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Research Article

Biological and Chemical Investigation of the Soft Coral *Lobophytum pauciflorum* Collected from the Egyptian Red Sea

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ABSTRACT
Soft corals of the genus *Lobophytum*, a marine invertebrate, have shown diverse biological activities as antiinflammatory, cytotoxic, and antibacterial. Our research is concerning with biological screening and discovering bioactive substances from the Red Sea soft coral *Lobophytum pauciflorum*. *In vitro* cyclooxygenase inhibitory activity using COX-1 and COX-2 kits and antimicrobial screening were carried out for n-hexane, dichloromethane, ethyl acetate and methanol fractions. The isolated compounds were elucidated using different spectroscopic methods including nuclear magnetic resonance and mass spectrometry. Also n-hexane fraction was subjected to GC/MS analysis. Bioassay guided fractionation resulted in isolation and characterization of two bio-active metabolites nephthenol (**2**) and gorgost-5-ene-3β-ol (**3**) with significant *in vitro* antiinflammatory activity against COX-1 and COX-2 compared to Indomethacin and Celecoxib. Four other compounds were also isolated: Heptadecan-1-ol (**1**), palmitic acid (**4**), stearic acid (**5**) and batilol (**6**). The isolated compounds showed antimicrobial activity ranging from 25 µg/ml to 50 µg/ml against the tested microorganisms. The fatty acid constituents of the *n*-hexane fraction were identified by GC/MS analysis; results revealed the presence of hexadecanoic acid, methyl ester as major saturated fatty acid and 7,10-hexadecadienoic acid, methyl ester as major unsaturated fatty acid.

Keywords: *Lobophytum pauciflorum***,** gorgost-5-ene-3β-ol**,** nephthenol, COX-1, COX-2 and GC/MS.

INTRODUCTION

Marine environment is considered as a reservoir for bioactive secondary metabolites, which are chemically and biologically differ from those metabolites of terrestrial origin. Many of marine organisms produce these metabolites as a mean of defense against predators[1-3]. Soft corals of the genus *Lobophytum*, a marine invertebrate of the subclass Alcyonaria, is rich source of diterpenes, lipids, sesquiterpenes and hydroxylated steroids[4]. Cembrane diterpenes previously isolated from *Lobophytum* species; have shown diverse biological activities as ichthyotoxic[5], cytotoxic[6-8], antiarthritic, antiinflammatory[9], antibacterial[10] and Ca-antagonist[11]. A sample of *Lobophytum pauciflorum* was collected for chemical investigation and for discovering bioactive substances from the Red Sea marine organisms. Bioassay guided fractionation of the bio-active fractions resulted in isolation and characterization of two bio-active metabolites nephthenol (**2**) and gorgost-5-ene-3β-ol (**3**) together with other four compounds heptadecan-1-ol (**1**), palmitic acid (**4**), stearic acid (**5**) and batilol (**6**) from the dichloromethane and ethyl acetate soluble successive fractions.

MATERIAL AND METHODS

General Experimental Procedures

The UV spectra were acquired in methanol using Thermo Scientific NanoDrop 2000C UV-Vis spectrophotometer. GC/MS of fatty acid analysis; HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector. Using capillary column, TR-FAME (Thermo 260 M142 P) (30 m, 0.25 mm ID, 0.25υm Film) (70% Cyanopropyl– Polysilphphenylene siloxane). The IR spectra were measured using JASCOFT/IR-300E spectrophotometer. ^{1}H, ^{13}C NMR and DEPT-Q spectra were recorded at 25°C with a Bruker 400 MHz NMR spectrophotometer. Jeol mass spectrophotometer, 70 ev was used for mass analysis. Column chromatography was carried out with silica gel (70-230 mesh size) and polyamide (50-160µm). All chemical reagents were purchased from Sigma-Aldrich and used without further purification.

