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ORIGINAL ARTICLE

Anti-Inflammatory, Antioxidant, Cytotoxic Activities, and Sesquiterpenoid Contents of *Paralemnalia thyrsoides*

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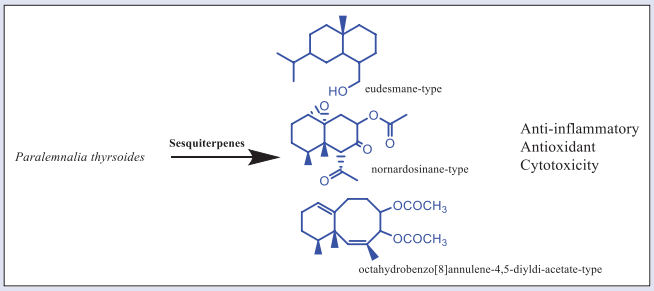
ABSTRACT

Background and Objectives: *Paralemnalia thyrsoides* is an octocoral species of the family Nephtheidae. It has established as platform for the production of a varied array of sesquiterpenoids such as africanane, nardosinane, and neolemnane -type compounds. Antiviral and cytotoxic effects of sesquiterpenes from *P. thyrsoides* were reported. **Materials and Methods:** The animal sample of *P. thyrsoides* was repeatedly extracted with organic solvents. Then, the animal extract was fractionated and purified employing different planar chromatographic methods. The chemical structures of all isolated metabolites were identified by employing spectroscopic tools (ultraviolet [UV], IR, and nuclear magnetic resonance) along with MS. The anti-inflammatory activity (membrane stabilization%), and histamine release inhibitory effect, the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, along with cytotoxic activity against four-cancer cells: hepatocellular carcinoma (Hep G-2), colon (HCT-116), prostate (PC-3), and breast (MCF-7) cancers were evaluated. **Results:** Three new sesquiterpenoids along with two known ones and a gorgostane steroid. The three new sesquiterpenoids were identified as eudesma-1, 2, 15-trihydroxy-3-en-7-one (1), eudesma-1, 2, 15-trihydroxy-5-ene (2), and nardosinanol J (3). Whereas the known compounds were identified as lemnolin A (4) and gorgostane (5). The *in-vitro* assays results revealed that the total organic extract showed anti-inflammatory activity (membrane stabilization %) with IC_{50} of 88.3 ± 1.2 compared to positive control (indomethacin with IC_{50} of 17.02 ± 1.2 µg/ml) and strong histamine release inhibitory effect with IC_{50} of 17.94 ± 1.08 compared to a positive control (diclofenac with IC_{50} of 17.94 ± 1.26 µg/ml). It also showed that the total organic extract has antioxidant activity using DPPH assay with an IC_{50} of 157.5 ± 4.24µg/ml. Moreover, the total organic extract has strong inhibitory activities against Hep G-2 with an IC_{50} of 12.1 ± 1.1 µg/ml, HCT-116 with an IC_{50} of 13.4 ± 1.8 µg/ml, PC-3 with an IC_{50} of 28.6 ± 2.7 µg/ml, and good inhibitory activity against MCF-7 with an IC_{50} of 49.0 ± 3.9 µg/ml. **Conclusion:** The observed bioactivity and the variety of carbon skeletons isolated warrants further work on the constituents of *P. thyrsoides*.

Key words: *Alcyonacea*, anti-inflammatory, chromatography, spectroscopy, nephtheidae, norsesquiterenoids

SUMMARY

- A Red Sea specimen of the soft coral *Paralemnalia thyrsoides* was chemically explored. After extraction, fractionation, and purification, five secondary metabolites were isolated. The compounds isolated include four sesquiterpenes and a common marine sterol. The anti-inflammatory, cytotoxicity, and antioxidant activity were evaluated.



