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Nourhan Hisham Shady
Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, New Minia, Egypt

Usama Ramadan Abdelmohsen
Department of Pharmacognosy Faculty of Pharmacy, Minia University, Minia, Egypt

Safwat Ahmed
Department of Pharmacognosy, Faculty of Pharmacy, Seuz Canal University, Ismailia, Egypt

Mostafa Fouad
Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt

Mohamed Salah Kamel
Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt

Correspondence
Nourhan Hisham Shady
Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, New Minia, Egypt

Phytochemical and biological investigation of the red sea marine sponge *Hyrtios* sp

Nourhan Hisham Shady, Usama Ramadan Abdelmohsen, Safwat Ahmed, Mostafa Fouad and Mohamed Salah Kamel

Abstract
Three compounds including indole-3- carbaldehyde (1) 5, 6-dibromoindole- 3-carboxaldehyde (2) and Ergosterol (3) were isolated from Red Sea sponge *Hyrtios* sp. The structures of compounds 1-3 were determined by spectroscopic analyses including 1D and 2D NMR, and HR-ESI-MS experiments as well as comparison to literature. These results demonstrate that sponges are rich sources for natural products with new pharmacological activities and relevance to drug discovery, some biological investigations were carried out to the total extract and some fractions of *Hyrtios* sp as anti-inflammatory, anti-pyretic, analgesic activities and showed that the sponge *Hyrtios* has significant biological activities.

Keywords: Indole alkaloids, *Hyrtios* sp, ergosterol, sponge, Indole-3-carbaldehyde

1. Introduction
Marine sponges are among the oldest metazoans with fossil records dating back to the time of the Precambrian-Cambrian boundary [1, 2]. More than 6,000 species of sponges have been described so far that inhabiting a wide variety of marine and fresh water systems, such as tropical reefs, deep sea, polar region, as well as freshwater rivers and lakes [3-5]. The phylum Porifera is classified into *Hexactinellida* (glass sponges), *Calcarea* (calcareous sponges), and *Demospongiae* (demosponges), of which the last class *Demospongiae* is described with the most comprehensive and diverse species and covers an estimated 85% of all known living species [6, 7]. These sessile animals are prolific producers of a huge diversity of secondary metabolites that have been discovered over the past years [8-12]. Marine sponges of the genus *Hyrtios* (Demospongiae class, Dictyoceratida order, Thorectidae family) continue to be a rich source of structurally diverse natural products with different chemical classes include sesterterpenes, acyclic triterpenes, sterols, macrolides and indole alkaloids [13-17]. Members of the genus *Hyrtios* showed activity as Neuronal Nitric Oxide Synthase (nNOS) Selective Inhibitors [18], and against *Plasmodium falciparum*, *Trypanosoma brucei* subsp. *rhodesiense*, *Trypanosoma cruzi*, hepatitis A virus (HAV) [19], significant antiphospholipase A2 activity [20], inhibitory effect against *Mycobacterium tuberculosis* [21]. A great variety of simple and substituted indole derivatives, including halogenated indoles, bisindoles and tryptamine derivatives. In the present study, *Hyrtios sp* collected from the Red Sea – Egypt was chemically investigated and two indole alkaloids were isolated including a known indole alkaloid (1) in addition to naturally new indole alkaloid (2) and Ergosterol (3).

2. Materials and methods
2.1 Sponge Material
The sponge material used in this work is the marine sponge *Hyrtios* sp. and it was collected by Prof. Dr. Safwat Ahmed (Suez Canal University) from the Egyptian coasts of the Red Sea at Sharm el-Sheikh using scuba diving at a depth of 30 ft. The collected material was immediately frozen and kept at -20 °C until investigation. The sponge biomass was identified by Dr. R. van Soest (Institute of Systematic Population Biology, Amsterdam University, the Netherlands). A voucher specimen was kept in the collections of the Zoological Museum of the University of Amsterdam, under registration number ZMAPOR19762. Another voucher sample was deposited under the numbers SAA-61 at the Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University, Egypt.

2.2 Materials for Biological Study
2.2.1 Chemicals
1. Carboxymethylcellulose, (El-Nasr pharmaceutical and chemical co. Egypt.)

