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Comparative Assessment of the Antibacterial Potential of Four Soft Corals Collected from the Red Sea (Egypt) Against Selected Pathogenic Bacteria

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Abstract: The objective of this current research is to investigate the antibacterial properties of crude extracts derived from various soft coral species Specimens were collected off the coast of Hurghada city in Egypt's Red Sea region. The antimicrobial activity of *Sinularia leptoclados, Sarcophyton ehrenbergi*, *Nephthya molle,* and *Dendronephthya hemprichi* was evaluated against several harmful microorganisms. The well-cut diffusion technique was employed to ascertain the level of activity. The determination of minimal inhibitory concentrations (MIC) and minimal bacterial concentration (MBC) was conducted using the dilution method. These concentrations were assessed against *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 9027, *Aeromonas hydrophila* ATCC 13037, and *Staphylococcus aureus* ATCC6538. The antibacterial potential of the crude extract obtained from a mixture of methanol/dichloromethane was 10.0 for *S. leptoclados* and ranged from 8.0 ± 0.58 to 14.0 ± 0.65 for *S. ehrenbergi*, and 10.0 ± 0.46 to 24.0 ± 0.68 for *D. hemprichi*. The *D. hemprichi* extract showed the highest antimicrobial activity which was carried out through MIC and MBC testing. Additionally, a partial characterization of these substances was conducted utilizing (GC-MS). The findings indicated the presence of varied bioactive substances, however, cis-vaccenic acid was considered the major compound in this species. Our findings revealed the potent antibacterial of *Dendronephthya hemprichi* compared to the other species.
Keywords: *Sinularia leptoclados*; *Sarcophyton ehrenbergi*; *Nephthya mole; Dendronephthya hemprichi*; GC-MS.

1. Introduction

Multiple aspects of ecosystem structure and function are influenced by the level of biodiversity present in the system [1]. Potentially bioactive chemicals have an important role in drug development, available in aquatic environments [2]. Due to their rich supply of high-quality proteins and other micronutrients beneficial to human health, marine invertebrates have long been consumed as a food source [3]. Scientists have started collecting, isolating, and studying phytochemicals from these species because of their potential health advantages. There is enormous potential for medication discovery and development in the over 40,000 chemicals identified from marine creatures since the second part of the last century. Cytarabine was one of the first marine-derived medications licensed by the FDA in 1969 for the treatment of leukemia [4]. Nowadays, various medications and drug candidates have been produced from marine natural compounds and are either in preclinical testing or clinical studies [5]. The soft corals develop unique secondary metabolites to be protected from their hostile habitats [6]. Twenty-two percent of the new marine metabolites isolated from marine invertebrates up until 2012 were from soft coral [7]. Marine natural compounds derived from soft coral have a wide variety of molecular structures, some of which have medicinal qualities [8]. *In vivo* and *in vitro* testing have shown that several of these substances have potential activity, which has led to their entry into both preclinical and clinical research. The principal source of natural goods came from either the *Xeniidae*, *Nephtheidae*, *Alcyoniidae*, or *Clavulariidae* family [9]. From 2015 to 2020, around 179 novel steroids were isolated from soft corals around the world [5]. The majority of these new steroids were classified as hydroxysteroids. Anticancer, antibacterial, and anti-inflammatory were the three categories of obvious bioactive activity that came from these newly found metabolites [5].

The objective of the current study is to assess the antibacterial activity of four soft coral species from Egypt's Red Sea coral reef. Subsequently, the most potent (active) extract was further analyzed by GC-MS to characterize the bioactive constituents that may be responsible for the antibacterial activity.

2. Materials and methods

2.1. Study area

The National Institute of Oceanography and Fisheries (NIOF) selected the front region for its location, which offers natural protection against most human-related activities, such as

diving and fishing. This area remains largely unaffected by anthropogenic influences or human actions but does experience sedimentation processes as a result of water flow and northward currents. The study site, which is five kilometers north of Hurghada City, was divided into three areas; No 1. Northern reefs, No. 2, Shabrur reefs, and No 3 Crescent reefs, at latitudes of 27˚17'13"N and longitudes of 33˚46'43"E, locations were distinguished by rapid sedimentation rates.