Abbreviations used: COSY: Homonuclear Correlation Spectroscopy; DEPT: Distortionless enhancement by polarization transfer; HepG-2: Hepatocellular carcinoma; HMBC: Heteronuclear multiple-bond correlation spectroscopy; HSQC: Heteronuclear single-quantum correlation spectroscopy; HCT-1116: Human colon cancer cell line; IR: Infrared; MS: Mass spectroscopy; MCF-7: Breast cancer; NMR: Nuclear magnetic resonance; NOESY: Nuclear Overhauser effect spectroscopy; PC-3: Prostate adenocarcinoma; PTLC: Preparative thin layer chromatography; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TLC: Thin Layer Chromatography; UV: Ultraviolet.

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INTRODUCTION

Seventy years have passed since the first successful attempts to separate natural compounds from marine creatures, and since that time marine organisms have continued to provide us with diverse organic compounds that possess remarkable prospects on the medical and environmental levels.[1,2] Soft corals (coelenterata, *Octocorallia*, and *Alcyonacea*) are fleshy benthic marine animals capable of producing secondary compounds that enable these animals to defend themselves against the high level of predation present in the marine environment.[3]

Members of the genus *Paralemnalia* have established as platform for the production of a varied array of natural bioactive sesquiterpenoids such as africanane, nardosinane, neolemnane -type compounds,[4-17] in addition to norsesquiterpenoids of nardosinane-type skeleton.[6,8,11,12] Several reported bioassay results on the secondary natural compounds from the genus *Paralemnalia* had displayed diverse biological applications such as antiviral [11-13] and cytotoxic.[4-17] Our ongoing project focuses on the

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exploration of the secondary content of marine organisms from the Red Sea environment. In addition, the cytotoxic activity of the isolated compounds is assessed. In the current work, a specimen of the alcyonacean *Paralemnalia thyrsoides* was collected, extracted, and chromatographed for pure compounds. Eudesma-1,2,15-trihydroxy-3-en-7-one (1), eudesma-1,2,15-trihydroxy-5-ene, (2) and nardosinanol J (3) are three previously unreported sesquiterpenes, in addition to the known sesquiterpene lemnolin A (4) and the cyclopropane-containing steroid gorgostane (5) were isolated.

MATERIALS AND METHODS

General

Nuclear magnetic resonance (NMR) analyses were conducted at Mansoura University (NMR Unit Center, Faculty of Science) using Bruker Avance III (400 Hz).

Animal specimen

In June 2019, a specimen of *P. thyrsoides* was collected from the Red Sea (Jeddah, Saudi Arabia). A voucher specimen (PLT19-01) was deposited at the Marine biology department, Faculty of Marine Sciences, KAU.

Extraction and isolation

The freeze-dried *P. thyrsoides* (500 g) specimen was extracted with 2 × 3 L of $CHCl_3$/MeOH (1:1, v/v; 3 times; 24 h/patch; rt). The extract was completely dried to provide 16.3 g of viscous oil. 9.0 g of the obtained oil was well mixed with silica gel and prepared for chromatographic fractionation (normal phase Si gel column). The column fractionation started with petroleum ether and the polarity was increased gradually by adding increased percentages of chloroform in pet. ether and the EtOAc in pet. ether. A total of 552 fractions (50 ml each) were obtained. Normal phase Si gel TLC and visualizing reagents (UV_{254} and *p*-anisaldehyde) aided the process of fractions categorization. Promising fractions were further purified using Si gel preparative thin-layer chromatography (PTLC). Fraction A eluted which had eluted with $CHCl_3$: EtOAc (9: 1) was further purified on PTLC and pet. ether: EtOAc (9:1; greenish color with *p*-anisaldehyde reagent, *Rf* 0.25) to give compound 5. Fraction B which had eluted with $CHCl_3$: EtOAc (7: 3) was further purified on PTLC and pet. ether: EtOAc (1:1; purple color with *p*-anisaldehyde reagent, *Rf* 0.44) to give compound 4. Fraction C which had eluted with $CHCl_3$: EtOAc (1:1) was initially purified on Sephadex LH-20 column and methanol and finally on PTLC and pet. ether: EtOAc (6:4; blue color with *p*-anisaldehyde reagent, *Rf* 0.61) to give compound 3. Fraction D which had eluted with $CHCl_3$: EtOAc (2:8) was initially purified on sephadex LH-20 column and methanol and finally on PTLC and $CHCl_3$: MeOH (9.7:0.3; grey color with *p*-anisaldehyde reagent, R_f 0.41) to give compound 2. Fraction E which had eluted with $CHCl_3$: EtOAc (1:98) was purified on sephadex LH-20 column and methanol and finally on PTLC and CH_2Cl_2: EtOAc (9.8:0.2; purple color with *p*-anisaldehyde reagent, *Rf* 0.40) to give compound 1.