2. Indomethacin (Liometacen®), El-Nile co., Egypt.
3. Normal saline 0.9% (El-Nasr pharmaceutical and chemical co. Egypt).
4. Carrageenan (Sigma, USA) as a factor inducing edema.
5. Acetyl salicylic acid (Aspegic®), Ameria Pharmaceutical Industry, Egypt.

2.2.2 Animals

The animals used in this study were Male albino rats weighing (200 ± 50) and mice (30 ± 5g), they were obtained from the animal house of faculty of Medicine, Assiut, Egypt and housed and bred under standardized environmental conditions, and fed with standard diet and water.

2.3 Methods for biological study

2.3.1 Evaluation of the anti-inflammatory activity by carrageenan-induced paw edema method

The total extract and different fractions of the sponge *Hyrtios* sp. were evaluated for their anti-inflammatory activity using the yeast-induced paw edema method [22]. Male albino rats (200 ± 50 g) were randomly divided into six groups (four animals per group). The specified doses 350 mg/kg of the total extract and the fractions as well as the standard drug Indomethacin (8 mg/kg) were suspended in 0.5% CMC solution.

The negative Control group was given vehicle (0.5% CMC solution) alone, while the tested total extract, fractions and the reference drug were administrated orally one hr before carrageenan injection (0.01 ml, 1% W/V in normal saline, sc.) as phlogistic of the inflammation and were injected into the sub-planter tissue of the right hind paw.

The paw thickness (cm) was measured using a Vernier caliper immediately at 0, 0.5, 1, 2, 3, 4, 5 hrs. The percentage inhibition of the rat paw edema was calculated as follows [23].

$$\%\ Inhibition = \frac{(control\ mean - treated\ mean)}{control\ mean} \times 100$$

2.3.2 Evaluation of the antipyretic activity

The total extract and different fractions of *Hyrtios* sp were evaluated for their anti-pyretic activity using yeast induced pyrexia method [24].

The test was performed on rats (200±50g) by subcutaneous injection (in the back, below the nape of the neck) of 20% aqueous suspension of yeast in a dose of 10 ml/kg to induce pyrexia and the site of the injection was massaged to spread the suspension beneath the skin. Rectal temperature of each animal was recorded by inserting a thermometer 2 cm into the rectum after 18 hours of the yeast injection, when the temperature reached its peak value. Animals that did not show a minimum increase of 0.5 °C in temperature were discarded.

The selected pyretic animals were grouped into six groups (four animals each). Group (6) was the negative control group and it was only given the vehicle (0.5% CMC solution). Groups (1-4) were administered the total extract and fractions in a dose of 300 mg/kg p.o. (suspended in 0.5% CMC solution). Group (5) was given acetylsalicylic acid 100 mg/kg p.o. The rectal temperature of each animal was again recorded immediately at 0, 0.5, 1, 2, 3, 4, and 5 hrs.

2.3.3 Evaluation of the analgesic activity

The total extract and different fractions, obtained from fractionation procedures of the total extract of *Hyrtios* sp. were evaluated for their analgesic activity using the hot plate method described by [25]. The mice (30 ± 5g) were treated with 300 mg/kg p.o. of the total extract and different fractions, suspended in 0.5% CMC solution. The animals were divided randomly to six groups, four groups received the tested extract and fractions, while the control group received the extract vehicle orally and the reference group animals were treated with 100 mg/kg ASA p.o. [26]. The temperature of the hot plate was regulated at 54 ˚C. Each mouse was placed on the hot-plate in order to obtain the animal's response to heat-induced paw.

Licking the hind paw or jumping was taken as an indicator of the animal's response to the pain. The time taken for each mouse to lick either hind paw or to jump up (i.e. reaction time) was noted and recorded in seconds. Each mouse served as its own control. Thus, before treatment, its reaction time was determined three times at 1 h interval. The mean of these three determinations constituted the "initial reaction time", i.e. the reaction time before the treatment of the mouse [27]. The reaction time is measured at 0, 0.5, 1, 2, 3, 4, and 5 hrs of treatment with all tested drugs.