2.2. Sampling of soft coral species

In the summer of 2021, a total of four types of marine soft corals were collected from different spots within the NIOF region using both SCUBA diving and snorkeling techniques. For each coral species, two colonies were acquired, with one colony designated for extraction and subsequent evaluation of its biological activity. The second colony was reserved to verify and confirm the coral's identification. In-situ photographs were taken of all the samples. Using an underwater slate, detailed records of the coral's morphology, surrounding environment, and habitat were documented. The samples were carefully collected using sharp scalpels and scissors. Parts of boulders adhering to the animal were in some instances removed and put into plastic bags. To prepare the soft coral specimens for systematic analysis, they were initially preserved in a solution of 4% formalin mixed with seawater for a duration of 24 hours. Subsequently, the specimens were thoroughly rinsed using fresh water and then stored in a solution of 70% ethanol. When the soft coral tissue arrived at the laboratory, sodium hypochlorite, also known as household bleach, was used to disintegrate it. This process effectively removed the tissue, leaving behind the carefully cleaned sclerite remnants, which were thoroughly rinsed with double distilled water. It's worth noting that all samples were obtained from a depth of five meters [10].

2.3. Identification of soft coral species

Dr. Hussien Nasr Mohamed from the Hydrobiology Lab at NIOF's Hurghada branch in Egypt graciously assisted in identifying the collected soft coral species. To facilitate this process, each sample was meticulously sliced into small squares, approximately 1 cm in size, using a scalpel. Subsequently, these squares were placed on a glass slide along with two drops of bleach. The sclerites have spread and the specimen has been studied using a microscope with a camera (AmScope 40X-1600X Lab Clinic Vet Trinocular Microscope with 5MP Camera, Model: T490A-5M) and a phase micrometer (Electronic Outside Micrometer 0-25 mm, Model: EB004079, US) after the bubbles have halted. When the sclerites became heavily pigmented and challenging to differentiate from the remaining tissue, a clearing process was initiated. This involved utilizing a combination of phenol and xylene to facilitate the distinction of the sclerites [11-13].

2.4. The process of generating crude extracts from soft corals

The samples were frozen and stored at -20°C before the extraction operation. Following this, the frozen soft coral samples were allowed to thaw, fragmented into smaller pieces, and then subjected to the extraction process at room temperature. To ensure thorough extraction, the macerated tissues were extracted in triplicate using methanol/dichloromethane (1:2, *v/v*) until no color was recovered. Using Whatman No. 1 filter paper, the extracts were combined, filtered, and dried with a rotary evaporator (IKA Rotary Evaporators, Model: RV 3, Germany) at 40°C [14]. The crude extracts were obtained after evaporation and were placed in a sterilized glass container and stored at refrigerator temperature (4°C).

2.5. Pathogenic bacterial indicators

All culture media and solutions underwent sterilization through autoclaving at 121°C for 15 minutes under pressure (15 psi.). The pathogenic bacterial indicators used in our study were *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10237, *Aeromonas hydrophila* ATCC 13037 and *Staphylococcus aureus* ATCC6538. The staff members of NIOF, Red Sea branch, Hurghada, Egypt, kindly donated these pathogens. The bacteria were grown in a nutrient broth (OXOID) medium and stored on nutrient agar plates. Initially, 10 mL of medium were inoculated with colonies derived from nutrient agar plates containing recently cultured cells of preselected pathogenic bacteria. These inoculated batches were subsequently incubated overnight at 37°C.

2.6. Antibacterial screening

The well diffusion method was run according to [15]. In summary, 0.1 mL of bacterial suspension in a sterile medium (with a concentration of 1.5 x 10^7 CFU/mL) was evenly spread on nutrient agar plates. After the agar solidified, wells with a diameter of 4 mm were carefully cut out. Subsequently, 50 µL of every instance (250 mg/mL) dissolved in Dimethyl Sulfoxide (DMSO) were placed into these wells. Control wells received only DMSO (20 mL). All plates were then left at 48°C for 1 hour and subsequently incubated at 37°C for 24 hours. The inhibition zones that formed around the wells had their diameters measured, and the average diameter was determined using data from three replicates [16].

