



## Chemical Investigation of the Red Sea Gorgonian Coral *Rumphella torta*

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### Abstract

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Natural products can provide new structures for medicinal products which can't be obtained from other sources such as combinatorial synthesis. Aquatic environment provides a countless and varied source for new drugs to combat major diseases. It also affords an ecological advantage that includes a diversity of marine plants and animals. Marine invertebrates are known to develop secondary metabolites that may have potential as candidates for new drugs. Recent studies show that octocorals, like gorgonians can generate secondary metabolites which have powerful pharmacological activities. In this work, chemical investigation of the gorgonian coral *Rumphella torta*, collected from the Red Sea led to isolation of eight known compounds which are firstly reported from the species, Cholesterol (1), four fatty acids, Myristic acid (2), Palmitic acid (3), Arachidic acid (4) and Stearic acid (5), Chimyl alcohol (6), Hexadecanoic acid 2, 3-dihydroxy-propyl ester (7) and Thymine (8). The structures of these isolated compounds were determined by spectroscopic methods, including 1D and 2D-NMR, as well as mass spectrometry and by comparison to the literature.

**Keywords:** Gorgonian coral, *Rumphella torta*, Chimyl alcohol, Ester, Thymine.

## 1. Introduction

The marine environment is an outstanding store house of new bioactive natural products, with structural and chemical properties not commonly found in terrestrial products (Liu, 2019). The marine entities also are considered a rich source of nutraceuticals and possible candidates for treatment of many human diseases (Malve, 2016). More than 70% of the planet's surface is aquatic environment

that owns unique biological and chemical characters that play a critical role in detection of multiple drug leads (Anjum *et al*, 2016). Many marine-living organisms are soft bodied and/or sessile. Consequently, they have produced toxic secondary metabolites as a defensive mechanism to protect themselves against predators (Eltamany, 2015). Due to its biodiversity and seasonal

variations in air and water temperatures, the Red Sea is considered a one of the most important sources for marine research (Abdelhameed *et al*, 2017). Numerous natural product classes were isolated from Red Sea marine organisms such as alkaloids, terpenes, sterols and steroidal glycosides, and other compounds that were previously mentioned in details (El-Ezz *et al.*, 2017). Gorgonians are Cnidarians, that means stinging celled animals. They belong to Alcyonacea Order, that are further classified into three Suborders: Holaxonia, Scleraxonia, and Calcaxonia. They are also members of Octocorals which are the subclass Octocorallia. These are corals with their polyp structure which typically have eight-fold symmetry or eight-branched tentacles. Like the soft corals, the Gorgonians are sessile colonial animals. There are over 1200 recognized Gorgonian species (Animal-World References, and Erhardt *et al*, 2005). The gorgonian coral *Rumphella torta* belongs to phylum Cnidaria, class Anthozoa, order Gorgonacea, suborder Holaxonia, family Gorgoniidae (Hayward *et al*, 1990). We report, in this study, the isolation and identification of eight compounds from the Gorgonian coral *Rumphella torta*. The isolated compounds are known but firstly reported from the species.

## 2. Materials and Methods

### 2.1. General experimental procedures

<sup>1</sup>H NMR (400 MHz), <sup>13</sup>C NMR (100 MHz), DEPT-135 and 2D NMR spectra were registered on a Varian AS 400 (Varian Inc., Palo Alto, CA, USA) using the residual solvent signal as an internal standard. High-resolution mass spectra were

recorded using a Bruker BioApex (Bruker Corporation) machine. Pre-coated silica gel G-25 UV254 plates were used for thin layer chromatography (TLC) (20 cm×20 cm) (E. Merck, Darmstadt, Germany). Silica gel (Purasil 60A, 230–400 mesh) was used for flash column chromatography (Whatman, Sanford, ME, USA).

### 2.2. Gorgonian coral material

The gorgonian coral *Rumphella torta* was collected by hand using SCUBA from Safaga in the Egyptian Red Sea. The soft coral material was immediately frozen and kept at -20°C until processed. The voucher specimen was deposited in the herbarium section of Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt under registration numbers (SAA-1). The identification and description of the soft coral was provided by Prof. Tarek A. Temraz, Department of Marine Science, Faculty of Science, Suez Canal University, Ismailia, Egypt.

