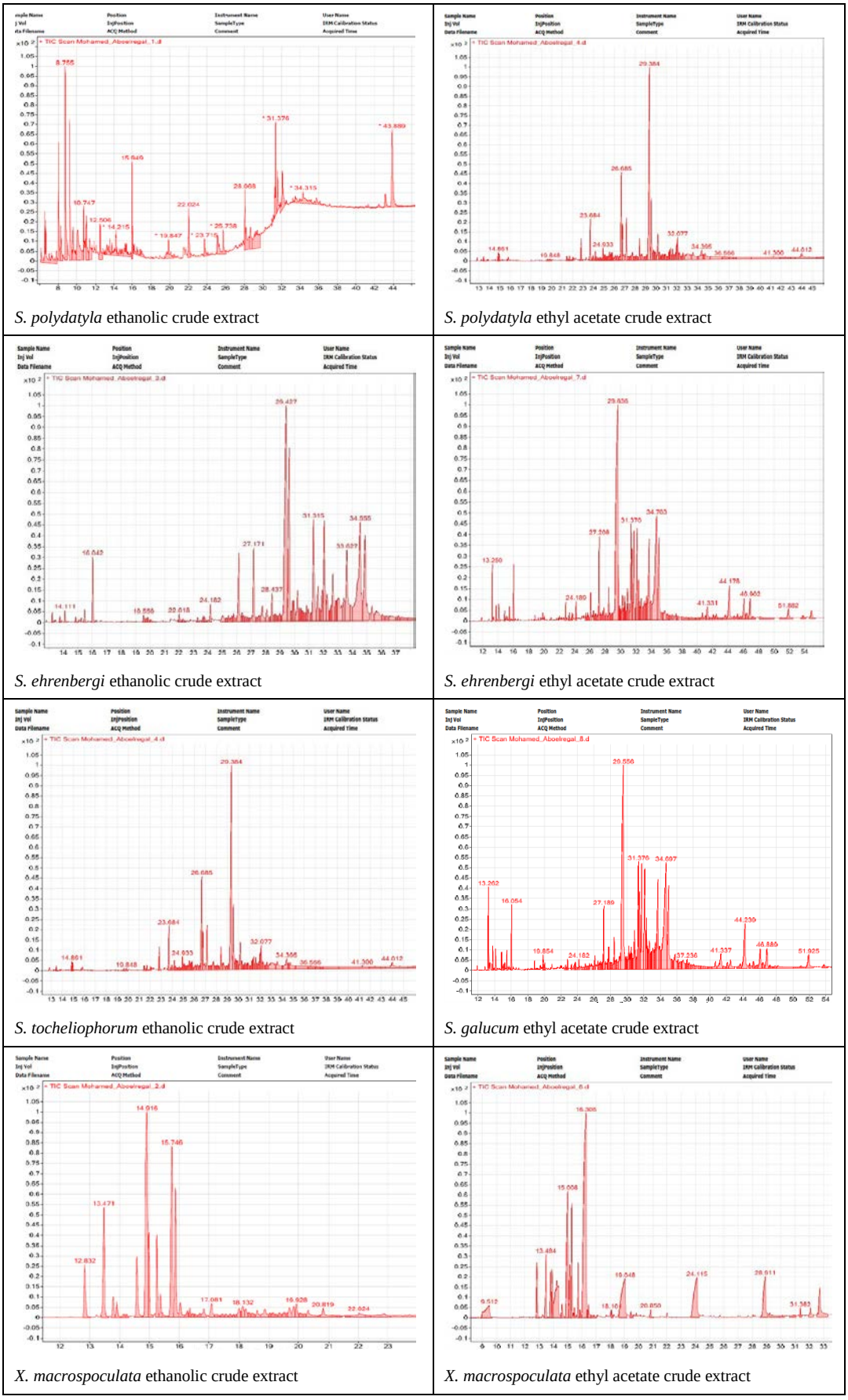
Table 5: MICs of extracts (mg/mL) from selected soft coral against the most previously affected *Penicillium* species [a= concentration of extract (mg/mL), while b= zone, mm].

