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RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Cytotoxic and Antileishmanial Activities of the Red Sea Soft Coral *Sarcophyton glaucum* Extract and Some of Its Isolates

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Abstract

Truthfully, natural products have been the principal productive source of advancing new drugs. The broad aim of this work was to conduct a phytochemical and biological study of the Red Sea soft coral *Sarcophyton glaucum*, Family: Alcyoniidae collected from Red Sea at the Egyptian coasts. The phytochemical investigation from the soft coral *S. glaucum* led to the isolation of ten compounds including: palmitic acid (**1**), stearic acid (**2**), (24*S*)-24-methyl cholesterol (**3**), batyl alcohol (**4**), heptadecanoic acid pentadecyl ester (**5**), sarcophine (**6**), (+)-7α, 8β-dihydroxydeepoxysarcophine (**7**), uracil (**8**), thymine (**9**) and a ceramide (**10**). As cancer is one of the most hazardous factors threatening the human life, in this study the potential *in-vitro* cytotoxicity of the soft coral *S. glaucum* extract and three marine isolates were measured against HepG2 and MCF7 using Sulphorhodamine-B (SRB) assay and all of the tested samples showed a good cytotoxic activities against both hepatic and breast cancer. The extract of *S. glaucum* was tested also against the protozoan parasite *Leishmania donovani*, using pentamidine and amphotericin B as controls and showed antileishmanial activity.

Keywords: *Sarcophyton glaucum*; cytotoxic activity; antileishmanial activity.

1. Introduction:

Marine life forms, comprising more than half of the entire worldwide organisms, offer an important source of possibly novel active compounds. Soft corals comprising an important group of marine organisms. There are about 35 species belonging to the soft coral genus *Sarcophyton*, and they are hard to be distinguished (**Verseveldt, 1982**). The soft coral *Sarcophyton* is a good source of different structures of diterpenes of cembrane type, sesquiterpenes, steroids, fatty acids and amino acids (**Jia *et al*., 2006**, **Lan *et al*., 2007**). Soft corals synthesize terpenoids as compounds used for chemical defense to avoid predatory fishes which attack their soft bodies (**Iwagawa *et al*., 1999**). So, many secondary metabolites isolated from soft corals have been proved to possess many different biological activities such as anti-microbial, anti-fungal, anti-tumor, anti-viral and anti-inflammatory (**Wei *et al*., 2013**).

In our study we are trying to discover biologically active compounds from Egyptian natural products, our group in focused on the isolation of the active compounds from the soft coral *S. glaucum*, and identification of the isolated compounds using different spectroscopic techniques. The potential *in-*

vitro cytotoxicity of the soft coral *S. glaucum* extract and three of the isolated compounds were measured against HepG2 and MCF7 and the extract was tested also against the protozoan parasite *Leishmania donovani*, using pentamidine and amphotericin B as controls.

2. Materials and Methods

2.1 Animal material:

About 5 Kg of *S. glaucum* were collected by hand using SCUBA from Safaga at the Egyptian Red Sea. It was immediately frozen and kept at (-24°C). A voucher specimen was deposited in the Egyptian Red Sea invertebrates collection at the Department of Pharmacognosy, Suez Canal University under registration number (SAA-54). The identification and description of the soft coral was provided by Dr. Tarek A. Temraz, Department of Marine Science, Faculty of Science, Suez Canal University, Ismailia, Egypt.

2.2. Extraction and fractionation of the soft coral and isolation of pure compounds:

The soft coral (5 kg) of the frozen chopped small pieces were extracted with MeOH- CH_2Cl_2 (1:1) (5L × 4) at room temperature to yield 200 g of dry extract. Fractionation using vacuum liquid chromatography (VLC) with gradient elution led to separation of different fractions. Using *n*-hexane, EtOAc, and MeOH gradient elution, F2 fraction (20 g) (25% EtOAc in hexane) was subjected to column chromatography of silica gel type, two fractions were obtained and subjected to different chromatographic separation techniques, including silica gel leading to isolation of **1** (20 mg), **2** (15 mg) and **3** (8 mg). Fractions F3 and F4 of 50%+75% EtOAc in hexane, 38 g was purified using silica gel, three subfractions were obtained and subjected to different chromatographic separation techniques, including sephadex LH-20 leading to isolation of **4** (30 mg), **5** (14 mg) **6** (50 mg) and **7** (15 mg). F5 Fraction of 100% EtOAc, 21 g was purified using silica gel column chromatography and sephadex LH-20 to yield **8** (20 mg) and **9** (15 mg). 27 g of fraction F6 (5% MeOH in EtOAc) was purified using silica gel column chromatography and sephadex LH-20 to yield **10** (25 mg).