### 2.3. Extraction and Fractionation of Marine Material

The frozen chopped small pieces of gorgonian coral *Rumphella torta* (4Kg weight) was extracted with methanol- CH<sub>2</sub>Cl<sub>2</sub> (1:1) (5L X 4) at room temperature. The combined extract was concentrated under vacuum resulting in 250 g residue. The crude extract (250 g) was slurred with silica gel and the mixture was transferred to a top of a sintered glass Büchner filter funnel (12 X 500 cm) packed with 500 g silica gel and connected to vacuum pump. Step gradient elution with a non-polar solvent (*n*-Hexane) with increasing the polarity using EtOAc then MeOH to give eight fractions (RT-1~ RT-8). Fraction RT-2 (20g) was

sequentially re-chromatographed over silica gel column using gradient elution of *n*-hexane: EtOAc to give three sub fractions RT-2-1~ RT-2-3. Compound **1** (10 mg) and compound **2** (6 mg) were obtained after further purification of sub fraction RT-2-1 on silica gel column using gradient elution of *n*-hexane: EtOAc. On the same way, further purification of sub fraction RT-2-2 on silica gel column using gradient elution of *n*-hexane: EtOAc gave compound **3** (5mg) and compound **4** (6mg). Also, compound **5** (4 mg) was separated from the sub fraction RT-2-3 after re-chromatographing over silica gel column using gradient elution of *n*-hexane: EtOAc. Fraction RT-3 (23g) was successively re-chromatographed over silica gel column using gradient elution of CHCl<sub>3</sub>: MeOH to give compound **6** (30mg) as a pure one and one sub fraction RT-3-1 that was chromatographed over Sephadex LH-20 column and eluted with CHCl<sub>3</sub>: MeOH (1:1) to give compound **7** (5 mg). Furthermore, re-chromatographing of the sub fraction RT-5 (15 g) over silica gel column using gradient elution of CHCl<sub>3</sub>: MeOH resulted in the two sub fractions RT-5-1 and RT-5-2. RT-5-1 was further chromatographed over Sephadex LH-20 column and eluted with CHCl<sub>3</sub>: MeOH (1:1) to give compound **8** (10 mg).

### 3. Results and Discussion

#### 3.1. Structure Elucidation of the Isolated Compounds

Compound **1** (figure 1) was obtained as white amorphous powder, and its molecular formula was determined to be C<sub>27</sub>H<sub>46</sub>O by HRESIMS (m/z 387.3642 [M+H]<sup>+</sup>) (calc.

387.3627), representing five degrees of unsaturation. The <sup>1</sup>H and <sup>13</sup>C-NMR spectral data of compound **1** are listed in (table 1). The <sup>1</sup>H-NMR spectrum, table (1), represented five methyl resonances, two of them are connected to sp<sup>3</sup> carbon at δ<sub>H</sub> 0.92 (s) and 0.68 (s) assigned to H<sub>3</sub>-18 and H<sub>3</sub>-19 respectively. The other three are connected to sp<sup>2</sup> carbon at δ<sub>H</sub> 0.80 (d, 6.1) assigned to H<sub>3</sub>-21 and at δ<sub>H</sub> 0.85 (d, 6.3) assigned to H<sub>3</sub>-26 and H<sub>3</sub>-27 which is in correspondence with the steroidal structure (**Kalinowski et al, 1984**). Also, a resonance at δ<sub>H</sub> 5.30 (m) of the most downfield chemical shift was assigned to H-6 which demonstrates that it is attached to an olefinic sp<sup>2</sup> carbon. The <sup>13</sup>C-NMR spectrum, table (1), showed 27 carbon resonances, which represented five methyl groups (CH<sub>3</sub>), eleven methylenes (CH<sub>2</sub>), eight methines (CH), and three quaternary carbons (C). The <sup>13</sup>C-NMR spectrum showed resonance for one oxygenated carbon at δ<sub>C</sub> 71.6 that could be attributed to the carbon C-3. Comparing the chemical shift values of H-3/C-3 with the data reported by (Kalinowski et al, 1984) supported the β configuration of the OH moiety at C-3. The signals of the side chain at C-17, (table 1), are comparable with those reported by (**Altena et al, 1999**). The above-mentioned are in good agreement with those reported for cholesterol (**Kalinowski et al, 1984**).