Biological Material

The soft coral *Lobophytum pauciflorum* Ehrenberg, 1834 (order Alcyonacea) was collected from Hurghada at the Egyptian Red Sea coast by scuba at depths of 2-3 m in March 2012. *L. pauciflorum* has a limited distribution throughout small patches in front of Hurghada Marine

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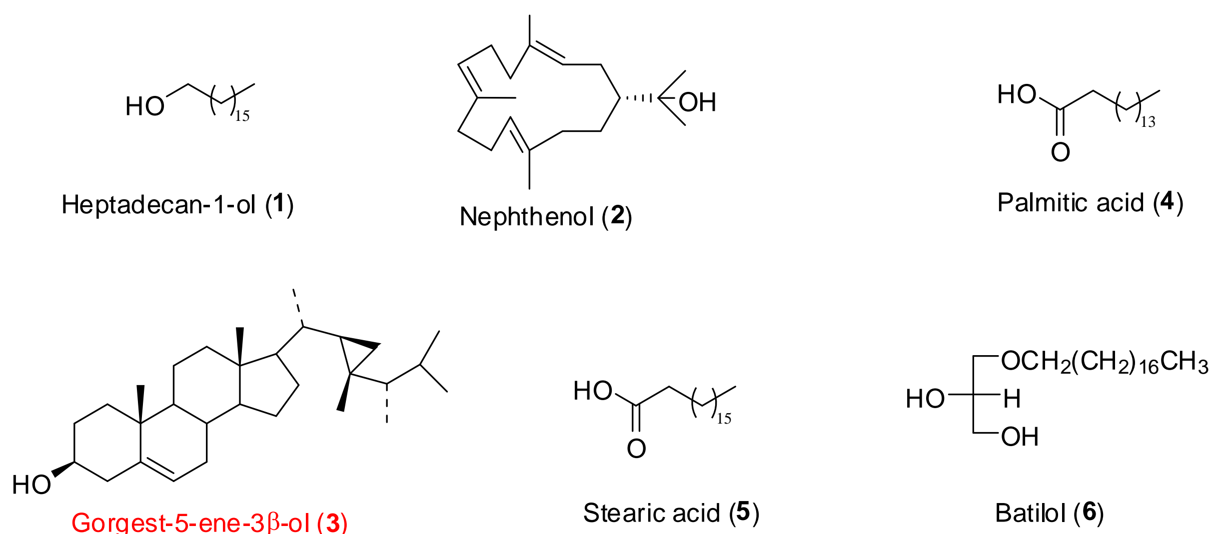

Figure 1: Structures of the isolated compounds.

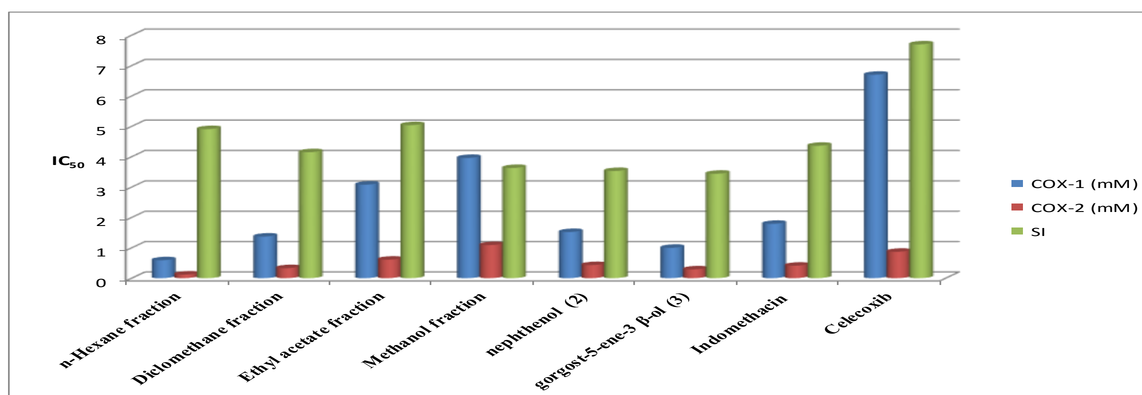

Figure 2: Results of *in vitro* antiinflammatory activity of *Lobophytum pauciflorum* fractions and isolated compounds (**2** and **3**) against COX-1 and COX-2".

Biological Institution (Natinal Institute of Oceanography and Fisheries). A specimen was collected by researchers in the National Institute of Oceanography and Fisheries, Red Sea Branch, Invertebrates Department. A voucher specimen has been deposited in the National Institute of Oceanography and Fisheries, Red Sea Branch, Hurghada, Egypt, (RT 10-2012).