Eudesma-1,2,15-trihydroxy-3-en-7-one (1)

Colorless oil, (0.9 mg, 0.00016%); IR υ_{max} (film) cm^{-1}: 3513, 3497, 2923, 2853, 1691, 1661, 1372, 1366; NMR data were recorded in $CDCl_3$ (^1H, 400 MHz; ^{13}C, 100 MHz): [Table 1]; HRESIMS (positive mode) m/z = 269.1746 $[M + H]^+$ (Calculated m/z = 269.1753 for $C_{15}H_{25}O_4$).

Eudesma-1,2,15-trihydroxy-5-en (2)

Colorless oil, (0.7 mg, 0.00014%); IR υ_{max} (film) cm^{-1}: 3482, 3385, 2920, 1664, 1374, 1367; NMR data were recorded in $CDCl_3$ (^1H, 400 MHz; ^{13}C, 100 MHz): [Table 1]; HRESIMS (positive mode) m/z = 255.1953 $[M + H]^+$ (Calculated m/z = 255.1960 for $C_{15}H_{27}O_3$).

Nardosinanol J (3)

Colorless oil, (0.8 mg, 0.00015%); IR υ_{max} (film) cm^{-1}: 2960, 2854, 1723, 1697, 1166, 1037; NMR data were recorded in $CDCl_3$ (^1H, 400 MHz; ^{13}C, 100 MHz): [Table 1]; HRESIMS (positive mode) m/z = 295.1537 $[M + H]^+$ (Calculated m/z = 295.1545 for $C_{16}H_{23}O_5$).

Biological evaluation

Anti-inflammatory activity

Membrane stabilization

Calculation of the membrane stabilization (percentage inhibition of hemolysis) was calculated as described by Shinde *et al.*[18]

Histamine release assay

The histamine release was estimated as shown by Venkata *et al.* 2012.[19]

Cytotoxicity assay

Mammalian cell lines

All employed cancer cells were obtained from VACSERA Tissue Culture Unit.

Cell line propagation

Cytotoxic assay experiment was conducted as demonstrated by Mosmann (1983).[20]

Antioxidant assay

By using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay the antioxidant activity of the extract was determined at Al- Azhar University (the Regional Center for Mycology and Biotechnology RCMB).

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Antioxidant assay experiment was conducted according to Yen and Duh, 1994.[21]

RESULTS AND DISCUSSION

Chemistry

Compound 1 was isolated as oily material. The molecular formula $C_{15}H_{24}O_4$ was indicated from HRESIMS, requiring four degrees of unsaturation. ^{13}C NMR spectrum displayed fifteen carbon atoms. The DEPT experiments aided the classification of carbons into 3 CH_3, 3 CH_2, 6 CH, and 3 nonprotonated carbons. The IR spectrum displayed absorption bands at 3513, 3497 (OH function), 2923, 2853 (CH stretching), 1691 (carbonyl function), 1661 (carbon-carbon double bond), and 1372 and 1366 (*gem*-dimethyl) cm^{-1}. ^1H, ^{13}C NMR, and HSQC spectral data clarified the existence of two secondary methyls resonating at δ_H/δ_C 1.03/18.8 and 0.71/14.4 ppm, one tertiary methyl signal at δ_C 19.6, a carbinol function (a primary alcohol) at δ_H/δ_C 4.11 and 3.30/61.9, two oxygenated methine groups at 4.02/73.7 and 4.15/69.5, a trisubstituted carbon-carbon double bond 5.70/111.3 and 156.7, along with carbonyl function at 211.7 ppm.