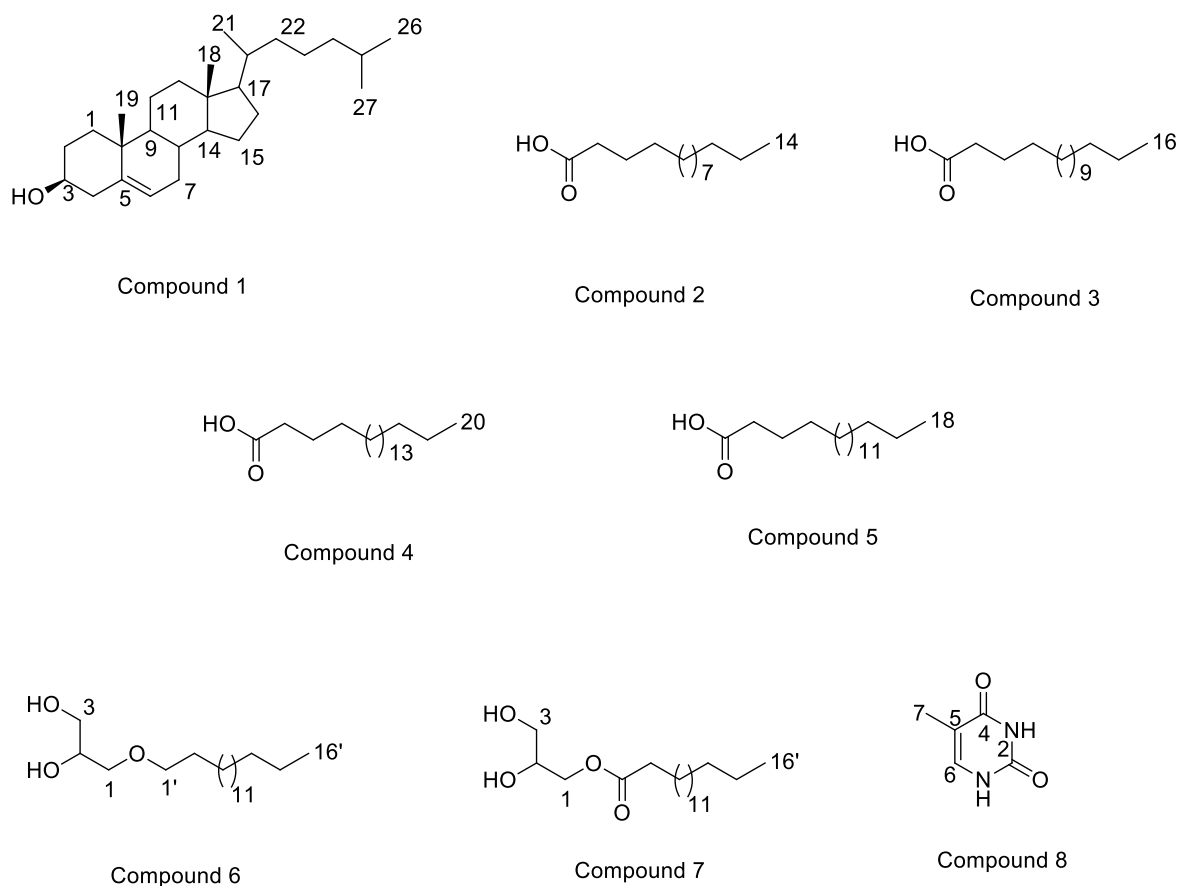
Compound **2** (figure 1) was obtained as white powder, and its molecular formula was determined

to be  $C_{14}H_{27}O_2$  by HRESIMS  $m/z$  227.2017  $[M-H]^-$  (calc. 227.2011). The  $^1H$ -NMR spectral data of compound **2** are listed in (table 2). The structure elucidation of compound **2** started with the analysis of its  $^1H$ -NMR exhibited the structure of saturated fatty acid. The NMR data of Compound **2** was compared with a reference data and found to be matched with myristic acid (**Dung et al, 2012**).

Compound **3** (figure 1) was obtained as white powder, and its molecular formula was determined to be  $C_{16}H_{31}O_2$  by HRESIMS  $m/z$  255.2330  $[M-H]^-$  (calc. 255.2324). The  $^1H$ -NMR spectral data of

compound **3** are listed in (table 2). The NMR data of Compound **3** was compared with a reference data and found to be matched with the saturated fatty acid, palmitic acid (**Sheng et al, 2012**).