	MICs (mg/mL)/Fungus					
	P. aurantiogriseum		*P. chrysogenum*		*P. oxalicum*	
Species/extract	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate
S. polydactyla	10^a	10^a	10^a	30^a	30^a	30^a
	9^b	9^b	9^b	9^b	11^b	9^b
S. gracile	50^a	10^a	100^a	10^a	30^a	50^a
	9^b	13^b	9^b	11^b	11^b	9^b
S. glaucum	30^a	10^a	10^a	10^a	30^a	50^a
	11^b	13^b	9^b	12^b	11^b	9^b
S. trocheliophorum	50^a	10^a	50^a	100^a	30^a	50^a
	11^b	12^b	9^b	9^b	10^b	10^b
S. ehrenbergi	30^a	30^a	50^a	50^a	50^a	50^a
	10^b	9^b	9^b	9^b	9^b	9^b
X. macrospiculata	10^a	30^a	30^a	30^a	100^a	30^a
	9^b	11^b	10^b	9^b	9^b	10^b

This trail proved the high efficiency of crude extracts under investigation as antifungal agents. Despite of, the crudes of the soft corals that were applied varied in their extract concentration and were not consistent with their antifungal activities; they are very promising based on their AUs and MICs values.

3.4. Composition of potent extracts using GC/MS spectroscopy

The current study was extended to determine the GC/MS profiles of the most potent extracts (from; *S. polydactyla, S. gracile, S. glaucum, S. trocheliophorum, S. ehrenbergi,* and *X. macrospiculata*) as antifungal agents either ethanolic or ethyl acetate crudes after confirming their high positive records as antifungal agents. The GC/MS chromatogram of them with their retention times (RT), molecular formula, molecular weight, and peak area are presented in Tables 6 & 7 and Figure 2.



S. polydatyla ethanolic crude extract

S. polydatyla ethyl acetate crude extract

S. ehrenbergi ethanolic crude extract

S. ehrenbergi ethyl acetate crude extract

S. tocheliophorum ethanolic crude extract

S. galucum ethyl acetate crude extract

X. macrospoculata ethanolic crude extract

X. macrospoculata ethyl acetate crude extract

Figure 2: Different mass spectra of ethanolic and ethyl acetate of the most potent extracts from soft coral species.

3.4.1. GC/MS profile of major components in the most effective ethanolic extracts

The GC/MS chromatogram of ethanol extract of *S. polydatyla* showed the presence of several active principle compounds. Eleven compounds were identified in this extract. However, the prevailing compounds were (Table 5); 6-octen-1-ol, 3,7-dimethyl-, (R)- (32.7%), 3,7-octadiene-2,6-diol, 2,6-dimethyl- (14.9%), caryophyllene (26.7%), trans-calamenene (42.3%), 8-epi-.gama.-eudesmol (22.1%), 1-heptatriacotanol (32.1%), hexadecanoic acid, ethyl ester (40.2%), 2-hexadecanol (15.3%), 5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)- (19.0%), diisooctyl phthalate (14.3%), and cholest-5-en-3-ol, 24-propylidene-, (3β)- (36.5%). The GC/MS chromatogram of ethanol extract of *X. macrospoculata* showed the presence of several active principle compounds. Six compounds were identified in this extract. However, the prevailing compounds were; naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)- (54.5%), isolongifolene, 7,8-dehydro-8a-hydroxy- (22.6%), cycloisolongifolene, 8,9-dehydro- (21.2%), -naphthalenemethanol, 1,2,3,4-tetrahydro-8-methyl- (12.5%), murolan-3,9(11)-diene-10-peroxy (27.3%), and nootkaton-11,12-epoxide (32.0%). The GC/MS chromatogram of ethanol extract of *S. ehrenbergi* showed the presence of several active principle compounds. Nine compounds were identified in this extract. However, the prevailing compounds were; cubedol (42.9%), tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethy (12.8%), (Z)6,(Z)9-pentadecadien-1-ol (37.2%), hexadecanoic acid, ethyl ester (55.5%), geranyl-a-terpinene (21.7%), 3,7-cyclodecadien-1-one (23.8%), 3,7-dimethyl-10-(1-methylethylidene)-, (E,E) (9.38%), isoaromadendrene epoxide (50.6%), and 3-oxo-10(14)-epoxyguai-11(13)-en- (34.2%). The GC/MS chromatogram of ethanol extract of *S. tocheliophorum* showed the presence of several active principle compounds. Seven compounds were identified in this extract. However, the prevailing compounds were; isolongifolene, 7,8-dehydro-8a-hydroxy (19.4%), 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) (10.5%), thunbergol (41.2%), androstan-17-one, 3-ethyl-3-hydroxy-, (5a) (10.5%), 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane (8.37%), isoaromadendrene epoxide (11.7%), and 4,8,13-cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl) (16.5%).

