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Original Article

Chemical investigation and isolation of some constituents from *Paralemnalia thyrsoides*

Aya Ali Ahmed Alassass *[1], Marwa Samy Abubakr [2], Seif-Eldin Nasr Ayyad [3], Abd-Elsalam Ebrahim Mohammed [2]

[1]Department of Pharmacognosy, Faculty of Pharmacy Horus University, Damietta 34518, Egypt
[2]Department of Pharmacognosy, Faculty of Pharmacy AlAzhar University, Cairo, Egypt
[3]Department of Chemistry, Faculty of Science, Damietta University, Damietta, Egypt

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ABSTRACT

Soft coral constituents are an important group of marine's invertebrates widely distributed in the coral reefs of the world ocean and have proven to be a biochemical warehouse for terpenes of them, examples of these soft corals are the animals belonging to genus *paralemnalia*. The organism of *Paralemnalia* thyrsoides was frozen immediately after collection and the freeze-dried organism was extracted sequentially with EtOAc. Phytochemical investigation of Egyptian octocoral *Paralemnalia thyrsoides* lead to the isolation of two compounds were identified as lemnolin A and gorgostane. Their structures were identified using 1D & 2D-NMR spectra and by comparison with data reported in the literature. The isolated compounds were evaluated for their antifungal activity against (*Aspergillus fumigatus* and *Candida albicans*) and antibacterial activity against (gram positive bacteria *Staphylococcus aureus*) and (gram-negative bacteria *Escherichia coli*), they showed week inhibitory activity.

Keywords: *Paralemnalia thyrsoides;* lemnolin A; gorgostane; antifungal; antibacterial.

INTRODUCTION

Marine organisms produce a wide array of fascinating terpenoid structures distinguished by characteristic structural features [1] Octocorals of the order Alcyonacea are among the most common members of many tropical coral reef habitats, including the Red Sea octocorals that may occupy up 25 % of the primary space [2] Soft corals of the genus *Paralmnalia* (Alcyonaceae) are well known to be rich in sources of sesquiterpenoids and norsesquiterpenoids [3] Soft corals belonging to the genus Paralemnalia have been proved to be a rich source of terpenoids [4] Terpenes comprise primary and secondary metabolites, all derived from the five carbon isoprene entity. Combination

Address for Correspondence: Aya Ali Ahmed Alassass, Department of Pharmacognosy, Faculty of Pharmacy, Horus University, Damietta 34518, Egypt; Email: Ayamnalgamal7@gmail.com

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and modifications of these isoprene units leads to a multitude of diverse structures with different chemical and biological properties. Terpenoids from higher plants are well studied and pharmacologically applied since centuries. However, it was not until the early to mid-20th century that their marine counterparts were explored Secondary metabolites. Several biologically active terpenoids turned out to possess biomedical potential and are thus already in preclinical or clinical development The anti-inflammatory potential of pseudopterosins is superior to that of standard drugs such as indomethacin [1]

MATERIALS AND METHODS

Materials.

Soft coral material: Soft bodied coral, *Paralemnalia thyrosides* (Order Alcyonacea, Family Nephtheidae was collected from the North of Jeddah Saudi Arabia Red Sea coast (21°29′31″N 39°11′24″E) at Jeddah, at a depth of 5-10 m (Jan. 2019). A voucher specimen (SC-2013-1) was deposited in the faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Chemicals and solvents:

Reagents for chromatographic & biological study: The solvents used for extraction, chromatographic separation and crystallization {methanol, Chloroform, Petroleum ether (EL-Nasr Company for Pharmaceutical Chemicals, Egypt)} were of reagent grade. Pre-coated TLC plates 20 x 20 cm, Silica Gel 60 F254, layer thickness 0.2 mm (Merck, Germany). Normal phase chromatography was carried out using silica gel G 60 (0.063-0.200 mm,) 70-230 meshastm (Merck, Germany) packed by the wet method in the stated solvent. Pre-coated TLC glass plates SIL G-25 UV254, 0.25 mm silica gel. Size exclusion gel "Sephadex LH-20, GE healthcare company, USA".