2.3. *In-vitro* cytotoxicity of the soft coral *S. glaucum* extract and some of its isolates:

The potential *in-vitro* cytotoxicity of the soft coral *S. glaucum* extract and the isolated compounds **6**, **7** and **10** was measured against HepG2 and MCF7 using Sulphorhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara **(Vichai and Kirtikara, 2006)**.

2.4 *In-vitro* antileishmanial assay of the soft coral *S. glaucum* extract:

The extract was tested against the protozoan parasite *Leishmania donovani*, using pentamidine and amphotericin B as controls using the method of **(Bharate *et al.*, 2008; Jain *et al.*, 2005)**.

3. Results and discussion

3.1. Identification and characterization of the isolated compounds:

The structure elucidation of the isolated compounds was deduced on the basis of spectroscopic methods, (El-MS, 1D and 2D NMR), physicochemical properties in addition to comparison with literature data and/or authentic samples. The isolated compounds were identified as:

Compound **1** was identified as palmitic acid **(Figure 1) (Di Pietro *et al*., 2020)**, as molecular formula was determined to be $C_{16}H_{32}O_2$ by El-mass spectrum as the molecular ion peak appeared at *m/z* 256.08 $[M]^+$. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 2.32 (2H, *m*, H-2), 1.63 (2H, *m*, H-3), 1.27 (24H, *s*, H-4: H-15), 0.84 (3H, *t*, *J*=8.0, H-16). ^{13}C-NMR (CD_3OD-$CDCl_3$): δ 173.8 (C-1), 34.3 (C-2), 31.8 (C-3), 22.6-29.6 (C-4: C-15), 14.0 (C-16).

Compound **2** was identified as stearic acid **(**Error! Reference source not found.**) (Di Pietro *et al.*, 2020).** molecular formula was determined to be $C_{18}H_{36}O_2$ by El-mass spectrum as the molecular ion peak appeared at *m/z* 284.21 $[M]^+$. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 2.35 (2H, *m*, H-2), 1.64 (2H, *m*, H-3), 1.27 (28H, *s*, H-4: H-17), 0.89 (3H, *t*, *J*=8.0, H-18). ^{13}C-NMR (CD_3OD-$CDCl_3$): δ 179.9 (C-1), 33.9 (C-2), 31.8 (C-3), 22.6-29.6 (C-4: C-17), 14.0 (C-18).

Compound **3** was identified as (24*S*)-24-methyl cholesterol **(Figure 1) (Rahelivao *et al*., 2017)**, molecular formula was determined to be $C_{28}H_{48}O$ by El-MS spectrum (*m/z* 400.26 $[M^+]$).^{1}H-NMR (CD_3OD-$CDCl_3$): δ 0.93 (1H, *m*, H-1a), 1.86 (1H, *m*, H-1b), 1.93 (1H, *m*, H-2a), 1.54 (1H, *m*, H-2b), 3.50 (1H, *m*, H-3), 2.23 (2H, *m*, H-4), 5.35 (1H, *m*, H-6), 2.01 (1H, *m*, H-7a), 1.13 (1H, *m*, H-7b), 0.87 (1H, *m*, H-8), 1.07 (1H, *m*, H-9), 0.93 (2H, *m*, H-11), 1.20

1.33 (2H, *m*, H-12), 1.20 (1H, *m*, H-14), 0.93 (2H, *m*, H-15), 1.38 (1H, *m*, H-17), 0.67 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 1.82 (1H, *m*, H-20a), 1.86 (1H, *m*, H-20b), 0.91 (3H, *m*, H-21), 1.26 (1H, *m*, H-22a), 1.82 (1H, *m*, H-22b), 1.19 (1H, *m*, H-23a), 1.56 (1H, *m*, H-23b), 1.58 (1H, *m*, H-24), 1.50 (1H, *m*, H-25), 0.77 (3H, *m*, H-26), 0.85 (3H, *m*, H-27), 0.68 (3H, *m*, H-28). ^{13}C-NMR (CD_3OD-$CDCl_3$): δ 37.3 (C-1), 31.6 (C-2), 71.7 (C-3), 42.3(C-4), 140.8 (C-5), 121.6 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.6 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.8 (C-18), 19.4 (C-19), 36.2 (C-20), 18.9 (C-21), 33.7 (C-22), 30.6 (C-23), 39.1 (C-24), 31.5 (C-25), 17.6 (C-26), 20.5 (C-27), 15.4 (C-28).