3. Extraction and isolation

The sponge *Hyrtios* sp. was collected and freeze dried, then reduced to fine powders. The powdered sponge was then macerated in MeOH: DCM (1:1), to give the total extract 46 gm. and then fractionated with ethyl acetate to give ethyl acetate fraction (6 g). The mother liquor was fractionated with butanol to give butanol fraction (15 g).The EtoAc-soluble residue of the alcoholic extract of the red sea sponge *H*yrtios sp *was* subjected to vacuum-liquid chromatography eluting using Pet-EtOAc gradient elution starting with Pet. Ether and the polarity was increased to Pet-EtOAc 1:1 then EtOAc 100% and finally MeOH 100%. Each polarity was concentrated under reduced pressure giving four subfractions. The four subfractions are: subfraction I (Pet–ether Fr.) (30 mg), subfraction II (Pet-EtOAc) (1:1) (2 g), subfr.III (EtOAc) (0.65 g) and subfr. IV (MeOH) (3 g).while subfraction II (2g) was subjected to sephadex LH-20 CC using methanol: water gradient elution starting from 10% till 100%. Sub fr II 30% was further purified by using HPLC with an acetonitrile (MeCN) and water solvent mixture complemented by 0.05% trifluoroacetic acid (10% MeCN/H_2O to 100% MeCN over 30 min at a flow rate of 5 ml/min), to afford compound 1 (1.4 mg).

Compound 2 has been isolated from Subfraction IV using silica gel column chromatography by using DCM: MeOH gradient elution. Subfr IV_2 is subjected to Sephadex CC using gradient elution 10-100% (MeOH: H_2O), Subfr IV_2 30% is further purified by using semi preparative HPLC using an acetonitrile (MeCN) and water solvent mixture complemented by 0.05% trifluoroacetic acid: (10% MeCN/H_2O to 100% MeCN over 30 min at a flow rate of 5 ml/min), to yield compound 2 (0.9 mg), compound 2 have a characteristic peak at a retention time 18.3 min.

Compound 3 has been isolated from subfr IV using silica gel column chromatography by using DCM: MeOH gradient elution. Sub Fraction IV_{15} was further purified by using semi preparative HPLC using an acetonitrile (MeCN) and water solvent mixture complemented by 0.05% trifluoroacetic acid: (10% MeCN/H_2O to 100% MeCN over 30 min at a flow rate of 5 ml/min), to yield compound 3 (2 mg), compound **3** has a characteristic peak at a retention time 14.8 min.

4. Results and discussion

Compound (1)

Compound 1 (1.4 mg) was purified as pale yellow amorphous

powder. It is soluble in methanol and water and insoluble in petroleum ether and chloroform. The HRESI-MS of Compound 1 showed exact mass at *m/z* 144.0449 $(M-H)^-$, (calculated mass 144.0449), corresponding to the molecular formula C_9H_6NO. The ^{1}H-NMR spectrum showed four protons resonances for the aromatic ABCE system at δ_H 7.21 (1H,td, *J*= 8.4, 1.3 Hz) corresponding to H-5, δ_H 7.28 (1H, td, *J*= 8.2, 1.3 Hz) corresponding to H-6, δ_H 7.50 (1H, br d, *J*= 7.9 Hz) corresponding to H-7, δ_H 8.08 (1H, br.d, *J*= 8.5 Hz) corresponding to H-4.In addition to three other proton signals firstly broad singlet proton at δ_H 8.28 (1H, br.s) corresponding to H-2, another singlet peak at δ_H 8.54 (1H, s) corresponding to NH-1, and a characteristic downfield singlet peak for aldehydic proton at δ_H 9.93 (1H,s) corresponding to H-8. From the previous HREMS, ^{1}HNMR data and from the data reported in literature it is suggested that compound **1** contains indole alkaloid nucleus [28]. In comparison the previous data with data reported in literature [16] compound 1 shown in figure (1), was identified as indole-3-carbaldehyde, and it is first reported in this sponge species.

^{1}HNMR spectral analysis of compound 1 is recorded in table (1).