Table 6: GC/MS data of major components in the most effective ethanolic extracts.

Crude extract	Name	RT (min)	Formula	Molecular weight	Probability (%)
S. polydatyla	6-Octen-1-ol, 3,7-dimethyl-, (R)-	8.768	$C_{10}H_{20}O$	156	32.7
	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	10.747	$C_{10}H_{18}O_2$	170	14.9
	Caryophyllene	12.506	$C_{15}H_{24}$	204	26.7
	trans-calamenene	14.203	$C_{15}H_{22}$	202	42.3
	8-epi-.gama.-eudesmol	16.023	$C_{15}H_{26}O$	222	22.1
	1-Heptatriacotanol	19.847	$C_{37}H_{76}O$	536	23.1
	Hexadecanoic acid, ethyl ester	22.036	$C_{18}H_{36}O_2$	284	40.2
	2-Hexadecanol	23.733	$C_{16}H_{34}O$	242	15.3
	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	28.080	$C_{22}H_{36}O_2$	332	19.0
	Diisooctyl phthalate	31.376	$C_{24}H_{38}O_4$	390	14.3
	Cholest-5-en-3-ol, 24-propylidene-, (3β)-	43.895	$C_{30}H_{50}O$	426	36.5
X. macrospoculata	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	12.832	$C_{15}H_{22}$	202	54.5
	Isolongifolene, 7,8-dehydro-8a-hydroxy-	13.471	$C_{15}H_{24}O$	220	22.6
	Cycloisolongifolene, 8,9-dehydro-	14.016	$C_{15}H_{22}$	202	21.2
	-Naphthalenemethanol, 1,2,3,4-tetrahydro-8-methyl-	15.746	$C_{12}H_{16}O$	176	12.5
	Murolan-3,9(11)-diene-10-peroxy	18.870	$C_{15}H_{24}O_2$	236	27.3
	Nootkaton-11,12-epoxide	22.034	$C_{15}H_{22}O_2$	234	32.0
S. ehrenbergi	Cubedol	14.111	$C_{15}H_{26}O$	222	42.9
	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethy	16.029	$C_{15}H_{24}O$	220	12.8
	(Z)6,(Z)9-Pentadecadien-1-ol	19.559	$C_{15}H_{28}O$	224	37.2
	Hexadecanoic acid, ethyl ester	22.024	$C_{18}H_{36}O_2$	284	55.5
	geranyl-a-terpinene	24.176	$C_{20}H32$	272	21.7
	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)	27.158	$C_{15}H_{22}O$	218	23.8
	Isoaromadendrene epoxide	29.384	$C_{15}H_{24}O$	220	9.38
	3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide	31.296	$C_{15}H_{18}O_4$	262	50.6
	Androstan-17-one, 3-ethyl-3-hydroxy-, (5a)	33.621	$C_{21}H_{34}O_2$	318	34.2
S. tocheliophorum	Isolongifolene, 7,8-dehydro-8a-hydroxy	14.867	$C_{15}H_{24}O$	220	19.4
	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	19.614	$C_{21}H_{36}O_4$	352	10.5
	Thunbergol	23.678	$C_{20}H_{34}O$	290	41.2
	Androstan-17-one, 3-ethyl-3-hydroxy-, (5a)	24.945	$C_{21}H_{34}O_2$	318	10.5
	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	26.660	$C_{33}H_{56}$	452	8.37
	Isoaromadendrene epoxide	29.366	$C_{15}H_{24}O$	220	11.7
	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)	32.090	$C_{20}H_{34}O_2$	306	16.5

3.4.2. GC/MS profile of major components in the most effective ethyl acetate extracts

The GC/MS chromatogram of ethyl acetate extract of *S. polydatyla* in ethyl acetate showed the presence of several active principle compounds. Three compounds were identified in this extract. However, the prevailing compounds were (Table 6); hexadecanol, 2-methyl (9.42%), dasycarpidan-1-methanol, acetate (ester) (12.1%) and tert-hexadecanethiol (10.5%).