2.7. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The least amount of extract required to prevent the growth of test pathogenic microorganisms was determined as MIC. For antimicrobial testing, the soft coral extract concentrations in Mueller Hinton broth varied from 0.05 to 2.00 mg/mL. In each case, 100 µL of the bacterium with a concentration of 1×10^7 CFU/mL was introduced, and the tubes were incubated at 37 ℃ for 24 hours. The final dilution at which no discernible bacterial growth was seen, as indicated by the absence of turbidity in the medium, was determined to be the Minimum Inhibitory Concentration (MIC). To determine the Minimum Bactericidal Concentration (MBC), A 5 µL drop of a 1:5 diluted sample was applied to Tryptic Soy Agar (TSA) media from each tube that had no turbidity apparent. After that, these cultures were kept at 37°C overnight. The lowest concentration at which bacterial growth was reduced by three logs was known as the MBC [17].

2.8. Characterization of crude extract

The extracts derived from the collected soft corals underwent GC-MS analysis. This analysis was conducted using

an (Agilent 7890A GC) instrument, which was equipped with an HP-5MS column measuring 30 meters in length, 250 micrometers in diameter, and 0.25 micrometers in thickness. A MS detector (Agilent 5975C) was employed for the preliminary identification of bioactive secondary metabolites in the *D. hemprichi* extract. The oven's initial temperature was established at 90°C and maintained for a duration of 1 minute, after which it was raised at a rate of 8°C per minute until it reached 300°C, where it was maintained for 30 minutes. The carrier gas, helium, was employed and flowed at a rate of 1.5 mL/min. The injector temperature was set to 290°C, and the sample injection volume was 1 µL in the splitless mode. The mass spectrum was operated at 70 electron volts (eV), covering a mass range from 60 to 600 atomic mass units (amu). The extracts were prepared using ethyl acetate, concentrated until they were completely dried, and subsequently reconstituted in an appropriate volume of ethyl acetate [18].

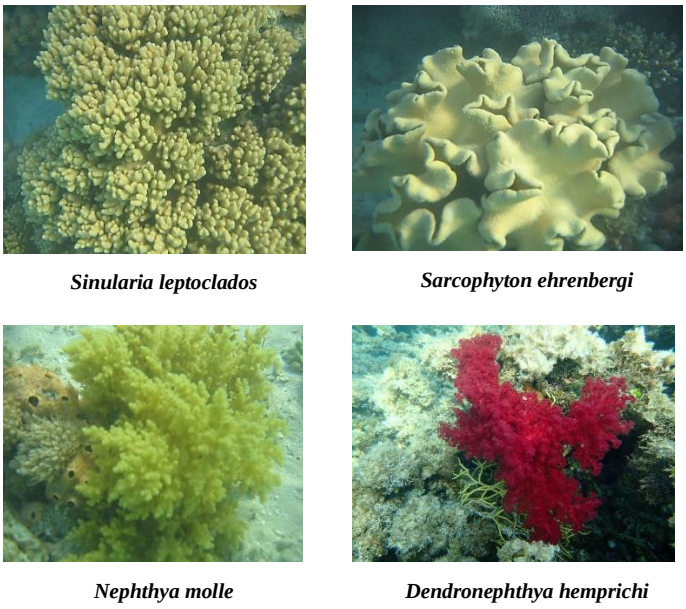


Figure 1: Photograph of Red Sea soft corals in their habitat.