The presence of several methyl groups included in the fifteen-carbon atom skeleton suggested that compound 1 belongs to the sesquiterpenes. Moreover, compound 1 is entirely a bicyclic sesquiterpene owing to the presence of a carbonyl function, a carbon-carbon double bond in a four- unsaturation sites skeleton. The presence of eudesmane-type sesquiterpene has proved after examining the methylation pattern in ^1H, ^{13}C NMR, DEPT, and HSQC spectra which showed a gem-dimethyl,

Table 1: ^{1}H and ^{13}CNMR spectral data for compounds 1[a], 2 and 3 (400 MHz)

1		δ_C	2			3		
δ_H	Mult., (*J* in Hz)		δ_H	Mult., (*J* in Hz)	δ_C	δ_H	Mult. (*J* in Hz)	δ_C
4.02	d (6.4)	73.7	3.50	d (5.2)	71.8	3.20	m	62.1
4.15	m	69.5	3.40	dt (7.6, 6.4)	68.9	1.89-1.97	m	25.3
5.70	dd (6.4, 2.0)	111.3	1.57	m	31.5	1.26	m	28.2
			1.31	m		1.16	m	
-	-	156.7	2.06	m	33.9	1.93	m	29.4
1.42	m	36.3	-	-	121.0	-	-	43.7
1.66	m	29.7	5.40	d (4.0)	114.1	2.90	s	71.2
1.53	m							
2.04	m	60.7	1.65	m	34.2	-	-	202.3
-	-	211.7	1.72	m	24.9	5.37	m	69.4
			1.47	m				
2.12	m	38.4	1.42	m	32.4	2.28	m	29.2
1.97	m		1.35	m		2.12	m	
-	-	29.3	-	-	34.4	-	-	63.3
2.31	m	35.3	1.55	-	29.9	-	-	206.8
1.03	d (6.8)	18.8	0.89	d (6.8)	22.8	2.29	m	31.4
0.71	d (6.8)	14.4	0.86	d (6.8)	22.7	-	-	-
0.91	s	19.6	0.90	s	21.8	0.87	d (6.8)	16.2
4.11	dd (11.6, 2.0)	61.9	4.32	dd (11.6, 2.8)	62.8	1.04	s	18.2
3.30	d (11.6)		4.20	dd (11.6, 6.4)				
						-	-	174.0
						2.22	s	21.2

[a]All compounds were measured in $CDCl_3$

a tertiary methyl, and the methyl group equivalent (i.e., the carbinol function). Though compound 1 has eudesmane-sesquiterpene carbon skeleton, which is decorated with alkyl substitution at positions 4, 7, and 10 however, the locations of three hydroxyls, a carbonyl, and a carbon-carbon double bond are still not accounted. The presence of an unsubstituted isopropyl function and a tertiary methyl (angular methyl) emphasized the presence of hydroxylation at Me-15 [Figure 1]. ^{1}H-^{1}H COSY spectrum established the presence of two methyl sequences, in addition to two isolated methylenes and the angular methyl. The first sequence started with the proton signal that appeared at δ_H 5.7 (H-3) which is correlated δ_H 4.15 (H-2), the latter proton is correlated with that resonating at chemical shift 4.02 (H-1). The second sequence started with the signal due to the CH proton resonating at 1.42 which is correlated with the CH_2 signals that appeared at 1.66 and 1.53 (H-6), the latter proton signals are correlated with the CH proton at 2.04 (H-7). The later proton signal is correlated with H-11 resonating at δ_H 2.31, which in turn is correlated with two methyl-proton signals resonating at 1.03 and 0.71 ppm [Figure 2]. The previous extensive investigation of the COSY spectrum along with the detected heteronuclear multiple-bond correlation spectroscopy (HMBC) correlations between the angular methyl proton signals appeared at δ_H 0.91 with the CH_2 carbon signal at δ_C 36.4 (C-1), the CH carbon signal at δ_C 36.3 (C-6), and the signal at 73.7, and the correlation observed between H-7 and the carbonyl at δ_C 211.7 (C-8) and C-6 confirmed the location of the carbonyl group at C-8, the hydroxyl functions at C-1, C-2 and C-15 and the carbon-carbon double bond at C-3-C-4 [Figure 2]. Hence, compound 1 can be identified as eudesma-1, 2,15-trihydroxy-3-en-8-one [Figure 1].