Extraction and Isolation

Lobophytum pauciflorum (1 Kg fresh weight) was soaked in methanol (2L X 3) and the methanol extract was collected and further evaporated under reduced pressure to give a total extract of semisolid brown residue (30 gm). The methanol extract was successively partitioned using n-hexane, dichloromethane, ethyl acetate and n-butanol then was concentrated under reduced pressure and screened for their antibacterial effect and in vitro anti-inflammatory activity. The bio-active dichloromethane and ethyl acetate fractions (10 gm) were further fractionated on silica gel column using n-hexane-ethyl acetate-methanol in a gradient elution manner to give 110 fractions (codes Fr1-Fr110). Similar fractions were pooled together after TLC screening. NMR-guided analysis revealed that sub-fractions (14-17), (19-27), (45-48), (49-56), (58-66) and (90-100) had to be followed. Firstly Sub-Fr14 was chromatographed on silica gel column using n-hexane; increasing polarity with ethyl acetate affording 50 fractions. Sub-fractions (Fr40-Fr49) were combined and further chromatographed on silica gel column using petroleum ether; increasing polarity with ethyl acetate affording 50 fractions. These steps afforded compound **1** (10 mg). Further Sub-Fr19-27 gave pure compound **2** (30 mg) and Sub-Fr45-48 was purified using sephadex LH-20 chromatographic column and dichloromethane: n-hexane as solvent system in a ratio of (1:1) to yield compound **3** (25 mg). Sub-Fr49-56 was purified by applying on silica gel column using solvent system n-hexane, ethyl acetate in a gradient elution to obtain compound **4** (5 mg). Sub-Fr58-66 was purified by applying on silica gel column using solvent system n-hexane, ethyl acetate in a gradient elution to afford compound **5** (8 mg). Finally Sub-Fr90-100 was purified by applying on sephadex LH-20 chromatographic column using a solvent system n-hexane: dichloromethane

Table 1: Results of GC/MS analysis of the fatty acid methyl esters of *Lobophytum pauciflorum*.

Peak No.	Identified compound	*RT	**RRT	% Area
1	Methyl tetradecanoate	18.58	0.78	3.74
2	Tetradecanoic acid, ethyl ester	19.69	0.83	1.23
3	1,1,3-Trimethylurea	20.87	0.88	9.26
4	1,5,9-Cyclotetradecatriene, 1,5,9-trimethyl-12-(1-methylethenyl)	22.69	0.96	3.72
5	Hexadecanoic acid, methyl ester (Methyl palmitate, compound 4)	23.71	1	25.01
6	Methyl hexadec-9-enoate	24.52	1.03	2.41
7	Hexadecanoic acid, ethyl ester (Ethyl palmitate, compound 4)	24.68	1.04	9.39
8	7,10-Hexadecadienoic acid, methyl ester	25.51	1.08	5.38
9	Hexadecanoic acid, 15-methyl-, methyl ester	26.08	1.09	1.87
10	*Z,Z*-10,12-Hexadecadien-1-ol acetat	26.37	1.11	3.66
11	Octadecanoic acid, methyl ester (Methyl stearate, compound 5)	28.41	1.19	6.84
12	Urea	29.03	1.22	8.57
13	Octadecanoic acid, ethyl ester (Methyl stearate, compound 5)	29.28	1.23	3.02
14	8,11-Octadecadienoic acid, methyl ester	30.13	1.27	1.02
15	5,8,11,14-Eicosatetraenoic acid, methyl ester	35.81	1.51	3.35
16	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis (1-methylethenyl)-, [1*S*-(1α, 2β,4β)]	36.22	1.53	11.53
	Total identified compounds			100

*RT= Retention Time

**RRT= Relative Retention Time to Hexadecanoic acid, methyl ester

Table 2: Results of antimicrobial screening of different fractions from *Lobophytum pauciflorum* against different microorganisms.