Compound (2)

Compound (2) (0.9 mg) was isolated as brown amorphous powder. It is soluble in methanol and water and insoluble in petroleum ether. The HRESI-MS of compound **2** revealed the presence of a cluster of ionic peaks at 323.8635: 325.8635: 327.8635 with a ratio of 1:2:1 this indicating the presence of 2 bromine atoms with exact mass at *m/z* 325.8635 $(M+Na)^+$, corresponding to the molecular formula $C_9H_5Br_2NONa$ (calculated mass is 325.8636). Furthermore, the presence of a cluster ionic peak at 245.9530: 247.9530 with a ratio 1:1 indicating the loss of one bromine atom. The ^{1}H-NMR spectrum summarized in Table (1) showed 4 protons resonances at δ_H 7.87 (1H,s) corresponding to H-7, δ_H 8.00 (1H,s) corresponding to H-4, δ_H 8.54 (1H,s) corresponding to H-2 and a downfield singlet at δ_H 9.80 (1H,s) corresponding to an aldehydic proton at H-8. The previous data suggested that compound 2 contain bromo-indole nucleus. Finally from the mass and ^{1}H-NMR data and in comparison with the data reported in the literature [29, 30, 31] it is indicated that compound 2 is 5, 6-dibromoindole-3-carboxaldehyde shown in figure (1). This compound is a new natural product.

Compound (3)

Compound (3) (2 mg) was isolated as white powder. It is soluble in EtOAc, hot methanol, and insoluble in chloroform. The ^{1}H-NMR spectrum of compound 3 showed 6 methyl signals at δ_H 1.08 (3H,d, *J* =6.6 Hz) Corresponding to H-21, δ_H 0.96 (3H,s) corresponding to H-19, δ_H 0.94 (3H, d, *J* =6.6 Hz) corresponding to H-28, δ_H 0.87 (3H,d, *J*=6.6 Hz) corresponding to H-27, δ_H 0.86 (3H, d, *J*= 6.9 Hz) corresponding to H-26 and finally at δ_H 0.68 (3H,s) corresponding to H-18. In addition to proton to, a characteristic multiplet proton at 3.53 (1H,m) corresponding to H-3 and four olefinic protons at δ_H 5.54 (1H, m) corresponding to H-6, δ_H 5.39 (1H,m) corresponding to H-7, δ_H 5.28 (1H,dd, *J=7.9,15.3*Hz) corresponding to H-22, δ_H 5.10 (1H, dd, *J=8.8,15.3* Hz) corresponding to H-23. On the other hand the ^{13}C NMR and DEPT spectra revealed the prescence of 28 signals including 7 methylene carbons peaks at δ_C 40.9 (C-4), δ_C 39.0 (C-16), δ_C 38.3 (C-1), δ_C 32.0 (C-2), δ_C 28.1 (C-12), δ_C 22.8 (C-11), δ_C 20.8 (C-15), and four characteristic downfield olefinic peaks at δ_C 135.7 (C-22), δ_C 131.8 (C-23), δ_C 119.0 (C-6), δ_C 116.5 (C-7).Other methin carbon signal have been inspected at δ_C 69.38 (C-3) suggested the presence of oxygenated methin.

Furthermore four quaternary carbons were revealed at δ_C 140.7 (C-5), δ_C 140.4 (C-8), 37.7 (C-10), 40.9 (C-13), other methin carbon signals at δ_C 55.6 (C-14), δ_C 54.3 (C-17), δ_C 46.2 (C-9), 42.8 (C-24), δ_C 40.4 (C-20), δ_C 32.9 (C-25). Finally characteristic methyl carbon peaks showed at δ_C 20.6 (C-27), δ_C 19.4 (C-26), δ_C 19.1 (C-21), δ_C 17.2 (C-28), δ_C 15.7 (C-19) and δ_C 11.5 (C-18). From the previously mentioned spectral data and in comparison with the data reported in the literature [32, 33, 34] compound 3 was identified as ergosterol shown in figure (1). This compound is first isolated from this sponge. It was previously isolated from marine sponge *Axinella cannabina* [33] and also from the marine sponge *Pseudaxinella Lunacharta* [34]. ^{1}HNMR, ^{13}C-NMR and DEPT spectral analysis of compound 3 are recorded in tables (2-3).