Compound 2 was isolated as colorless oily substance. The molecular formula was calculated as $C_{15}H_{26}O_3$, determined by HRESIMS, requiring four degrees of unsaturation. ^{13}C NMR spectrum displayed fifteen carbon atoms those were classified by DEPT procedures into three CH_3, four CH_2, six CH, and two non-protonated carbons. The IR spectrum displayed absorption bands at 3482, 3385 (OH function), 2920, 2816 (CH stretching), 1664 (carbon-carbon double bond), and 1374 and 1367 (*gem*-dimethyl) cm^{-1}. ^{1}H, ^{13}C NMR, and HSQC spectral data clarified the existence of two secondary methyls that appeared at δ_H/δ_C 0.89/22.8 and at 0.86/22.7 ppm, one tertiary methyl signal at 0.90/21.8, a carbinol function (a primary alcohol) at 4.32 and 4.20/62.8, two oxygenated methine groups at 3.50/71.8 and 3.40/68.9, and a trisubstituted carbon-carbon double bond at 5.40/114.1 and 121.0 ppm. The presence of several methyl groups included in the fifteen-carbon atom skeleton suggested that compound 2 belongs to the sesquiterpenes. Moreover, compound 2 is entirely a bicyclic sesquiterpene owing to the presence of a carbon-carbon double bond in a three-unsaturation sites structure. The presence of eudesmane-type sesquiterpene has proved after examining the methylation pattern in ^{1}H, ^{13}C NMR, DEPT and HSQC spectra which showed a gem-dimethyl, a tertiary methyl, and the methyl group equivalent (i.e., the carbinol function). Though compound 2 has eudesmane-sesquiterpene carbon skeleton, which is decorated with alkyl substitution at positions 4, 7, and 10 however, the locations of three hydroxyls, and a carbon-carbon double bond are still not accounted. A similar treatment to the 2D NMR spectral data (1H-1H COSY and HMBC) revealed a similar compound to that of [Figure 2]. Two differences have been observed, for compound 2, no carbonyl function and the location of the double bond is in position 5. Therefore, compound 2 can be identified as eudesma-1,2,15-trihydroxy-5-en [Figure 1].

Compound 3 was isolated as colorless oily substance. The molecular formula was calculated as $C_{16}H_{22}O_5$, determined by HRESIMS, requiring six unsaturation. The IR spectrum revealed bands at 1733 (carbonyl ester), 1697 (carbonyl), 2960, 2854 (CH stretching), and 1166 and 1037 (epoxy) cm^{-1}. ^{13}C NMR spectrum displayed sixteen carbon atoms those were classified with the aid of DEPT procedures into four CH_3, three CH_2, four CH, and five nonprotonated carbons. ^{1}H, ^{13}C and HSQC NMR displayed signals due to two carbonyl functions resonating at δ_C 206.8 and 202.3, an ester function at δ_H/δ_C 2.22/21.2 and 174.0 ppm, two quaternary methyls δ_H/δ_C 2.29/31.4, and 1.04/18.2, a secondary