Compound **4** (figure 1) was obtained as white powder, and its molecular formula was determined to be  $C_{20}H_{39}O_2$  by HRESIMS  $m/z$  311.2950  $[M-H]^-$  (calcd 311.2950). The  $^1H$ -NMR spectral data of compound **4** are listed in (table 2). The structure elucidation of compound **4** started with the analysis of its  $^1H$ -NMR exhibited the structure of saturated fatty acid. The NMR data of Compound **4** was



**Figure 1: Chemical structures of the isolated compounds: cholesterol (1), myristic acid (2), palmitic acid (3), arachidic acid (4), stearic acid (5), ((2R)-1-(hexadecyloxy) propane-2, 3-diol) chimyl alcohol (6), 1-Mono palmitin (7), (5-Methyl-1H-pyrimidine-2,4-dione) Thymine (8).**

**Table 1:  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) for compound 1 in  $\text{CDCl}_3$ .**

| Compound 1 |            |  |    |            |  |
|------------|------------|--|----|------------|--|
| No         | $\delta_C$ | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) | No | $\delta_C$ | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) |
| 1          | 38.1       | 0.93 (1H, m),<br>1.87 (1H, m)                  | 15 | 24.8       | 0.93 (1H, m),<br>0.97 (1H, m)                  |
| 2          | 32.9       | 1.99 (1H, m),<br>1.55 (1H, m)                  | 16 | 21.6       | -  |
| 3          | 71.6       | 3.55 (1H, m)                                   | 17 | 56.7       | 1.35 (1H, m)                                   |
| 4          | 42.7       | 2.22, 2.33<br>(2H, d, $J=13.1$ )               | 18 | 12.3       | 0.92 (3H, s)                                   |
| 5          | 142.3      | -  | 19 | 19.9       | 0.68 (3H, s)                                   |
| 6          | 121.5      | 5.30 (1H, m)                                   | 20 | 32.5       | 1.82, 1.86 (1H, m)                             |
| 7          | 40.2       | 1.94 (1H, m),<br>1.13 (1H, m)                  | 21 | 19.4       | 0.80 (3H, d, $J=6.1$ )                         |
| 8          | 36.3       | 0.92 (1H, m)                                   | 22 | 36.8       | 1.25 (1H, m),<br>1.82 (1H, m)                  |
| 9          | 50.8       | 1.07 (1H, m)                                   | 23 | 24.8       | 1.03 (1H, m),<br>1.56 (1H, m)                  |
| 10         | 37.2       | -  | 24 | 40.0       | 1.99 (1H, m),<br>1.56 (1H, m)                  |
| 11         | 23.2       | 0.90 (2H, m)                                   | 25 | 28.5       | 1.51 (1H, m)                                   |
| 12         | 24.5       | 1.1, 1.34 (2H, m)                              | 26 | 23.0       | 0.85 (3H, d, $J=6.3$ )                         |
| 13         | 38.1       | -  | 27 | 23.0       | 0.85 (3H, d, $J=6.3$ )                         |
| 14         | 57.2       | 1.0 (1H, m)                                    |    |            |  |

**Table 2:  $^1\text{H}$  (400 MHz) for compounds 2, 3, 4, and 5 in  $\text{CDCl}_3$ .**

| Compound 2              |  | Compound 3              |  | Compound 4              |  | Compound 5              |  |
|-------------------------|--|-------------------------|--|-------------------------|--|-------------------------|--|
| No                      | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) | No                      | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) | No                      | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) | No                      | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) |
| 1                       | -  | 1                       | -  | 1                       | -  | 1                       | -  |
| 2                       | 2.34 (2H, t, $J=7.6$ )                         | 2                       | 2.34 (2H, t, $J=7.6$ )                         | 2                       | 2.35 (2H, t, $J=7.6$ )                         | 2                       | 2.29 (2H, t, $J=7.6$ )                         |
| 3                       | 1.60 (2H, m)                                   | 3                       | 1.62 (2H, m)                                   | 3                       | 1.56 (2H, m)                                   | 3                       | 1.58 (2H, m)                                   |
| 10<br>( $\text{CH}_2$ ) | 1.30~1.25 (20 H, m, H4~H13)                    | 12<br>( $\text{CH}_2$ ) | 1.29~1.25 (24 H, m, H4~H15)                    | 16<br>( $\text{CH}_2$ ) | 1.30~1.25 (32 H, m, H4~H19)                    | 14<br>( $\text{CH}_2$ ) | 1.19~1.27 (28 H, m, H4~H17)                    |
| 14                      | 0.88 (3H, t, $J=7.3$ )                         | 16                      | 0.87 (3H, t, $J=7.3$ )                         | 20                      | 0.88 (3H, t, $J=7.3$ )                         | 18                      | 0.81 (3H, t, $J=7.3$ )                         |

compared with a reference data and found to be matched with arachidic acid (**Termsarasab et al, 2012**).