Sample	*Pseudomonas aeruginosa*	*Staphyllococcus aureus*	*Bacillus subtilis*	*Mycobacterium pheli*	*Sarcina lutea*	*Candida albicans*
n-Hexane fraction	13*	13	15	13	14	9
Dichloromethane fraction	12	13	13	12	10	10
Ethyl acetate fraction	12	15	15	10	14	12
Methanol fraction	11	10	13	12	12	10
Standards;						
Tetracycline	31	28	30	30	30	------
Amphotericin B	------	------	------	------	------	20
DMSO	0.0	0.0	0.0	0.0	0.0	0.0

* Diameter of the zone of inhibition (mm).

The test was done using Agar diffusion method.

Standards were used in concentration (5mg/ml) while alcoholic extract in concentration (10 mg/ml).

Well diameter: 6 mm, 100 µl of each concentration was tested.

in a ratio (1:1) to yield compound **6** (5 mg). Preparation of fatty acid methyl esters and GC/MS analysis of the prepared fatty acid methyl esters were done according to previous literature[12].

Structure elucidation of isolated compounds

Structural elucidation of the isolated compounds was based on EI-MS, ^{1}H NMR, ^{13}C NMR and DEPT-Q spectroscopic data.

Compound, (**1**): white powder; IR (CH_2Cl_2) ν_{max} 3437.49, 2852.24, 1704.76, 1465.63, 1294.97 and 941.09 cm^{-1}; EI-MS gave a molecular ion peak at *m/z* 256.39 corresponding to $[M]^+$; with characteristic fragments at 265.39, 227.35, 213.31, 185.26, 157.25, 143.22, 97.20, 83.21, 73.14 and 555.14. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.98 (2H, t, *J*=8.0 Hz, CH_2), 2.21 (2H, t, *J*=8.0 Hz, CH_2), 1.54 (br.), 1.18 (s, -$(CH_2)_n$-), 0.81 (3H, t, *J*= 8.0 Hz, CH_3). DEPT-Q ($CDCl_3$, 100 MHz) δ: 14.11 (CH_3), 22.69 (CH_2), 25.03 (CH_2), 25.94 (CH_2), 28.65 (CH_2), 29.26 (CH_2), 29.36 (CH_2), 29.68 (CH_2), 31.92 (CH_2), 34.41 (CH_2), 64.40 (CH_2). These data are in agreement with the literature data[13] of heptadecan-1-ol; compound (**1**) was identified as heptadecan-1-ol isolated for the first time from *Lobophytum pauciflorum*.

Compound, (**2**): yellow oil; IR (CH_2Cl_2) ν_{max} 3430.74, 2971.77, 2928.38, 1712.48, 1668.12, 1159.01, 1445.39 and 1379.82 cm^{-1}; EI-MS gave a molecular ion peak at *m/z* 290.26 corresponding to $[M\text{-}H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.10 (1H, t, *J*=8.0 Hz, CH), 4.97 (1H, t, *J*=8.0 Hz, CH), 4.93 (1H, t, *J*=8.0 Hz, CH), 1.18 (6H, s, 2CH_3), 1.55 (3H, s, CH_3), 1.56 (6H, s, 2CH_3). DEPT-Q ($CDCl_3$, 100 MHz) δ: 15.26 (CH_3), 15.49 (CH_3), 15.53 (CH_3), 23.99 (CH_2), 24.65 (CH_2), 27.42 (CH_3), 27.61 (CH_3), 28.27 (CH_2), 28.42 (CH_2), 37.71 (CH_2), 38.83 (CH_2), 39.40 (CH_2), 48.44, 73.80 (q), 124.89, 125.76, 126.03, 132.89 (q), 133.15 (q) and 133.98 (q). These data are the same as previously reported data[14-16] of nephthenol diterpene previously isolated from *Lobophytum pauciflorum*.

Compound, (**3**): white powder; IR (CH_2Cl_2) ν_{max} 3424.96, 2939.95, 2871.49, 1719.23, 1458.89 and 1376.93 cm$^{-1;}$ EI-MS gave a molecular ion peak at *m/z* 426.65 corresponding to $[M]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: -0.01 (1H, m), 0.31

Table 3: Minimum Inhibitory Concentration (MIC) of the isolated compounds against different microorganisms using broth microdilution method.