Gentamycin (Alexandria Pharmaceutical Co., Egypt) used as control for antibacterial activity and ketoconazole (SEDICO Pharmaceutical Co., Egypt) used as control for antifungal activity. Microorganism; *Aspergillus fumigatus* (RCMP 002008) and *Candida albicans* (RCMP 005003) (1) ATCC 10231), *Aspergillus fumigatus* (RCMP 002008) and *Candida albicans* (RCMP 005003) (1) ATCC 10231).

Reagents for phytochemical screening: Alcoholic α-naphthol 15%, concentrated sulfuric acid, ferric chloride 5%, alcoholic potassium hydroxide 5%, hydrochloric acid 20%, silver nitrate 0.02 N, potassium dichromate 0.02N, hydrogen peroxide 20 V, alcoholic benzidine 1%, , potassium hydroxide 5%, concentrated ammonia solution, alcoholic 3,5-dinitrobenzoic acid, picric acid 1%., sodium hydroxide 10%, modified Dragendorff's reagent, Mayer's reagent, Wagner's reagent and vanillin sulfuric acid 1% [5].

Methods.

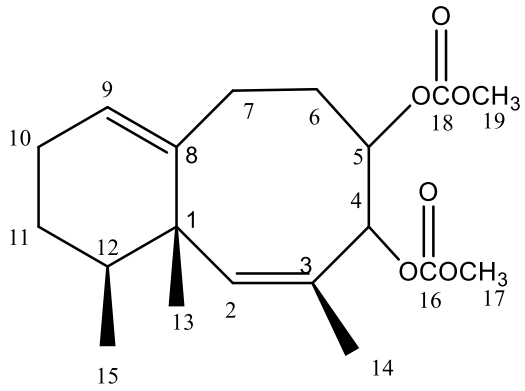
General experimental procedure: Both 1D and 2D NMR experiments were measured on Bruker spectrometer operating at 400 and 600 MHz. All spectra were recorded at 27 °C and samples were dissolved in deuterated solvents; DMSO-d_6 and CD_3OD, and the solvent's peak was used as internal reference for signals adjustment. Mass spectra were recorded on Thermo Scientific LTQ Orbitrap; equipped with ESI and MALDI sources. Thin-layer chromatography was carried out on silica gel F_{254} aluminum sheet (20 x 20 cm, Fluka). The UV of 254 and 360 nm were used to visualize the compounds on TLC. The spots were stained by spraying with 1% vanillin/ sulfuric followed by heating for 1 - 2 min. Analytical grade Solvents (Fischer Chemicals) were through the process of isolation and purification.

Extraction and fractionation

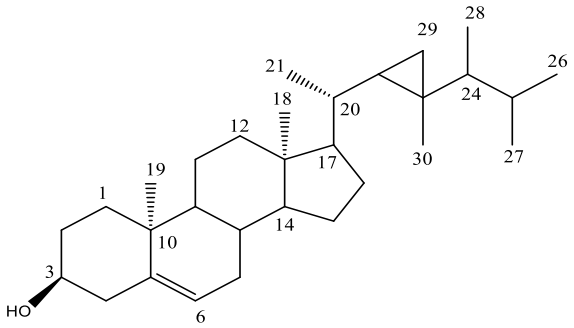
Extraction of soft coral: The dried soft bodied coral sample, *Paralemnalia thyrosides* (560 gm) was minced and extracted with 2 x 3 L of $CHCl_3$/MeOH (1:1, v/v), three times, 24 h for each batch, at room temperature. The extract was concentrated under reduced pressure to provide 114.0 g of viscous oil. Part of the residue (9 gm) was homogenized with silica gel and poured on the top of the column [Silica gel column (500 g, 80 × 2.5 cm)].

The column was eluted with gradients from petroleum ether followed by increasing concentrations of chloroform to yield 200 fractions then followed by increasing concentrations of ethyl acetate to yield 352 fractions (each 50 ml), which were combined into 100 main fractions. Similar fractions were pooled, according to their TLC behavior; appropriate solvent system and methanol/sulfuric acid as spray reagent and anizaldhyde spray reagent were employed. If the material was not homogeneous, preparative TLC of silica gel was applied using the appropriate solvent system for purification Normal-phase Si gel [using gradient elution of chloroform: ethyl acetate].