Compound **4** was identified as batyl alcohol **(Figure 1) (Sun *et al.*, 2015)**, molecular formula was determined to be $C_{21}H_{44}O_3$ by El-MS spectrum as *m/z* 345.21 $[M+H]^+$. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 3.67 (1H, *dd*, *J*=3.6, 11.4 Hz, H-1a), 3.58 (1H, *dd*, *J*= 5.4, 11.4 Hz, H-1b), 3.84 (1H, *m*, H-2), 3.43 (2H, *m*, H-3), 3.43 (2H, *m*, H-1`), 1.55 (2H, *m*, H-2'), 1.25 (30H, *brs*, H-3`:H-17`), 0.85 (3H, *t*, *J*= 6.6 Hz, H-18`). ^{13}C-NMR (CD_3OD-$CDCl_3$): δ 71.8 (C-1), 72.3(C-2), 64.2 (C-3), 70.6 (C-1`), 31.9 (C-2`), 22.6:29.7 (C-3`:C17`), 14.0 (C-18`).

Compound **5** was identified as heptadecanoic acid pentadecyl ester **(Figure 1) (Pedpradab *et al.*, 2012)**, molecular formula was determined to be $C_{32}H_{64}O_2$ by El-MS spectrum as *m/z* 480.55 $[M+H]^+$. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 4.04 (2H, *t*, *J* = 6.8 Hz, H-1`), 2.28 (2H, *t*, *J*=6.4 Hz, H-2), 1.60 (4H, *m*, H-3, H-2`), 1.26 (50H, *s*, H-4:H-16, H-3`:H-14`), 0.84 (6H, *t*, *J*=6.8, H-17, H-15`).$^{13}$C-NMR ($CD_3OD$-$CDCl_3$): $\delta$ 174.1 (C-1), 64.5 (C-1`), 34.5 (C-2), 32.0 (C-3, C-2`), 22.8-29.8 (C-4:C-16, C-3`:C-14`),14.2 (C-17, C-15`).

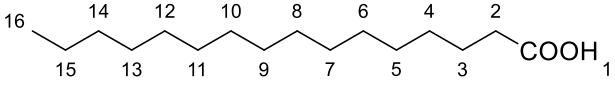
Compound **6** was identified as sarcophine **(Figure 1) (Abdel-Wahhab *et al.*, 2012)**, molecular formula was determined to be $C_{20}H_{28}O_3$ by HRESIMS as *m/z* 317.2142 $[M+H]^+$. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 5.51 (1H, *dd*, *J*=10.0, 1.5 Hz, H-2), 4.97 (1H, *dd*, *J*=10.0, 1.0 Hz, H-3), 2.30 (2H, *m*, H-5), 1.63 (2H, *m*, H-6), 2.61 (1H, *m*, H-7), 1.03, 1.86 (2H, *m*, H-9), 2.14 (2H, *m*, H-10), 5.07 (1H, *br t*, *J*=5.1 Hz, H-11), 1.98 (2H, *m*, H-13), 2.69 (2H, *m*, H-14), 1.78 (3H, *s*, H-17), 1.83 (3H, *s*, H-18), 1.21 (3H, *s*, H-19), 1.55 (3H, *s*, H-20). ^{13}C-NMR (CD_3OD-$CDCl_3$): δ 162.3 (C-1), 78.6 (C-2), 120.4 (C-3), 143.8 (C-4), 37.2 (C-5), 25.1 (C-6), 61.2 (C-7), 59.8 (C-8), 38.8 (C-9), 23.1 (C-10), 124.7 (C-11), 135.4 (C-12), 36.2 (C-13), 27.4 (C-14), 122.7 (C-15), 174.6 (C-16), 8.8 (C-17), 17.0 (C-18), 16.0 (C-19), 15.3(C-20).