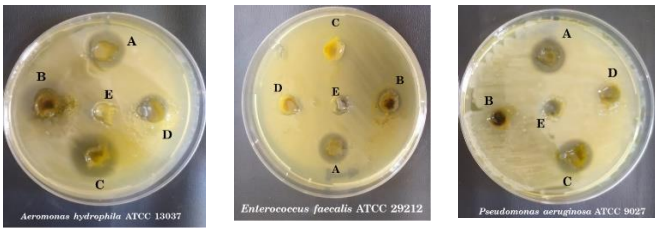


Figure 2: Effect of soft coral extracts; A: *Dendronephthya hemprichi*, B: *Sinularia leptoclados*, C: *Sarcophyton ehrenbergi*, D: *Nephthya molle* and E: control on different types of pathogenic bacteria.

2.9. Statistical analysis

The information was provided as the mean over three replicates with the standard error (SE) shown. The XLSTAT tool (version 5.03) was used for statistical analysis. ANOVA (one-way analysis of variance) was used to assess the results, and a statistically significant value of $p < 0.05$ was used to determine its significance.

3. Results

The soft corals were reported from selected sites in front of the NIOF, Red Sea, Egypt. The four species belonged to two families; *Sinularia* and *Sarcophyton* belong to family Alcyoniidae), while *Nephthya* and *Dendronephthya* (Family: Nephtheidae) were recorded. However, four Red Sea soft corals were identified as *Sinularia leptoclados*, Sarcophyton *ehrenbergi*, *Nephthya molle*, *and Dendronephthya hemprichi* (Fig. 1).

3.1. Antimicrobial activity

The antibacterial effects of four popular soft coral species from the Red Sea on Gram-positive (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC6538), Gram-negative (*Pseudomonas aeruginosa* ATCC 9027 and *Aeromonas hydrophila* ATCC 13037) and pathogenic yeast *Candida albicans* ATCC 10237. The raw extracts obtained from the four soft coral species exhibited varying levels of activity against the selected pathogen isolates. Using a jangka sorong, the diameter of the inhibition zone that developed around the wells was measured to determine the antibacterial activity.

The inhibition zone of each treatment has a different diameter and irregular shapes. To record the observations, we measured both the horizontal and vertical diameters of the inhibition zones formed around the well. (Table 1) lists the precise widths of the inhibition zones. The results of antibacterial activities of the four extracts of sponges (at concentrations of 250 mg/mL) against selected microbes.

Table 1: Antibacterial activity of four selected soft corals extracts by well diffusion assay against pathogenic microbes at the concentration 250 mg/mL.

Soft corals	Inhibition zone (mm)				
	E. faecalis	*S. aureus*	*P. aeruginosa*	*A. hydrophila*	*C. albicans*
S. leptoclados	10.0 ± 0.46	0.0	0.0	10.0 ± 0.65*	0.0
S. ehrenbergi	0.0	10.0 ± 0.85	8.0 ± 0.58	14.0 ± 0.65	0.0
N. molle	0.0	0.0	0.0	12.0 ± 0.28*	0.0
D. hemprichi	14.0 ± 023	24.0 ± 0.68*	12.0 ± 0.85	10.0 ± 0.46*	0.0

Data are expressed as mean ± SE (n = 3), where * was considered statistically significant at $p < 0.05$.

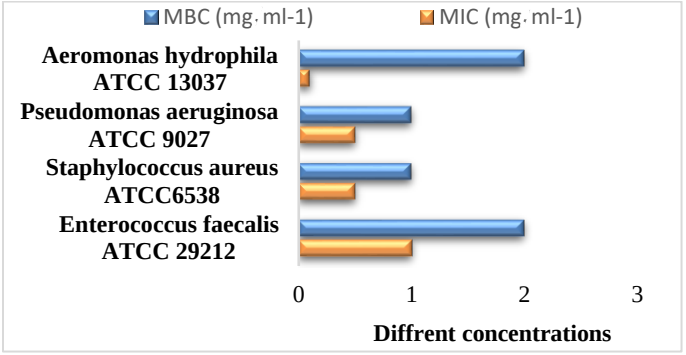


Figure 3: MIC and MBC of *Dendronephthya hemprichi* against different Gram-positive and Gram-negative bacteria.