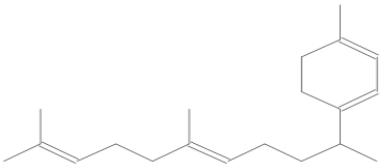
The GC/MS chromatogram of ethyl acetate extract of *X. macrospiculata* showed the presence of several active principle compounds. Four compounds were identified in this extract. However, the prevailing compounds were; naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl), (1S-cis) (27.5%), isolongifolene, 7,8-dehydro-8a-hydroxy, isolongifolene, 7,8-dehydro-8a-hydroxy (20.6%), isoshyobunone (13.0%), and hexaethylene glycol (55.3%). The GC/MS chromatogram of ethyl acetate extract of *S. ehrenbergi* showed the presence of several active principle compounds. Six compounds were identified in this extract. However, the prevailing compounds were; alloaromadendrene (12.7%), geranyl-a-terpinene (12.9%), 3,7-cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E) (13.5%), 4,8,13-cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl) (28.9%), 3-oxo-10(14)-epoxyguai-11(13)-en-6,12-olide (12.1%), and campesterol (25.6%). The GC-MS chromatogram of ethyl acetate extract of *S. galucum* showed the presence of several active principle compounds. Six compounds were identified in this extract. The prevailing compounds were; alloaromadendrene (12.2%), tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl (12.8%), 3,7-cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E) (13.0%), 4,8,13-cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl) (28.6%), 3-oxo-10(14)-epoxyguai-11(13)-en-6,12-olide (23.7%), and campesterol.(31.8%).

Table 7: GC/MS data of major components in the most effective ethyl acetate extracts.

Crude extract	Compound name	RT (min)	Formula	Molecular weight	Probability (%)
S. polydatyla	-Hexadecanol, 2-methyl	23.715	$C_{17}H_{36}O$	256	9.42
	Dasycarpidan-1-methanol, acetate (ester)	29.396	$C_{20}H_{26}N_2O_2$	326	12.1
	tert-Hexadecanethiol	32.090	$C_{16}H_{34}S$	258	10.5
X. macrospiculata	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)	13.477	$C_{15}H_{22}$	202	27.5
	Isolongifolene, 7,8-dehydro-8a-hydroxy	15.002	$C_{15}H_{24}O$	220	20.6
	Isoshyobunone	16.275	$C_{15}H_{24}O$	220	13.0
	Hexaethylene glycol	24.084	$C1_2H_{26}O_7$	282	55.3
S. ehrenbergi	Alloaromadendrene	13.244	$C_{15}H_{24}$	204	12.7
	Geranyl-a-terpinene	24.207	$C_{20}H_{32}$	272	12.9
	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)	27.201	$C_{15}H_{22}O$	218	13.5
	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)	29.618	$C_{20}H_{34}O_2$	306	28.9
	3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide	34.654	$C_{15}H_{18}O_4$	262	12.1
	Campesterol	44.178	$C_{28}H_{48}O$	400	25.6
S. galucum	Alloaromadendrene	13.250	$C_{15}H_{24}$	204	12.2
	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl	6.048	$C_{15}H_{24}O$	220	12.8
	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)	27.165	$C_{15}H_{22}O$	218	13.0
	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)	29.526	$C_{20}H_{34}O_2$	306	28.6
	3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide	31.358	$C_{15}H_{18}O_4$	262	23.7
	Campesterol	44.209	$C_{28}H_{48}O$	400	31.8

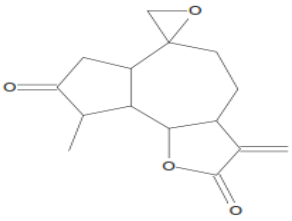
Furthermore, the structures of the most common compounds are illustrated in Figure 3. However, the prevailing compounds were: fatty acids and their derivatives (tetradecanoic acid, ethyl ester and methyl ester of hexadecanoic acid, methyl ester of pentadecanoic acid, methyl ester of octadecenoic acid, methyl ester of tridecanoic acid, Trans-13-octadecenoic acid, methyl ester, and octadecatrienoic acid), terpenoid (Nootkaton-11,12-epoxide, caryophyllene, geranyl-a-terpinene, etc.), steroid (corticosterone, cis-calamenene, etc.), and others.