Compound **7** was identified as (+)-7α, 8β-dihydroxydeepoxysarcophine **(Figure 1), (Wei Bie *et al.*, 2008),** molecular formula was determined to be $C_{20}H_{30}O_4$ by HRESIMS as *m/z* 335.2180 $[M+H]^+$. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 5.79 (1H,*d*, *J*= 10.2 Hz, H-2), 4.85 (1H, *d*, *J*= 10.2 Hz, H-3), 2.30 (1H, *m*, H-5a), 2.02 (1H, *m*, H-5b), 1.84 (1H, *m*, H-6a), 1.29 (1H, *m*, H-6b), 3.29 (1H, *d*, *J*=10.5 Hz, H-7), 1.73 (1H. *m*, H-9a), 1.46 (1H.*dt*, *J*= 9.9, 9.5 Hz, H-9b), 2.16(1H, *m*, H-10a), 1.97(1H, *m*, H-10b) 5.10 (1H, *dd*, *J*=6.5, 6.7 Hz, H-11), 2.16(1H, *m*, H-13a), 2.04(1H, *m*, H-13b), 2.65(1H, *m*, H-14a), 2.01 (1H, *m*, H-14b), 1.76 (3H, *s*, H-17), 1.81 (3H, *s*, H-18), 0.99 (3H, *s*, H-19), 1.59 (3H, *s*, H-20). ^{13}C-NMR(CD_3OD-$CDCl_3$): δ 163.2 (C-1), 79.3 (C-2), 120.7 (C-3), 144.2 (C-4), 35.4 (C-5), 26.9 (C-6), 72.7 (C-7), 75.6 (C-8), 36.9 (C-9), 23.6 (C-10), 125.3 (C-11), 134.7 (C-12), 36.5 (C-13), 26.8 (C-14), 122.6 (C-15), 175.2 (C-16), 8.9 (C-17), 16.5 (C-18), 24.2 (C-19), 15.3 (C-20).

Compound **8** was identified as uracil **(Figure 1) (Sun *et al.*, 2015)**. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 11.00(1H, *brs*, NH-1), 10.80 (1H, *brs*, NH-3), 5.41 (1H, *d*, *J*=7.6 Hz, H-5), 7. 35 (1H, *d*, *J*=7.6 Hz, H-6). ^{13}C-NMR (CD_3OD-$CDCl_3$): δ 152.1 (C-2), 164.9 (C-4), 100.8 (C-5), 142.8 (C-6).

Compound **9** was identified as thymine **(Figure 1) (Sun *et al.*, 2015)**. ^{1}H-NMR (CD_3OD-$CDCl_3$): δ 10.54 (1H, *brs*, NH-1), 10.95 (1H, *brs*, NH-3), 7.23 (1H, *s*, H-6), 1.72 (3H, *s*, H-7). ^{13}C-NMR (CD_3OD-$CDCl_3$): δ 151.5 (C-2), 164.9 (C-4), 107.7 (C-5), 137.7 (C-6), 11.8 (C-7).

Compound **10** was identified as a ceramide **(Figure 1) (Abdelhameed *et al.*, 2018)**. The mass spectrum showed a molecular ion peak at *m/z* (706 $[M+Na]^+$, 684 $[M+H]^+$), according to the results of **10** methanolysis with methanolic hydrochloric acid followed by EI-MS analysis of **10** FAME, afforded a molecular ion peak at *m/z* 398 $[M]^+$, indicating C23 fatty acid methyl esters. ^{1}H-NMR (C_5D_5N): δ 8.58 (1H, *d*, *J*=8.9 Hz, NH), 4.43 (1H, *dd*, *J*=10.8, 5.2 Hz, H-1a), 4.49 (1H, *dd*, *J*=10.8, 4.6 Hz, H-1b), 5.12 (1H, *m*, H-2), 4.35 (1H, *m*, H-3), 4.27 (1H, *m*, H-4), 4.61 (1H, *m*, H-2`),0.86 (6H, *m*, -$CH_3$), 1.27 (*s*, (-$CH_2$)$_n$). $^{13}$C-NMR ($C_5D_5N$): $\delta$ 62.0 (C-1), 52.9 (C-2), 76.7 (C-3), 73.0 (C-4), 175.2 (C-1`) 72.4 (C-2`), 14.2 (CH_3), 22.4 (CH_2).