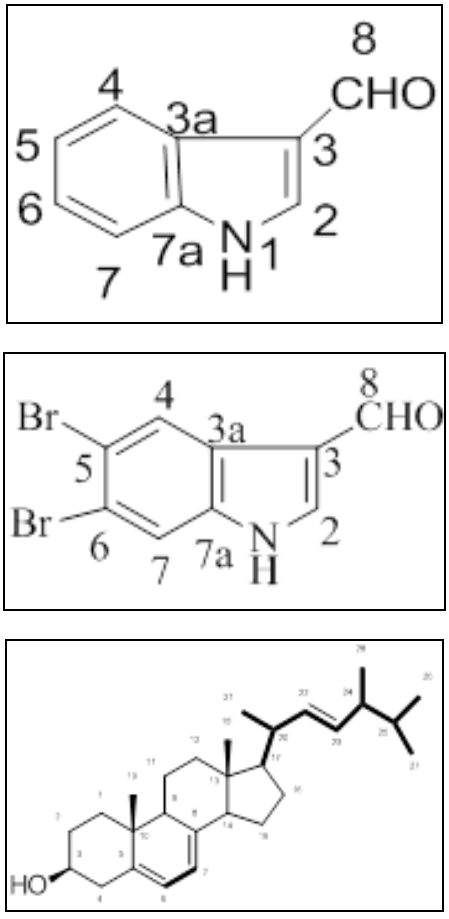

Fig 1: structure of compounds (1-3).

Table 1: ^{1}H-NMR spectral data of compound 1-2.

H	Compound 1 (600 MHz, DMSO-*d6*).	Compound 2 (600 MHz, CD_3OD).
H-2	8.28 (1H, br, s)	8.54 (1H,s)
H-4	8.08 (1H,br.d, *J*=8.5)	8.00 (1H,s)
H-5	7.21(1H,td, *J*= 8.4, 1.3)	-
H-6	7.28 (1H, td, *J*=8.2,1.3)	-
H-7	7.50 (1H,br.d, *J*=7.9)	7.87 (1H,s)
H-8	9.93 (1H,s)	9.80 (1H,s)
NH-1	8.54 (1H,s)	-

Table 2: Significant ^{1}H-NMR spectral data of compound 3.

Assignment	Chemical shift (ppm)	Multiplicity	*J (Hz)*
H-3	3.53	(1H,m)	-
H-6	5.54	(1H,m)	-
H-7	5.39	(1H,m)	
H-22	5.28	(1H,dd)	*J*= (7.9,15.3 Hz)
H-23	5.10	(1H,dd)	*J*= (8.8,15.3 Hz)

Table 3: ^{13}C NMR and DEPT data of compound 3 (150 MHz, CD_3OD).

Assignment	Chemical shift (ppm) multiplicity
C-1	38.3 (CH_2)
C-2	32.0 (CH_2)
C-3	69.3 (CH)
C-4	40.9 (CH_2)
C-5	140.7 (C)
C-6	119.0 (CH)
C-7	116.5 (CH)
C-8	140.4 (C)
C-9	46.2 (CH)
C-10	37.7 (C)
C-11	22.8 (CH_2)
C-12	28.1 (CH_2)
C-13	40.9 (C)
C-14	55.6 (CH)
C-15	20.87 (CH_2)
C-16	39.0 (CH_2)
C-17	54.3 (CH)
C-18	11.5 (CH_3)
C-19	15.7 (CH_3)
C-20	40.4 (CH)
C-21	19.1 (CH_3)
C-22	135.7 (CH)
C-23	131.8 (CH)
C-24	42.8 (CH)
C-25	32.9 (CH)
C-26	19.4 (CH_3)
C-27	20.6 (CH_3)
C-28	17.2 (CH_3)

5. Anti-inflammatory activity

The results mentioned in Tables (4-5) showed that the oral administration of the total extract and some fractions of the sponge *Hyrtios* sp. produced a notable decrease in the carrageenan – induced paw edema. Fraction 1 (butanol), fraction 4 (total extract) and fraction 2 (ethyl acetate) showed important anti-inflammatory effects that were comparable to the positive control (indomethacin). They significantly decreased paw edema after 1 hr till the end of the experiment in comparison with the non-treated control group which remained the highest edematous one. Besides, the highest inhibition of paw swelling (50.6%) produced by fraction 1 (butanol) was observed at 5 hrs. versus 51.9% by indomethacin.