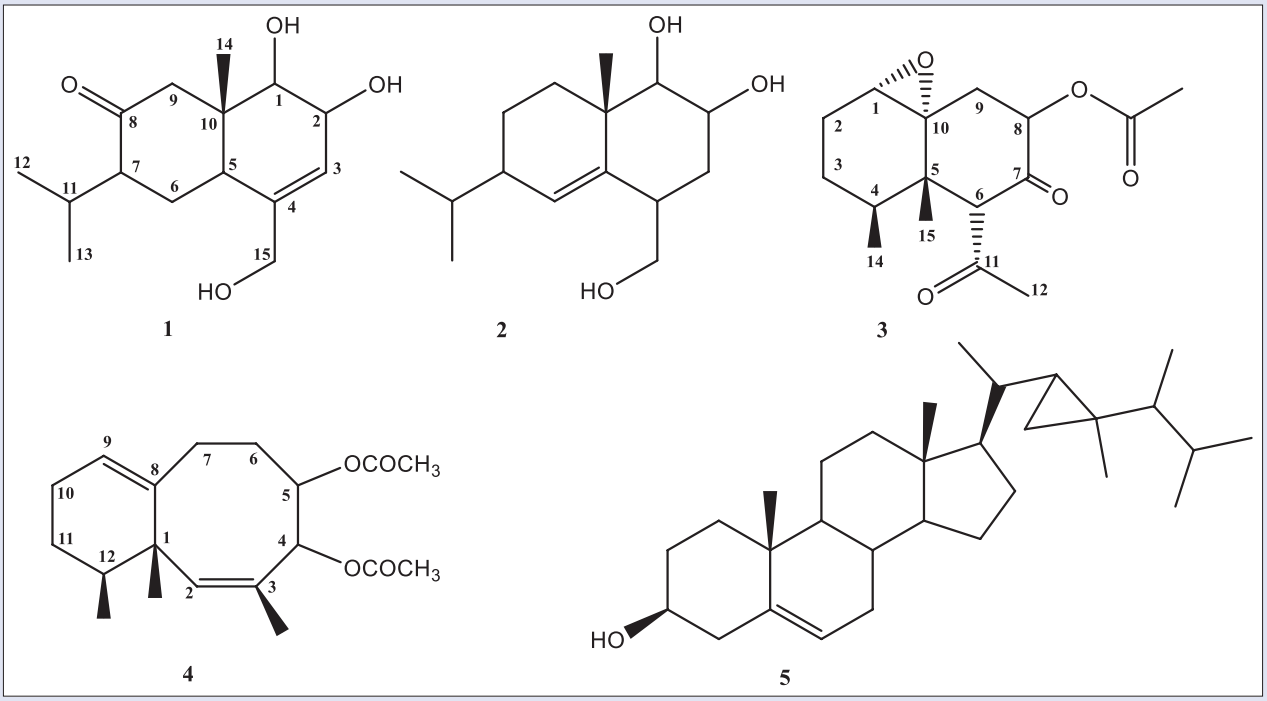

Figure 1: Structures of compounds isolated from *Paralemnalia thyrsoides*

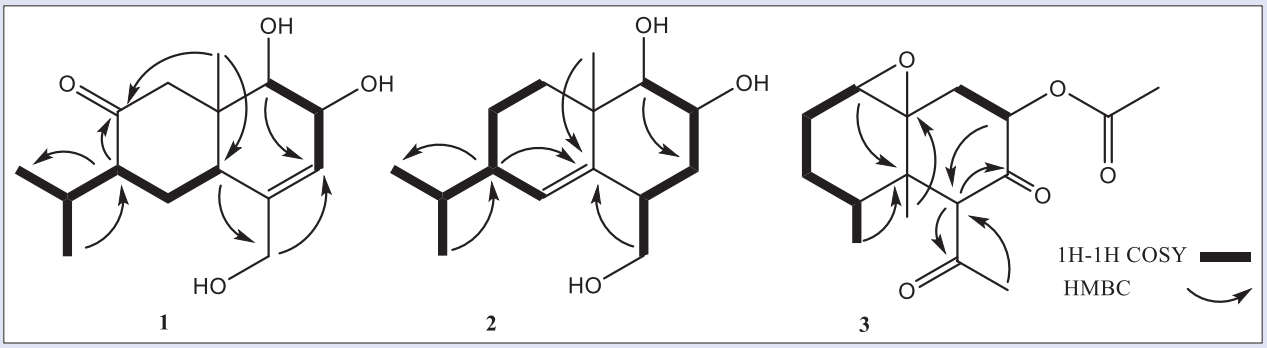

Figure 2: Selected H-H COSY and heteronuclear multiple-bond correlation spectroscopy correlation of the compounds 1–3

methyl at 0.87/16.2, and resonances due to oxirane ring δ_H/δ_C 3.20/62.1 and 63.3 ppm. Taking into consideration six degrees of unsaturation, three carbonyl functions, together with the absence of any absorption due to carbon-carbon unsaturation, compound 3 is a fourteen-carbon atoms tricyclic-sesquiterpenoid. The nature of the sesquiterpenoids has been assigned as a nornardosinane- type, based on the methylation pattern and comparison with similar published compounds.[22] 1H-1H COSY spectrum exhibited two proton sequences, the first started with the oxirane proton resonating at 3.20 (H-1) and ended by the methyl protons resonating at 0.87 (H-14) [Figure 2 and Table 2], the second sequence started with the CH proton appeared at 5.37 (H-7) and ended by the CH_2 protons resonating at 2.28 and 2.12 (H-9). Investigation of the HMBC spectrum established the location of oxirane ring through the correlations of H-1 with C-2, C-5, and C-9, the esterification position through the correlation of H-8 with C-6, C-7, and C-10, and the location of the acetyl group through the correlation between H-12 with C-5 and C-6. Compound 3 was identified as nardosinanol J [Figure 1].

In addition to the three new compound, two known compounds were isolated and identified as lemnolin A (4)[17] and gorgosterol (5).[23]

Biological activities