The previous data revealed that the extracts of current corals differ in their chemical composition besides the potency of the active metabolites. The GC/MS profiles of the potent extracts from either ethanolic or ethyl acetate crudes were obtained, and it was found that the most found compounds were: fatty acids and their derivatives, terpenoid (nootkaton-11,12-epoxide, caryophyllene, geranyl-a-terpinene, etc.), steroid (corticosterone, cis-calamenene, etc.), and others. As the same, several workers employed the GC/MS of *Spongia officinalis* extracts conducting the major constituents were fatty acids and their esters (hexadecanoic acid and octadecanoic acid) which have antimicrobial effect (Abou-Elela *et al.*, 2009; Ibrahim *et al*., 2012).

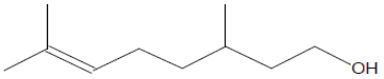


Geranyl-a-terpinene

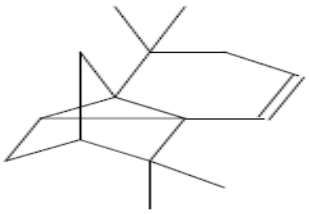
-Hexadecanol, 2-methyl



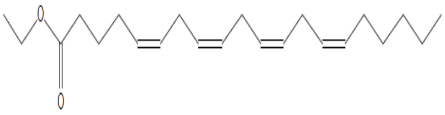
3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide



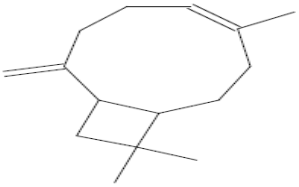
6-Octen-1-ol, 3,7-dimethyl-, (R)-(called also; Citronellol)



Cycloisolongifolene, 8,9-dehydro-

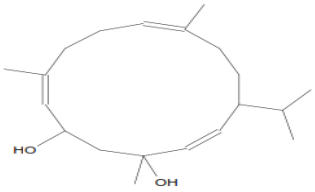


5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-



Caryophyllene

Hexadecanoic acid, ethyl ester



4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)

trans-13-Octadecenoic acid, methyl ester

9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)

Tridecanoic acid, methyl ester

Pentadecanoic acid, methyl ester

Hexadecanoic acid, methyl ester

Figure 3: Structures of the most common compounds in the potent extracts.

Furthermore, the GC/MS patterns conducted by several workers (Ibrahim *et al.*, 2018; Ibrahim *et al.*, 2020a; Ibrahim *et al.*, 2020b) were rather in the same direction, which reported that the major constituents of the effective extracts were: fatty acids (tridecanoic acid, hexadecanoic acid, pentadecanoic acid, oleanoic acid, etc.) and their esters, terpenoids, carotenoids, other compounds. These findings also observed most of these bioactive constituents had antimicrobial activities.

Most fatty acids found in the crudes were palmitic acid, oleanolic acid and octadecanoic acid, while fatty acids derivatives were ethyl ester of pentadecanoic, hexadecanoic, tetradecanoic, otadecanoic acids and methyl ester of hexadecanoic, nonadecanoic and tetradecanoic acids. Similarly, many significant constituents such as: palmitic acid, stearic acid, myristic acid, phenols, acetogenins, terpenes, labdane diterpenes, brominated hydroquinones, phlorotannins, and tropodithietic acid, which may produce antibiosis against fungi (Agoramoorthy *et al*., 2007; Balamurugan *et al*., 2013).