On the other hand, the total extract caused significant decrease in the induced inflammation that increased gradually along the experiment to reach 46.7% after 5 hrs, while fraction 2 (ethyl acetate) showed mild inhibition of the induced inflammation that increased gradually along the experiment to reach 44.1% after 5 hrs. On the other hand, fraction 3 (Pet-EtOAc) displayed weak anti-inflammatory properties.

The significant anti-inflammatory effect of fraction 1 (butanol) is probably attributed to its content of alkaloids as it rich with alkaloids which posses anti-inflammatory activity [35], also the significant anti-inflammatory effect of the total extract is probably attributed to their content of steroids and terpenoids in addition to the alkaloids.

6. Antipyretic activity

The results mentioned in Table (6) showed that the oral administration of the total extract and some fractions of *Hyrtios* sp. produced a marked decrease in the rectal temperature of rats. The total extract treated group significantly decreased the rectal temperature from 1 hr till reached its maximum at 5 hrs compared to the standard drug acetyl salicylic acid. Fraction 3 (Pet-EtOAc) showed marked decreases in the rectal temperature from 3 hrs till reached its maximum effect at 5 hrs, followed by fraction 2 (EtOAc) which showed mild decrease in the rectal temperature at 5 hrs. On the other hand fraction 1 (butanol) showed week antipyretic activity compared to the standard treated group. The significant antipyretic activity of the total extract and Pet-EtOAc treated groups may be attributed to their high content of steroids and triterpenoids.

7. Analgesic activity

The results mentioned in Table (7) showed that the oral administration of some fractions of *Hyrtios* sp. produced mild analgesic activity. The total extract showed the highest analgesic activity as it produced a remarkable increase in hot plate reaction times comparable to other fractions. While other fractions, fraction 1 (butanol), fraction 2 (EtOAc) and fraction 3 (Pet-EtOAc) showed weak analgesic activity.

Table 4: Results of the anti-inflammatory activity of the total extract and different fractions of *Hyrtios* sp**.**

Group no.	Treatment	Paw swelling (thickness) in cm. (Mean ± S.D.)						
		Pretreatment	30 min.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
1	Butanol	0.73 ±0.02	0.71 ± 0.02	0.62 ±0.02***	0.57 ± 0.05**	0.53 ± 0.04***	0.43 ± 0.04	0.38 ± 0.02
2	EtOAc	0.73 ± 0.02	0.71 ± 0.02	0.67 ± 0.02**	0.62 ± 0.02***	0.56 ± 0.04***	0.47 ± 0.02	0.43± 0.02
3	Pet-EtOAc	0.72 ± 0.02	0.71 ± 0.02	0.71 ± 0.03*	0.71 ± 0.02**	0.71± 0.02	0.71± 0.02**	0.68 ±0.02**
4	Total extract	0.73 ± 0.02	0.71 ± 0.06	0.68 ± 0.02*	0.61± 0.04****	0.47 ± 0.06***	0.47 ± 0.02	0.41 ± 0.02
5	Indomethacin	0.72 ± 0.02	0.71 ± 0.04	0.61 ± 0.06**	0.51 ± 0.06***	0.41 ± 0.02	0.37 ± 0.02	0.37 ± 0.02
6	control	0.72 ± 0.029	0.72 ± 0.02	0.73 ± 0.02	0.73 ± 0.025	0.73 ± 0.02	0.77 ± 0.02	0.77 ± 0.02

(*p $\leq$0.05, **p$\leq$0.01, *** p$\leq$0.001) compared to the control group, data represented as mean ± S.D in each group, n=4 rats in each group

Table 5: Inhibition of paw edema for the total extract and different fractions of *Hyrtios* sp**.**