The *in vitro* assays results revealed that the total organic extract of *P. thyrsoides* showed anti-inflammatory activity (membrane stabilization%) with IC_{50} of 88.3 ± 1.2 compared to positive control (indomethacin with IC_{50} of 17.02 ± 1.2 µg/ml) and strong histamine release inhibitory effect with IC_{50} of 17.94 ± 1.08 compared to a positive control (diclofenac with IC_{50} of 17.94 ± 1.26 µg/ml).

It also showed that the total organic extract of *P. thyrsoides* has antioxidant activity using DPPH assay with IC_{50} of 157.5 ± 4.24µg/ml compared to a positive control (ascorbic acid with IC_{50} of 14.2 ± 0.24µg/ml). Results also showed that the total organic extract has strong inhibitory activities against HepG-2 with an IC_{50} of 12.1 ± 1.1 µg/ml, colon cancer IC_{50} of 13.4 ± 1.8 µg/ml, PC-3 with an IC_{50} of 28.6 ± 2.7 µg/ml, and good inhibitory activity against MCF-7 with IC_{50} of 49.0 ± 3.9 µg/ml. Vinblastine sulfate was employed as a positive control with IC_{50} values of 2.93 ± 1.05, 3.5 ± 6.36, 42.2 ± 1.10, and 5.9 ± 0.9 for HepG-2, HCT-116, PC-3, and MCF-7, respectively.

CONCLUSION

The Red Sea soft coral *P. thyrsoides*, was found to be a source of different sesquiterpenoids and norsesquiterpenoid carbon skeletons. The obtained *in vitro* assays results clarified that the total organic extract of *P. thyrsoides* showed strong anti-inflammatory activity (membrane stabilization %) and strong histamine release inhibitory effect. Furthermore, it exhibited antioxidant activity and significant cytotoxic effects against HepG-2 and colon cancer cell lines.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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