Preparative TLC using different concentration of petroleum ether: ethyl acetate for purification to afford compounds (**1** & **2**)



Compound 1



Compound 2

Preliminary Phytochemical Screening: the crude extract was subjected to crude extract which were phytochemically screened in order to detect the chemical constituents according to standard conventional protocol described by Harborne, the tests were performed for flavonoids, tannins, triterpenes, saponins, steroids and alkaloids [6]

Determination of antibacterial and antifungal activity: A single colony of the test strain was taken and added to 20 mL of NB medium. This medium was incubated at 37 C ± 1 C, 160 rpm for 12 h. It was then added to bacterial suspension to prepare a bacterial concentration at 105 CFU/ml. The bacterial solution was used for the following antibacterial assay. Each sample was dissolved in DMSO at maximum concentration. 89 µL of NB medium, 10 µL of bacteria suspension, and 1 µL of DMSO with or without each sample were added into each well of a 96-well plate. Also, Gentamycin used as a positive control for antibacterial activity and ketoconazole used as a positive control for antifungal activity. The plate was incubated at 37 C ± 1 C, 1160 rpm for 12 h [7]

RESULTS AND DISSECTION

Isolated compounds: The column chromatography of methanolic extract of *Paralemnalia thyrsoides* over normal phase gel resulted in the purification of two compounds (**1** and **2**). The structures of isolated compounds were identified based on 1D- and 2D-NMR experiments and comparison with those previously reported about these compounds in literature. **Compound 1** was isolated as a colorless oil (5 mg).

Its C^{13} NMR spectra showed 19 carbon signals (**Table 1 & 2**) that attributed to five quaternary carbons {two olefinic carbons (C-3 and C-8) at δ_C 127.0 and 139.2, two esterified carbons (C-16 and C- 18) at δ_C 170.4 and 170.6 and C-1 at δ_C 44}; five methines {two oxygenated carbons (C-4 and C-5) at δ_C 72.6 and 74.,1 attached to H-4 and H-5 at δ_H (6.72 and 5.12, dd, *J*= 9.6 and 5.2 Hz) two olefinic carbons (C-2 and C-9) at δ_C 138.9 and 126.5 attached to H-2 and H-9 at δ_H (5.25 and 5.6) and C-12 at δ_C 38.2 attached to multiplet signal appeared at δ_H 1.7}, four methylenes and five methyls. (C-13, C-14, C-15, C17 and C-19 at dc 23.7, 20.1, 21.3, 20.8 and 20.9, respectively attached to five singlet signals at δ_H 1.02, 1.58, 0.86, 2.07 and 2.01, respectively). Its molecular formula was determined as $C_{19}H_{28}O_4$ by the GC-MS at m/z 338.2323.

The full assignments of ^{1}H- and ^{13}C-NMR (**Table 1**) spectra were facilitated by comparison with those of sesquiterpene and further confirmed by HSQC, HMBC and COSY spectra. Accordingly, compound 1, was concluded to be Lemnolin A. Further confirmation was done by comparing the data with those in the literature [8]

Table (1) ^{1}HNMR, ^{13}CNMR& APT spectral data of compound 1

position	^{1}H	^{13}C	APT
1	----------	44.0	C
2	5.25, s	138.93	CH
3	----------	127.0	C
4	6.72	72.57	CH
5	5.12, dd J=9.6 , 5.2 Hz	74.08	CH
6		28.6	CH_2
7		29.7	CH_2
8	----------	139.2	C
9	5.6,m	126.47	CH

10		25.1	CH_2
11		26.7	CH_2
12	1.7, m	38.2	CH
13	1.02	23.74	CH_3
14	1.58	20.15	CH_3
15	0.86	21.26	CH_3
16	----------	170.93	C
17	2.07	20.81	CH_3
18	----------	170.46	C
19	2.01	20.89	CH_3

Table (2) ^{1}HNMR, ^{13}CNMR& APT spectral data of compound 2.