Tested Compounds	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*	*Mycobacterium pheli*	*Sarcina lutea*	*Candida albicans*
Heptadecan-1-ol (1)	50 µg/ml	50 µg/ml	50 µg/ml	25 µg/ml	50 µg/ml	50 µg/ml
Nephthenol (2)	ND[a]	ND	ND	ND	ND	50 µg/ml
Gorgost-5-ene-3*β*-ol (3)	ND	ND	ND	ND	ND	50 µg/ml
Palmitic acid (4)	ND	ND	ND	ND	ND	ND
Stearic acid (5)	ND	ND	50 µg/ml	ND	ND	ND
Batilol (6)	ND	ND	25 µg/ml	25 µg/ml	ND	50 µg/ml

[a] ND: Not detected at the highest measured concentration of 500 µg/ml.

(1H, m), 0.36 (1H, m), 0.59 (1H, m), 0.80 (3H, s), 0.90 (3H, s), 1.10 (15H, s), 3.65 (1H, m), 5.47 (1H, br). DEPT-Q ($CDCl_3$, 100 MHz) δ: 11.9 (CH_3), 14.3 (CH_3), 15.7 (CH_3), 19.4 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 21.1 (CH_2), 21.3 (CH_2), 24.5 (q), 28.1 (CH_2), 28.2 (CH_2), 31.5 (CH), 31.6 (CH_2), 31.7 (CH), 31.8 (CH_2), 32.0 (CH), 36.2 (CH), 37.2 (CH_2), 39.8 (CH_2), 39.8 (q), 39.9 (q), 42.2 (CH_2), 50.0 (CH), 50.1 (CH), 50.7 (CH), 56.7 (CH), 71.7 (CH), 121.8 (CH), 140.7 (q). These data are in consistence with reported data of gorgost-5-ene-3β-ol; compound (**3**) was identified as gorgost-5-ene-3β-ol previously isolated from *Lobophytum pauciflorum*[17]. It was reported from soft coral gorgonian *Isis hippuris*[18], *Sarcophyton trocheliophorum*[19] and from *Heteroxenia fuscescens*[20].

Compound, (**4**): white powder; IR (CH_2Cl_2) v_{max} 3382.53, 2923.56, 2853.17, 1735.62, 1465.63 and 1179.26 cm^{-1}; EI-MS gave a molecular ion peak at *m/z* 257.39 corresponding to $[M+H]^+$ with characteristic fragments at 257.41, 229.37, 196.34, 153.27, 129.19, 97.19, 83.17 and 57.16. ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.34 (2H, t, *J*=8.0 Hz, CH_2), 1.63 (2H, t, *J*=8.0 Hz, CH_2), 1.26 (s, $-(CH_2)_n-$), 0.87 (3H, t, *J*= 8.0 Hz, CH_3). DEPT-Q ($CDCl_3$, 100 MHz) δ: 14.11 (CH_3), 22.69 (CH_2), 24.70 (CH_2), 29.07 (CH_2), 29.24 (CH_2), 29.36 (CH_2), 29.44 (CH_2), 29.59 (CH_2), 29.65 (CH_2), 29.67 (CH_2), 29.68 (CH_2), 31.92 (CH_2), 34.05 (CH_2), 179.60 (q). From previous literature[17] compound (**4**) identified as palmitic acid; previously isolated from *Lobophytum pauciflorum*.

Compound, (**5**): white powder; IR (CH_2Cl_2) v_{max} 3390.24, 2922.59, 2853.17, 1708.62, 1463.71, 1294.00 and 1117.55 cm^{-1}; EI-MS gave a molecular ion peak at *m/z* 284.45 corresponding to $[M]^+$ with characteristic fragments at 284.45, 256.42, 227.36, 129.21, 97.20, 83.18, 73.07 and 55.13. ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.27 (2H, t, *J*=8.0 Hz, CH_2), 1.56 (2H, m), 1.23 (2H, m), 1.18 (s, $-(CH_2)_n-$), 0.79 (3H, t, *J*= 8 Hz, CH_3). DEPT=Q ($CDCl_3$, 100 MHz) δ: 14.13 (CH_3), 22.70 (CH_2), 24.29 (CH_2), 29.07 (CH_2), 29.25 (CH_2), 29.37 (CH_2), 29.44 (CH_2), 29.60 (CH_2), 29.65 (CH_2), 29.67 (CH_2), 29.70 (CH_2), 31.93 (CH_2), 34.00 (CH_2), 179.68 (q). Comparing these data with literature[13]; compound (**5**) identified as Stearic acid; isolated for the first time from *Lobophytum pauciflorum*.