Compound **5** (figure 1) was obtained as white powder, and its molecular formula was determined to be  $C_{18}H_{35}O_2$  by HRESIMS  $m/z$  283.2644  $[M-H]^-$  (calcd 283.2637). The  $^1H$ -NMR spectral data of compound **5** are listed in (table 2). The structure elucidation of compound **5** started with the analysis of its  $^1H$ -NMR exhibited the structure of saturated fatty acid. The NMR data of Compound **5** was compared with a reference data and found to be matched with stearic acid (**Sultana et al, 2020**).

Compound **6** (figure 1) was obtained as white powder, and its molecular formula was determined to be  $C_{19}H_{40}O_3$  by HRESIMS  $m/z$  317.3056  $[M+H]^+$  (calcd 317.3056). The  $^1H$  and  $^{13}C$ -NMR spectral data of compound **6** are listed in Table (3). The  $^1H$ -NMR spectrum, table (3), displayed signals at  $\delta_H$  0.87 (3H, t,  $J=7.2$  Hz,  $H_3-16'$ ), 1.26 (overlapped H, m), 1.63 (2H, m,  $H_2-2'$ ) and 1.53 (2H, m,  $H_2-3'$ ) that are corresponding to aliphatic hydrocarbons. Moreover,  $^1H$  NMR spectrum showed characteristic resonances of a 1, 1'-dioxy-2,3diol unit of the hydrocarbon chain at  $\delta_H$  3.55 (1H, m, H-1), 3.47 (1H, t,  $J=2.8$ , H-1'), 3.86 (1H, m, H-2) and 3.65 (1H, m, H-3). The  $^{13}C$ -NMR spectrum, (table 3), showed 19 carbon resonances that was identified as one methy at  $\delta_C$  13.9 ( $C_{16}$ ), seventeen methylenes, three of them are oxymethylenes at  $\delta_C$  72.3, 64.1 and 70.2 (C-1, C-3 and C-1' respectively) in addition to one oxymethine at  $\delta_C$  71.7 (C-2).

The above data are in good agreement with that reported for (2R)-1-(hexadecyloxy) propane-2, 3-diol (chimyl alcohol) (**Ouijano et al 1994, Chao et al, 2007**).

Compound **7** (figure 1) was obtained as white powder, and its molecular formula was determined to be  $C_{19}H_{38}NaO_4$  by HRESIMS  $m/z$  353.2669  $[M+Na]^+$  (calcd 353.2668). The  $^1H$  and  $^{13}C$ -NMR spectral data of compound **7** are listed in table 3.

The  $^1H$ -NMR spectrum, (table 3), displayed signals at  $\delta_H$  0.80 (3H, t,  $J=6.6$  Hz,  $H_3-16'$ ), 1.19 (overlapped H, m), 2.28 (2H, t,  $H_2-2'$ ) and 1.63 (2H, q,  $H_2-3'$ ) that are corresponding to aliphatic hydrocarbons. Moreover,  $^1H$  NMR spectrum showed characteristic signals at  $\delta_H$  4.11 (1H, m, Ha-1), 4.12 (1H, m, Hb-1), 3.86 (1H, m, H-2), 3.35 (1H, dd, Ha-3), 3.62 (1H, dd, Hb-3). The  $^{13}C$ -NMR spectrum, (table 3), showed 19 carbon resonances that was identified as one methy at  $\delta_C$  14.2 ( $C_{16}$ ), sixteen methylenes, two of them are oxymethylenes at  $\delta_C$  70.4 and 63.4 (C-1 and C-3 respectively) in addition to one oxymethine at  $\delta_C$  62.5 (C-2). Also, one ester signal is identified at  $\delta_C$  70.4 (C-1').

The above data and discussion are in good agreement with the data reported for 1-Mono palmitin (**Jumina et al, 2018**).

Compound **8** (figure 1) was obtained as white powder. The  $^1H$  and  $^{13}C$ -NMR spectral data of compound **8** are listed in (table 4).