In addition, Duh *et al.* (2002) discovered four new diterpenes from the soft coral; *Cespitularia hypotentaculata*, besides six new cadinene sesquiterpenoids, xenitorins A-F, were isolated from the soft coral; *X. puertogalerae.* Moreover, Duh *et al.* (2004) discovered new cytotoxic steroids from both soft corals *Dendronephthya gigantean* and *Lemnalia cervicorni*. Liang and Fang (2006) obtained diterpenes from some *Sarcophyton* species. Sponges are also a good source of unusual sterols. The sulphated and alkaloidal sterols showed potential antimicrobial activity. The existence of terpenoids in sponges is widespread. Most of these compounds exhibit bioactivities. The marine sponge *Tethya aurantia* produces the ether lipids (2*S*)-1-(hexadecyloxy) propane-2, 3-diol, (2*S*)-1-(16 methylheptadecyloxy) propane-2, 3-diol (Ibrahim *et al*., 2017). Moreover, *Spongia officinalis* is well kwon as a source of terpenoids. Antifungal and antibacterial activities have been recorded by the tetracyclic furanoditerpenes isolated from sponge *S. officinalis* (Abou-Elela *et al*., 2009).

3.5. Comparing efficacy of palmitic acid and commercial antifungal drug (Treflucan)

Later, we carried out a trail on the effect of selected fatty acids (especially on palmitic acid as a potent example of bioactive constituents present in extracts) in comparison to antifungal drug (Treflucan) against both *A. niger* and *P. auratiogriseum*,, which they were chosen based on results of MICs in Tables 3 and 4. Data shown in Table 8, revealed that low concentrations (10 and 20 mg/mL) of both palmitic acid and Treflucan did not exhibit any activity against *A. niger*. This appeared because the extracts actually had other constituents not palmitic acid alone. From 50 to 150 mg/mL of both antifungal agents, the AU activity raised from 3.1 to 6.3 against *A. niger*. Only 150 mg/mL showed moderate activities recorded by palmitic acid (AU = 2.3) and Treflucan (AU = 3.1) against *P. auratiogriseum*, respectively. Surprisingly, most of these components, especially palmitic, stearic and tridecanoic acids, methyl ester, had been proven to possess antifungal activities (Rajeswari *et al.,* 2013; Revathi *et al.,* 2014; Kaur *et al.,* 2016).

Table 8: Comparison of (palmitic acid) as representative for the most effective compounds detected in the extracts detected by GC/MS analysis and commercial antifungal drug (Treflucan; Fluconazole).

Agent concentration (mg/mL)	Antifungal activity (AU)/*A. niger*		Antifungal activity (AU)/*P. auratiogriseum*	
	Palmitic acid	Treflucan	Palmitic acid	Treflucan
10	- ve	- ve	- ve	- ve
25	- ve	- ve	- ve	- ve
50	3.1	3.1	- ve	- ve
75	4.0	4.0	- ve	- ve
100	4.0	5.1	- ve	- ve
150	6.3	6.3	2.3	3.1

4. Conclusion

Data obtained during the current study confirmed the idea of the marine invertebrates' usage, particularly soft coral and sponge species, as promising sources for antifungal medication. However, our results proved that both of ethanolic and ethyl acetate extracts of soft corals (*S. polydactyla, S. gracile, S. glaucum, S. trocheliophorum, S. ehrenbergi*, and *X. macrospiculata*) were the most effective extracts that exhibited the most potent antifungal activity (AU). Moreover, the most effective extracts were tested to determine the MICs against the most affected fungi and the lowest effective MIC was 10 mg/mL against several pathogenic fungi. Furthermore, the GC/MS analysis for the most effective extracts showed that fatty acids and their derivatives, terpenoid, steroid, and others were the major constituents. Data also proved the efficacy of palmitic acid as a potent example included in the bioactive constituents present in the extracts, especially when it was compared to Treflucan (a commercial antifungal drug).

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Authors' Contributions

Study Design- Hassan A.H. Ibrahim and EL-Sayed M. El-Morsy; Data Collection- Mohamed S. Amer, Aml Z. Farhat and Mohamed Abu El-Regal; Data Interpretation- Hassan A.H. Ibrahim and EL-Sayed M. El-Morsy; Manuscript Preparation and Literature Search- Marwa T. Mohsien, Mohamed S. Amer and Aml Z. Farhat; Manuscript Revision and Supervision- Hassan A.H. Ibrahim and EL-Sayed M. El-Morsy

Conflict of Interest Disclosure

The above-mentioned manuscript has not been published before and is not under consideration for publication anywhere else. The publication of this article was approved by all authors, as well as by the responsible authorities.

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