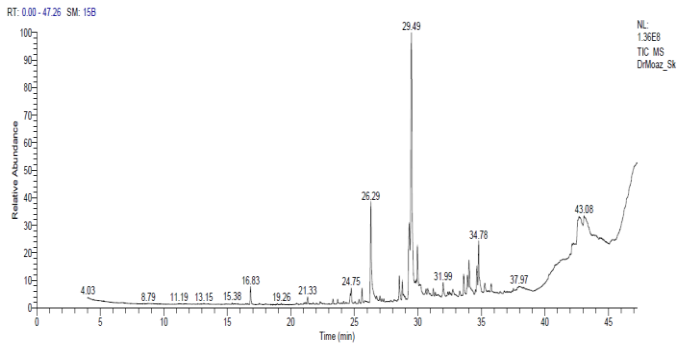


Figure 4: GC-MS total ion chromatogram (TIC) of *Dendronephthya hemprichi* extract.

The results of the antimicrobial activity of soft corals extracts against pathogenic microbes indicated that *Dendronephthya hemprichi* has the potent antibacterial capacity compared with other extracts. This species revealed a broad-spectrum antibacterial property against Gram-negative and Gram-positive bacteria (Figs. 2, 3).

3.1.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Dendronephthya hemprichi extract

(Fig. 3) showed the MIC and MBC values of *D. hemprichi* bioactive substances against bacterial cultures. The MIC values for *E. faecalis* ATCC 29212 and *A. hydrophila* ATCC 13037 were 0.1 mg/mL, whereas for *S. aureus* ATCC6538, and *P. aeruginosa* ATCC 9027 were 0.5 mg/mL. The MBC values for *S. aureus* ATCC6538 and *P. aeruginosa* ATCC 9027 were 1.0 mg/mL, whereas *E. faecalis* ATCC 29212, and *A. hydrophila* ATCC 13037 were 2.0 mg/mL.

3.2. GC-MS analysis of Dendronephthya hemprichi extract

The characterization of bioactive substances in *Dendronephthya hemprichi* was performed using GC-MS analysis (Fig. 4). By examining these chemicals' mass spectra along with gas chromatography, it was possible to identify these molecules (GC). The active compounds are shown in (Table 2), together with information on each one's retention time (t_R), molecular formula (MF), molecular weight (MW), and concentration or peak area (%). The GC-MS analysis of *D. hemprichi* revealed the presence of several bioactive chemicals (Fig. 5), such as 1-Dodecanamine, N, N-dimethyl- **(1)**, 1-Hexadecanol **(2)**, 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione **(3)**, Hexadecanoic acid, methyl ester **(4)**, 1-Eicosanol **(5)**, 10-Octadecenoic acid, methyl ester **(6)**, 9,12-Octadecadienoic acid (Z, Z)- **(7)**, cis-Vaccenic acid **(8)**, Glycidyl oleate **(9)**, Isochiapin b **(10)**, and stigmast-5-en-3-ol, (3β,24S) **(11)**.

4. Discussion

It is common knowledge that marine soft corals provide a rich source of physiologically active substances. Among these molecules, a significant number of steroid compounds can be found. Both the structures and the activity of these compounds have been applied to the process of finding and developing new drugs [5]. The kinds and levels of bioactive components that are present in marine species have a significant impact on their amazing resistance to a wide variety of threats and illnesses, including but not limited to, oxidative stress, inflammatory conditions, and parasite infections, amongst a great number of others [19]. As a diversified range of marine habitats, coral reefs are recognized for harboring a broad range of marine secondary metabolites. Antitumor, antibacterial, antiviral, and antifungal actions are only a few of the many biological functions attributed to the bioactive chemicals found in marine corals [10].

Table 2: Tentative identification of bioactive constituents in *Dendronephthya hemprichi* extract using GC-MS.