Position	**^{1}H**	**^{13}C**	**APT**
1	1.98, m 1.20, m	37.26	CH_2
2	1.96, m 2.12, m	31.67	CH_2
3	3.66, m	71.82	CH
4	2.36, m 2.41, m	42.31	CH_2
5	----------	140.77	C
6	5.49, br.s	121.74	CH
7	1.65, m 1.72, m	31.92	CH_2
8	1.52, m	31.97	CH
9	1.05, m	50.16	CH
10	----------	39.77	C
11	0.97, m 1.12, m	21.09	CH_2
12	1.28, m 2.13, m	39.86	CH_2
13	----------	42.78	C
14	1.12, m	56.75	CH
15	1.40, m 1.96, m	28.24	CH_2
16	1.41, m 2.14, m	28.21	CH_2
17	1.08, m	50.13	CH
18	0.81, s	11.91	CH_3
19	1.14, s	19.41	CH_3
20	1.70, m	35.30	CH
21	0.98, d, 6.8 Hz	20.54	CH_3
22	0.30, m	32.14	CH
23	----------	25.81	C
24	0.40, m	50.82	CH
25	1.69, m	31.46	CH
26	1.08, d, 6.4 Hz	21.56	CH_3
27	0.98, d, 6.8 Hz	21.19	CH_3
28	0.91, d, 6.4 Hz	15.48	CH_3
29	1.62, m 1.13, m	21.31	CH_2
30	1.03, s	14.28	CH_3

Compound 2 was obtained as a white powder (6 mg), and was deduced to have the molecular formula $C_{30}H_{50}O$ by positive mode HRESI-MS; $[M+H-H2O]^+$ m/z 409.3857 (calcd. 409.3834). The ^{1}H NMR data of compound 2 showed characteristic signals at δ_H -0.13 (1H, H-30), δ 0.30 and 0.40 (1H each, m, H-22, H-24), and at δ 1.03 (1H, H-30), thus requiring the presence of a cyclopropyl ring. It also showed signals for seven methyls at δ 0.66 (3H) and δ 0.81 – 1.01 (18H). The spectra also

showed signal at δ 3.66 (1H, bs, H-3) indicate the presence of methine proton attached to hydroxyl group. The spectra also showed signal for olefinic proton at δ 5.49 (1H, br s, H-6). The characteristic signals due to a cyclopropyl moiety were identical with those of gorgosterol with the signals for seven methyls were also consistent with a gorgosterol skeleton [9]

The forementioned data indicated the presence of gorgosterol type sterol with a hydroxyl group at C-3 and double bond between C-5 and C-6. The ^{13}C NMR and DEPT-135 confirmed the proposed structure and showed seven methyls signals, ten methine groups, and four quaternary carbons.

The ^{13}C NMR spectrum indicated the presence of one methine carbon bearing hydroxyl group at δ 71.8 and two olefinic carbons at δ 121.7 and at δ 140.7. From the above spectral data and discussion, the structure of compound 2 could be assigned as gorgosten-5(E)-3 β-ol. It was confirmed by comparison of its spectral data with published values [10]

Phytochemical Screening: The phytochemical analysis of all soft corals crude extracts was presented in (**Table 3**). The soft corals have been extracted using solvent dichloromethane: methanol 1:1 and then was assayed to detect secondary metabolites. The chemical constituent analysis of all crude extracts indicated the presence of saponins, polyphenols (tannins), steroids, triterpenoids, alkaloids, and flavonoids.

Table (3) Phytochemical analysis of all soft corals crude extracts.

Chemical Constituents	*Paralemnalia thyrsoides*
Saponins	-
Polyphenols (tannins)	+
Steroids	-
Triterpenoids	+
Alkaloids	+
Flavonoids	-

Antifungal assay: The total extract of *Paralemnalia thyrsoides* and the two isolated compounds showed very weak activity against *Aspergillus fumigatus* (RCMP 002008) and *Candida albicans* (RCMP 005003) (1) ATCC 10231).

Antibacterial assay: The total extract of *Paralemnalia thyrsoides* and the two isolated compounds showed very weak activity against gram positive bacteria *Staphylococcus aureus* (RCMP 010010) and gram-negative bacteria *Escherichia coli* (RCMP 010052) ATCC 25955).

CONCLUSION

Two compounds were isolated and characterized from the $CHCl_3$/MeOH extract of ***Paralemnalia thyrsoides***. They were identified by based on 1D- and 2D-NMR experiments and comparison with those previously reported about these compounds in literature. The total extract and the two isolated compounds showed very weak activity against *Aspergillus fumigatus*, *Candida albicans*, gram positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli*.

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