The  $^1H$ -NMR spectrum, (table 4), displayed signals at  $\delta_H$  12.96 (1H, brs, NH-3) and  $\delta_H$  12.14 (1H, brs, NH-1) for two exchangeable imido protons. In addition,  $^1H$ -NMR spectrum showed one methine signal at  $\delta_H=7.24$  (1H, s, H-6) and one methyl signal at  $\delta_H$  1.94 (3H, s, H-7).

The  $^{13}C$ -NMR spectrum, (table 4), showed five carbon resonances that was identified as two imides carbonyl  $\delta_C$  166.2 (C-4) and  $\delta_C$  153.2 ppm. (C-2)

**Table 3:  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) for compounds 6 and 7 in  $\text{CDCl}_3$ .**

| Compound 6 |            |  | Compound 7 |                        |  |
|------------|------------|--|------------|------------------------|--|
| No         | $\delta_C$ | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) | No         | $\delta_C$             | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) |
| 1          | 72.3       | 3.55 (2H, m)                                   | 1          | 70.4 ( $\text{CH}_2$ ) | 4.11 (1H, m)<br>4.12 (1H, m)                   |
| 2          | 71.7       | 3.86 (1H, m)                                   | 2          | 65.2 (CH)              | 3.86 (1H, m)                                   |
| 3          | 64.1       | 3.65 (2H, m)                                   | 3          | 63.4 ( $\text{CH}_2$ ) | 3.53 (1H, dd)<br>3.61 (1H, dd)                 |
| 1`         | 70.2       | 3.47 (2H, t, $J=2.8$ )                         | 1`         | 174.0 (CO)             | -  |
| 2`         | 29.4       | 1.63 (2H, m)                                   | 2`         | 34.1 ( $\text{CH}_2$ ) | 2.28 (2H, t)                                   |
| 3`         | 25.9       | 1.53 (2H, m)                                   | 3`         | 24.9 ( $\text{CH}_2$ ) | 1.63 (2H, q)                                   |
| 4`-13`     | 29.4       | 1.26 (20H, m)                                  | 4`-13`     | 29.1-29.7              | 1.19 (20H, m)                                  |
| 14`        | 31.9       | 1.26 (2H, m)                                   | 14`        | 31.9 ( $\text{CH}_2$ ) | 1.19 (2H, m)                                   |
| 15`        | 22.5       | 1.26 (2H, m)                                   | 15`        | 22.7 ( $\text{CH}_2$ ) | 1.19 (2H, m)                                   |
| 16`        | 13.9       | 0.87 (3H, t, $J=7.2$ )                         | 16`        | 14.2 ( $\text{CH}_2$ ) | 0.80 (3H, t, $J=6.6$ )                         |

**Table 4:  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) for compound 8 in  $\text{C}_5\text{D}_5\text{N}$ .**

| Compound 8 |                        |  |
|------------|------------------------|--|
| No         | $\delta_C$             | $\delta_H$ (No. of H, mult., $J_{\text{Hz}}$ ) |
| 1          | -                      | 12.14 (1H, brs)                                |
| 2          | 153.2 (C)              | -  |
| 3          | -                      | 12.96 (1H, brs)                                |
| 4          | 166.2 (C)              | -  |
| 5          | 108.7 (C)              | -  |
| 6          | 137.8 (CH)             | 7.24 (1H, s)                                   |
| 7          | 12.3 ( $\text{CH}_3$ ) | 1.94 (3H, s)                                   |

signals. In addition, it showed one quaternary  $\delta_C$  108.7 (C-5), one methine  $\delta_C$  137.8 (C-6), and one methyl  $\delta_C$  =12.3 (C-7) signals.

The above data are in good agreement with the data reported for (5-Methyl-1H-pyrimidine-2,4-dione) Thymine (Guo-qiang *et al*, 2012).

#### 4. Conclusion

In the current paper, chemical investigation of the

secondary metabolites isolated from the crude extract of the Red Sea gorgonian coral *Rumphella torta*, resulted in the isolation of eight compounds, Cholesterol (1), Myristic acid (2), Palmitic acid (3), Arachidic acid (4) Stearic acid (5), chimyl alcohol (6), Hexadecanoic acid 2, 3-dihydroxy-propyl ester (7) and Thymine (8). these isolated compounds are known but firstly reported from the species.

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## Conflict of interest

There is no conflict of interest

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