No	t_R (min)	Peak area (%)	Compound name	MF	MW
1	16.82	1.91	1-Dodecanamine, N, N-dimethyl-	$C_{14}H_{31}N$	213
2	24.68	0.83	1-Hexadecanol	$C_{16}H_{34}O$	242
3	24.76	1.50	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276
4	25.60	1.69	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
5	28.54	2.89	1-Eicosanol	$C_{20}H_{42}O$	298
6	28.78	1.99	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
7	29.31	7.42	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280
8	29.49	32.02	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282
9	34.78	6.36	Glycidyl oleate	$C_{21}H_{38}O_3$	338
10	35.77	1.08	Isochiapin B	$C_{19}H_{22}O_6$	346
11	34.08	2.08	Stigmast-5-en-3-ol, (3β,24S)-	$C_{29}H_{50}O$	414

In our current study, we collected four distinct specimens of soft corals from the Red Sea in Egypt, and these specimens were identified as *Sinularia leptoclados*, *Sarcophyton ehrenbergi*, *Nephthya mole*, and *Dendronephthya hemprichi*. In a separate study, Afifi and his team collected samples of soft coral from, Saudi Arabia, along the Red Sea coastline. These collected extracts were subsequently subjected to screening to assess their ability to inactivate the growth of both clinical and naturally occurring marine bacteria. This screening was conducted using a three-fold dilution of a methanol/dichloromethane mixture (1:1, *v/v*) [10].

Methanol and dichloromethane are chosen as solvents for liquid-liquid extraction in this context because caffeine exhibits greater solubility in dichloromethane compared to many other solvents. This selectivity makes it an effective choice for extracting caffeine from a mixture [20]. In addition to killing or stunting the growth of coexisting microorganisms, phytochemicals released by higher species may act selectively against particular features expressed by the bacteria.

The bioactive components were extracted using organic solvents, and the results were uniformly positive. From the antibacterial assay, the extraction of *Dendronephthya hemprichi* was found to be the most effective in inhibiting all tested microorganisms excluding *C. albicans*. On antithesis, the extracts of *Sinularia leptoclados*, *Sarcophyton ehrenbergi*, and *Nephthya molle* were found to have the lowest effectiveness against all pathogens. *D. hemprichi* was the only soft coral tested

by Kelman and his team that showed antibacterial action against the test microorganisms [21]. *Dendronephthya* is one of the most extensively distributed genera of soft corals in the tropical coastal waters of the Indian Ocean, the Pacific Ocean, and those around Southeast Asia. It is classified as an Alcyonacean soft coral [22].

The chemical diversity and novelty of *Dendronephthya* species have made them a valuable resource for developing new or unique bioactive compounds [23]. According to our data, the primary components found in the extract of *Dendronephthya hemprichi* were organic acids, aldehydes, esters, carotene, and various derivatives of these compounds. Numerous researches have demonstrated the antibacterial, antioxidant, and cancer-preventative properties of these prominent chemicals [18, 24].

Dodecanamine, N, N-dimethyl ($C_{14}H_{31}N_2$) was also found to be the most abundant compound in the extract of *Aspergillus terreus* She05, which was isolated from Alexandria (Egypt) as reported by Abd El-Latif [25]. 1-Hexadecanol a fatty alcohol was extracted and identified from *Sida cordata* using GC-MS [26]. On the other hand, the *Euphorbia pulcherrima* extract (whole plant) showed the presence of various bioactive components, one of these compounds was 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione. This extract is used in the traditional medicine to treat skin conditions, gonorrhea, migraines, intestinal parasites, and warts [27].

The GC-MS analysis of the ethanolic extract from Sterculia quadrifida bark has indeed confirmed the presence of Hexadecanoic acid [28]. 1-Eicosanol is a natural product isolated from the *Hypericum carinatum*, which has antioxidant activity [29]. 10-Octadecenoic acid, methyl ester, and 9,12-Octadecadienoic acid (Z, Z)- have been shown to possess a wide variety of potential therapeutic applications including antibacterial, anticarcinogenic, antimalarial, anti-ulcer, and other pharmacological effects [14, 18, 30]. Cis-vaccenic acid is considered the major compound in the extract of *D. hemprichi*, which is known to have antibacterial activity and hypolipidemic effect [31].