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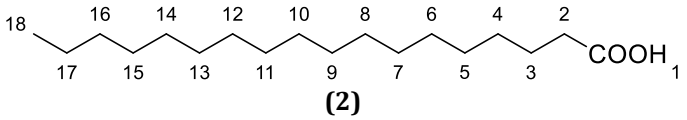

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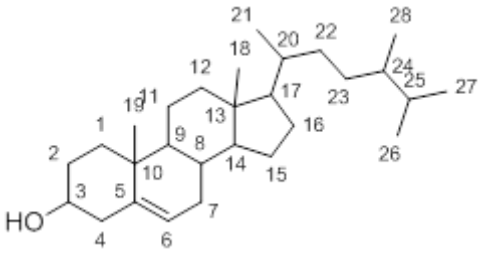

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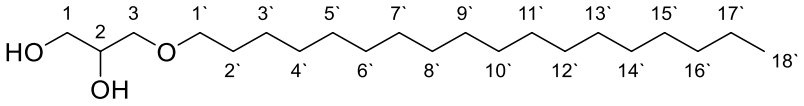

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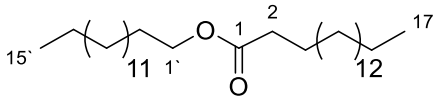

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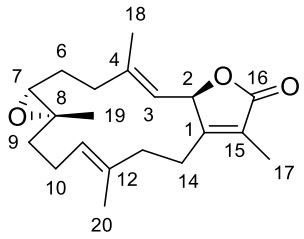

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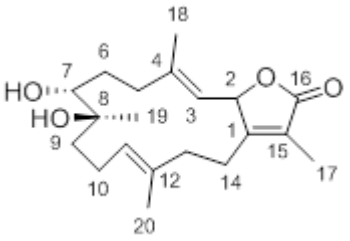

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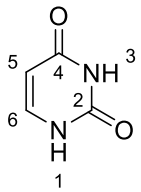

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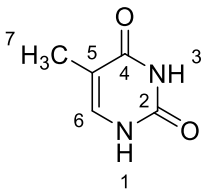

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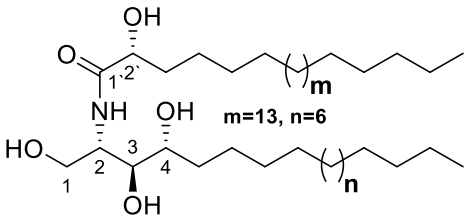

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Figure (1): Isolated compounds from *S. glaucum* extract.

Table 1: *In-vitro* cytotoxic effects (IC_{50}, µg/mL) of *S. glaucum* extract and some of isolated compounds on HepG2 and MCF7 cell lines.

Sample	HepG2	MCF7
Extract	19.3	18.7
6	10.4	8.33
7	4.88	3.98
10	10.5	11
Doxo	4.2	3.83

Table 2: Antileishmanial screening assays of the soft coral *S. glaucum* extract.

Sample	IC_{50} µg/mL	IC_{90} µg/mL
Extract	100	>100

IC_{50}: concentration causing 50% growth inhibition.
IC_{90}: concentration causing 90% growth inhibition.

3.2. *In-vitro* cytotoxicity of the soft coral *S. glaucum* extract and some of its isolates:

Screening of the total extract of *S. glaucum* and isolated compounds **6**, **7** and **10** resulted in promising cytotoxic activities against MCF-7 and HepG2 cell lines when compared to Doxorubicin as illustrated in **(Table 1)**.

3.3. *In-vitro* antileishmanial assay of the soft coral *S. glaucum* extract:

The extract was tested against the protozoan parasite *Leishmania donovani*, using pentamidine and amphotericin B as controls **(Table 2)** .This test indicated that this marine has antileishmanial activity.

4. Conclusion

The phytochemical examination of the soft coral *S. glaucum* including isolation and identification of: palmitic acid (**1**), stearic acid (**2**), (24*S*)-24-methyl cholesterol (**3**), batyl alcohol (**4**), heptadecanoic acid pentadecyl ester (**5**), sarcophine (**6**) (+)-7α, 8β-dihydroxydeepoxysarcophine (**7**), uracil (**8**), thymine (**9**) and ceramide (**10**). the soft coral *S. glaucum* extract and compounds **6**, **7** and **10** showed good anticancer activities against both hepatic and breast cancer. The extract of *S. glaucum* has antileishmanial activity.

5. Conflict of interest

The authors report no declaration of conflict of interest.

6. Funding

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