Compound, (**6**): white powder; IR (CH_2Cl_2) v_{max} 3422.06, 2920.66, 2852.20, 1730.80, 1636.30, 1462.74, 1247.72, 1121.40 and 1062.59 cm^{-1}; EI-MS gave a molecular ion peak at *m/z* 345.75 corresponding to $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.65 (2H, m), 3.45 (1H, m), 3.43 (1H, m), 3.39 (2H, m), 3.79 (1H, m), 1.52 (6H, m), 1.19 (24H, br s), 0.81 (t, *J*= 8.0 Hz). DEPT=Q ($CDCl_3$, 100 MHz) δ: 14.13 (CH_3), 22.70 (CH_2), 26.09 (CH_2), 29.37 (CH_2), 29.46 (CH_2), 29.59 (CH_2), 29.61 (CH_2), 29.68 ($2CH_2$), 29.67 ($2CH_2$), 29.70 ($5CH_2$), 31.93 (CH_2), 64.31 (CH_2), 70.40 (CH), 72.54 (CH_2). From previously reported data[13] and[21,22] compound (**6**) was identified as batilol; isolated for the first time from *Lobophytum pauciflorum*.

Antimicrobial screening

Antimicrobial screening were carried out using Agar diffusion method[23]. The bacterial stains used for antimicrobial screening were; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium pheli*, *Sarcina lutea* and one fungal strain *Candida albicans* from the Micro Analytical center, Cairo University for antimicrobial screening and from Microbiology Department, Faculty of Pharmacy, Beni-Suef University for determination of MIC. For further investigation, minimum inhibitory concentration (MIC) was determined using broth micro dilution method[24] using Muller Hinton broth, serial dilution of the tested compounds were used. MIC was stated as the lowest concentration of the compound resulted in no visible growth. The MIC was measured after 24 hours of incubation at 37ºC for bacteria and 25 ºC for fungus.

In vitro cyclooxygenase inhibitory activity

The capacity of the tested compounds to inhibit COX-1/COX-2 was measured using colorimetric COX (ovine) Inhibitor Screening Assay Kit (Kit catalog number 760111, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions and previous literature[25,26]. Different concentrations of tested compounds, Indomethacin and celecoxib were incubated with the enzymes for a period of 5 min at 25ºC. After the incubation period and the addition of colorimetric substrate and arachidonic acid, the absorbance was measured at 590 nm using plate reader. All measurements were done in duplicate.

Statistical analysis

IC_{50} value represents the compound concentration that is required to produce 50 % inhibition of COX-1 or COX-2 which is the mean value of two determinations where the deviation from the mean is‹10 % of the mean value Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}) were calculated for each sample.

RESULTS AND DISCUSSION

Table 4: Results of antiinflammatory activity of *Lobophytum pauciflorum* fractions; and compounds (**2** and **3**) against COX-1 and COX-2.

Sample	IC_{50}[a]		
	COX-1 (mM)	COX-2 (mM)	SI[b]
n-Hexane fraction	0.59	0.12	4.91
Diclomethane fraction	1.37	0.33	4.15
Ethyl acetate fraction	3.08	0.61	5.04
Methanol fraction	3.96	1.09	3.63
Nephthenol (2)	1.52	0.43	3.53
Gorgost-5-ene-3β-ol (3)	1.00	0.29	3.44
Sandards;			
Indomethacin	1.79	0.41	4.36
Celecoxib	6.7	0.87	7.70

[a] IC_{50} value represents the compound concentration that is required to produce 50 % inhibition of COX-1 or COX-2 which is the mean value of two determinations where the deviation from the mean is ‹10 % of the mean value.