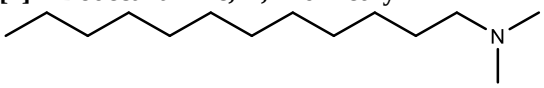
5. Conclusion

The GC-MS analysis serves as an initial step in gaining insight into the nature of active compounds within medicinal organisms. This analytical technique is valuable for laying the foundation for more in-depth research and investigation into the medicinal properties and potential applications of these active principles. The GC-MS results indicated that. It is possible to conclude that the *D. hemprichi* that was gathered from the Red Sea (Egypt) has different bioactive secondary metabolites that exhibited antibacterial activity against examined Gram-positive and Gram-negative bacteria. However, further research is required to separate and isolate the bioactive compounds that may be responsible for the antibacterial activity and to determine the toxicity profile of such substance(s).

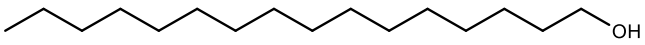
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

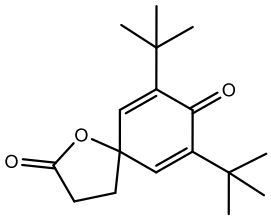
[1] 1-Dodecanamine, N, N-dimethyl-



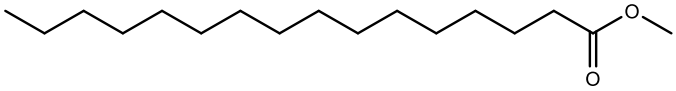
[2] 1-Hexadecanol



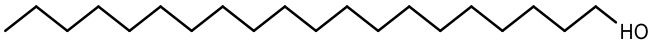
[3] 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione



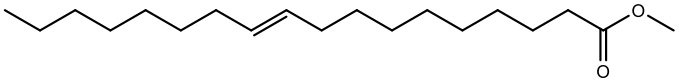
[4] Hexadecanoic acid, methyl ester



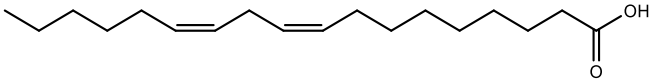
[5] 1-Eicosanol



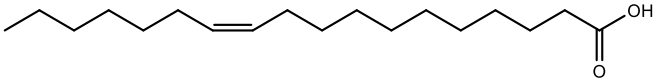
[6] 10-Octadecenoic acid, methyl ester



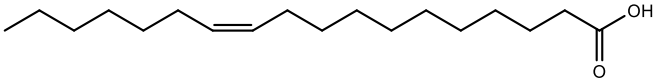
[7] 9,12-Octadecadienoic acid (Z, Z)-



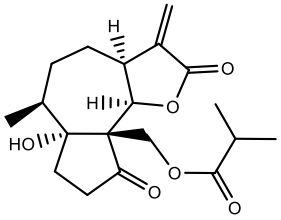
[8] cis-Vaccenic acid



[9] Glycidyl oleate



[10] Isochiapin B



[11] Stigmast-5-en-3-ol, (3β,24S)-

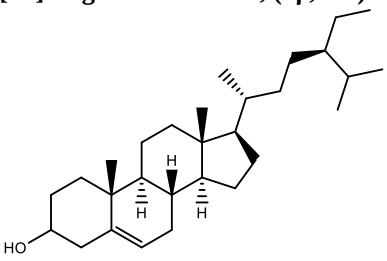


Figure 5: Chemical structures of identified bioactive compounds from *Dendronephthya hemprichi* extract using GC-MS analysis "See (Table 2) for more information."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

CRediT authorship contribution statement:

"Nagwa Mohamed El-Sawi: Supervision, Conceptualization, Writing–review and editing. *Moaz Mohamed Hamad*: Supervision, Conceptualization, Methodology, Visualization, Investigation, Data curation, Writing–original draft preparation, Writing–review and editing. *Mahmoud Hefny Gad*: Supervision, Conceptualization, Methodology, Visualization, Data curation, Writing–original draft preparation, Writing–review and editing. *Yasser Ali Ahmed Hefny*: Investigation, Formal analysis, Data curation, Writing–original draft preparation. All authors have read and agreed to the published version of the manuscript."

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