Group no.	Treatment	Dose (mg/kg)	Percentage of inhibition %				
			1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
1	Butanol	350	15.0	27.4	27.4	44.1	50.64
2	EtOAc	350	8.2	23.2	23.2	38.9	44.1
3	Pet-EtOAc	350	2.7	2.7	2.7	7.7	11.6
4	Total extract	350	6.84	35.6	35.6	38.9	46.7
5	Indomethacin	8	16.4	43.8	43.8	51.9	51.9
6	Control		-	-	-	-	-

Table 6: Results of the anti-pyretic activity of the total extract and different fractions of *Hyrtios* sp**.**

Group no.	Treatment	Rectal temperature (˚C)						
		Pretreatment	30 min.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
1	Butanol	39.72 ± 0.17	39.75 ± 0.12	39.725 ± 0.170	39.77 ± 0.09	39.7±0.08	39.775 ± 0.09	39.77 ± 0.09*
2	EtOAc	39.82 ± 0.09	39.57 ± 0.09*	39.65 ± 0.05*	39.675 ± 0.170	39.5 ± 0.21**	39.17 ± 0.25**	38.82 ± 0.35***
3	Pet-EtOAc	39.75 ± 0.12	39.8 ± 0.08	39.275 ± 0.17***	39.525 ± 0.170**	39.125 ± 0.17***	38.95 ± 0.19***	38.57 ± 0.55***
4	Total extract	39.72 ± 0.17	39.17 ± 0.15***	38.87 ± 0.12	38.25 ±0.20	38.075 ± 0.28	37.55 ± 0.44	37.77 ± 0.27
5	Acetyl salicylic acid	39.70 ± 0.16	38.95 ± 0.31**	38.125 ± 0.170	38.02 ± 0.25	37.4 ± 0.24	36.95± 0.34	37.07 ± 0.35
6	Control	39.60 ± 0.25	39.775± 0.09	39.8 ± 0.08	39.85 ± 0.129	39.85 ± 0.12	39.9 ± 0.16	39.92 ± 0.05

(*p ≤0.05, **p≤0.01, *** p≤0.001) compared to the control group, data represented as mean ± S.D in each group, n=4 rats in each group.

Table7: Results of the analgesic activity of the total extract and different fractions of *Hyrtios* sp**.**

Group no.	Treatment	Reaction time (sec)						
		pretreatment	30 min.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
1	Butanol	47.00 ± 5.88	5 1 ± 6.271	49 ± 4.32	47.5± 4.65	49.75 ± 3.30	47.75 ± 5.61	49.5 ± 3.316
2	EtOAc	47.25 ± 8.42	47.5± 6.60	48.5 ± 5.74	46.25 ± 4.11	47.5± 4.79	49 ± 3.651484	47.5 ± 3.10
3	Pet-EtOAc	44.50 ± 6.19	44.5 ± 5.06	47.75 ± 4.57	47± 5.88	46.25 ± 3.30	46.5 ± 3.511	48.25 ± 3.77
4	Total extract	43.00 ± 8.75	44.75± 7.13	75.25 ±5.12***	95.75 ± 12.91***	113.75 ± 20.61***	118.25 ± 16.33***	105.75 ± 12.89***
5	Diclofenac	41.00 ± 7.48	51.5 ± 5.25	75.5 ±6.244***	109.25 ± 34.9***	121.5 ± 37.27***	116.5 ± 23.51***	119.5 ± 20.56***
6	Control	47.00 ± 5.88	47.25 ± 8.42	44.75 ± 6.65	44.5 ± 6.19	44.25 ± 7.27	41 ± 7.48	45.25 ± 9.17

*p ≤0.05, **p≤0.01,*** p≤0.001) compared to the control group, data represented as mean± S.D in each group, n=4 mice in each group.

8. Conclusion

Phytochemical investigation of the marine sponge *Hyrtios* sp. yeilded three compounds one known but first reported in this species Indole-3-carbaldehyde (1), in addition to the naturally new compound 5, 6-dibromoindole-3-carboxaldehyde (2) and Ergosterol (3). On the other hand, the biological investigation of the total extract and some of its fractions showed that the sponge have significant anti-inflammatory, anti-pyretic and mild analgesic activity.

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