[b] Selective Index IC_{50} of COX-1/ IC_{50} of COX-2

In this study, six known compounds (Figure 1) were isolated from the marine soft coral and identified as heptadecan-1-ol **(1),** nephthenol **(2),** gorgost-5-ene-3β-ol **(3),** palmitic acid **(4),** stearic acid **(5)** and batilol **(6)**. The compounds heptadecan-1-ol **(1),** stearic acid **(5)** and batilol (**6**) were isolated from the first time from *Lobophytum pauciflorum*. GC/MS analysis (Table 1) shows that, the identified components of *Lobophytum pauciflorum* fatty acid methyl ester constituents represent 100%. The major saturated fatty acid was Hexadecanoic acid, methyl ester of *Lobophytum pauciflorum* fatty acid methyl ester constituent (25.01%). While the major unsaturated fatty acid was 7,10-Hexadecadienoic acid, methyl ester (5.38%). GC/MS analysis of *Lobophytum pauciflorum* fatty acid constituents also revealed the presence of the isolated compounds [palmitic acid (**4**) and stearic acid (**5**)]. Antimicrobial screening and minimum inhibitory concentration (MIC) of the isolated compounds against some bacterial strains were studied and the results exemplified in (Table 2 and 3; respectively). Antimicrobial screening (Table 2) shows that *n*-hexane and ethyl acetate showed moderate activity against *Bacillus subtilis*, *Staphyllococcus aureus* and *candida albicans* compared to the standards Tetracycline and Amphotricin B. MIC (minimum inhibitory concentration) (Table 3) shows that compound **4** (palmitic acid) have no activity against the tested bacteria, while the other five isolated compounds showed activity ranging from 25 µg/ml to 50 µg/ml. Compound **1** (Heptadecan-1-ol) shows inhibitory activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Sarcina lutea* and *Candida albicans* with MIC 50 µg/ml, *Mycobacterium pheli* with MIC 25 µg/ml. Compound **5** (Stearic acid) show antibacterial activity against *Bacillus subtilis* with MIC 50 µg/ml. Compound **6** (batilol) show antibacterial activity against *Bacillus subtilis*, *Sarcina lutea* with MIC 25 µg/ml and *Candida albicans* with MIC 50 µg/ml. The two metabolites nephthenol **2** and gorgost-5-ene-3β-ol **3** show only antifungal activity against *Candida albicans* with MIC 50 µg/ml.

In vitro cyclooxygenase (COX) inhibition assay

The capacity of the fractions and the two isolated compounds (**2** and **3**) to inhibit cyclooxygenase enzymes COX-1 and COX-2 was determined and the effectiveness of the tested samples were measured through determination of IC_{50} for both enzymes, the results illustrated in (Table 4 and Figure 2). Results show that; n-hexane, dichloromethane and compounds (**2** and **3**) showed significant antiinflammatory activity against COX-1 compared with positive control; Indomethacin with IC_{50} (0.59, 1.37, 1.52 and 1.00 mM; respectively). Also n-hexane, dichloromethane, ethyl acetate and the isolated compounds (**2** and **3**); showed significant antiinflammatory activity against COX-2 compared with Celecoxib the positive control with IC_{50} (0.12, 0.33, 0.61, 0.43 and 0.29 mM; respectively). The selectivity index (SI) of the tested samples was calculated as (COX-1 IC_{50}/COX-2 IC_{50}) it measures the selectivity towards inhibition of COX-2. The results for the n-hexane and ethyl acetate revealed that they are selectively inhibit COX-2 (SI= 4.91 – 5.04) compared to standards Celecoxib (SI= 7.70) and Indomethacin (SI= 4.36).

CONCLUSION

Biology-guided screening showed effective method for selection of biologically interesting extracts. In this study, six known metabolites were isolated depending on antimicrobial and antiinflammatory screening of *L. pauciflorum* collected from the Red Sea, Hurghada, Egypt. The structures of these compounds were fully characterized using different spectroscopic techniques. Compounds (**2** and **3**) showed significant anti-inflammatory activity against both COX-1 and COX-2. Re-collection of this soft coral in large quantities for further isolation of these active metabolites will provide us with additional deep studies of the mechanism of action and possible chemical modifications. Also, different fractions of the soft coral showed antimicrobial activity against the tested pathogens of Gram positive, Gram negative and fungal strains.

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