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GC-MS analysis of bioactive components in six different crude extracts from the Soft Coral (*Sinularia maxim*) collected from Ras Mohamed, Aqaba Gulf, Red Sea, Egypt

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ABSTRACT

The present investigation was carried out during summer 2016 to determine the possible bioactive components extracts with six solvents (Methanol, Methylene Chloride, EtOH, Ethyl Acetate, Acetone and Chloroform) from soft coral, *Sinularia maxima* using GC-MS analysis. Sixty-four compounds were identified from the six extracts. The prevailing compounds in these extracts were Alloaromadendrene (8.8); Hexadecanoic acid, methyl ester (9.4); batilol (12.5); Dimethyl Sulfoxide (2.4); n-Hexadecanoic acid (9.6); Heneicosane (11.6); Octacosanol (12.5). The compounds produced by the studied soft corals possess antimicrobial activity against a number of Gram-positive and Gram-negative bacteria, fungi and Cancer cell lines, in addition to other pharmaceutical activities.

INTRODUCTION

Biodiversity is a key biological parameter influencing multiple aspects of ecosystem structure and function **(Zakaria *et al.*, 2016; Zakaria *et al.*, 2018; El-Damhougy *et al.*, 2019).** There is a need to preserve biodiversity because of its importance for the continuity of human life life **(El-Naggar *et al.*, 2018 & 2019; Farrag *et al.*, 2019** and **Zakaria & El-Naggar, 2019)** Many marine organisms have been used as sources of medicines and primary products **(Hasaballah and El-Naggar 2017).** The Red Sea and its two gulfs (Aqaba and Suez) is one of the most important storehouses of global marine biodiversity **(El-Naggar *et al.*, 2017 and Mona *et al.*, 2019).**

Aquatic organisms are considered as a source for huge numbers of bioactive compounds and secondary metabolites that attract the attentions of biologists, pharmacist and chemists as a result of their potential activities as anticancer, antimicrobial, antiviral, anti-fungal and so on **(El-Naggar and Hasaballah, 2018; Elnagar *et al.*, 2018).** They are facing many of environmental challenges such as competition on space and nutrition, predation and self-defense etc., all these challenges stimulate aquatic organisms to produce secondary metabolites to cope it **(El-Damhougy *et al.*, 2017a; Ibrahim *et al.*,**

2017). The high diversity of soft corals in the Red Sea was confirmed by many studies **(Gohar, 1940; Mohammed, 2012).** Soft corals were addressed in little researches **(Reinicke, 1997; Benayahu *et al*., 2002; Benayahu and Perkol-Finkel, 2004)** due to their difficult taxonomy and the low number of experts who capable of identifying octocorals.

Sinularia comprises a group of soft corals (Phylum: Cnidaria, Class: Alcyonaria, and Family: Alcyoniidae). They are widely distributed from east Africa to the western Pacific, inhabiting the coral reefs or rocks in shallow water, but rarely forming large aggregates **(Chen *et al*., 2012).** Soft corals especially genus *Sinularia* were proven to be a rich source of Terpenoids. Although this genus has been well studied regarding bioactive constituents **(Jaclyn and Ciufolini 2011).** Also, *Litophyton arboreum* has resulted in the identification of a Secosteroidal derivative; 13,14-seco-22-norergosta-4,24(28)-dien-19-hydroperoxide-3-one (35). The specimens were collected from the Red Sea to the North of Jeddah, Saudi Arabia, at a depth of 5–10 m by scuba divers. The purified compound exhibited weak growth inhibitory activities against the human breast carcinoma MCF-7, colon cancer HCT116 and hepatic carcinoma HepG-2 cell lines, in comparisons with the standard anticancer drug doxorubicin **(Ghandourah *et al*., 2015).**

GC-Mass is important methods to identify organism compositions which most recently researches applied get to know important compounds, and there are some studies done by GC-Mass method **(El-Damhougy *et al*., 2017b). Badria *et al.* (1998)** discovered that gorgonian soft coral; *Lobophytum crasscopiculatum* tends to yield compound identified as Cembranolides which have antimicrobial activities. **Badria *et al*. (1998)** also studied the bioactivity-guided fraction of an alcohol extract of the soft coral *Sarcophyton glaucum* collected from the intertidal areas and fringing coral reefs near Hurghada, Red Sea, Egypt resulted in the isolation of a new lactone Cembrane Diterpene, Sarcophytolide. In antimicrobial assays, the isolated compound exhibited a good activity towards *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae*. **Kelman *et al*. (1998)** concluded that the antimicrobial activity detected in the extracts of a Red Sea soft coral, *Parerythropodium fulvum fulvum*, is specific rather than broad spectrum. This specificity may be important in order to enable certain bacteria to live in close association with their coral host. **Kelman *et al*. (2006)** isolated a range of compounds of different polarities from the soft coral, *Xenia macrospiculata*. One of these antibiotic compounds was identified as desoxyhavannahine, with a minimum inhibitory concentration (MIC) against a marine bacterium. The results of their study suggested that soft and hard corals have developed different means to combat potential microbial infections.

The current study was carried out to determine the GC-MS of soft coral *Sinularia maxima* extracts with six solvents (Methanol, Methylene Chloride, EtOH, Ethyl Acetate, Acetone and Chloroform).

MATERIALS AND METHODS

1. Sampling, preservation and identification

The soft coral *Sinularia maxima* specimens were collected during summer 2016 from Ras Muhammad National Park at Egyptian coast of Aqaba Gulf, South Sinai. The sampling was performed using SCUBA diving. Immediately upon sample collection, the

samples were cleaned with sea water and preserved in ice box containing ice cubes and a few pinches of table salt at -20°C until processing. The identification of collected soft coral were identified as possible to the nearest species according to the Great Barrier Reef Expedition **(Macfadyen, 1936)**, the SIBOGA Expedition **(Thomson and Dean, 1931),** Xeniidae of Red Sea **(Gohar, 1940),** Des Roten Meeres **(Reinicke, 1995),** Soft corals and sea fans **(Sheppard & Sheppard, 1991; Veron, 2000** and **Fabricious & Aldersdale, 2001).**

2. Preparation of extracts

In the laboratory, the frozen samples were left to defrost, broken down into small pieces. 10 g of macerated tissues were extracted by soaking in 50 ml of different six absolute solvents of (Methanol, Methylene Chloride, EtOH, Ethyl Acetate, Acetone and Chloroform) for 24 h at room temperature. These extracts were repeated three times until no color was obtained to ensure complete extraction. The combined extracts were filtered through Whatman no.1 filter paper and dried at 40°C using a rotary evaporator.

3. GC-MS analysis

GC-MS analysis was done by using Gas Hewlett Packard HP-5890 series II equipped with split/split less injector and a capillary column (30 m, 0.25 mm, 0.25 lm) fused with phenyl polysilphenylene siloxane. The injector and detector temperatures were set at 280 and 300 °C, respectively, and the oven temperature was kept at 80 °C for 1 min, rose to 300 °C at 20 °C/min. Helium was used as carrier gas at a constant flow of 1.0 ml/min. A volume of 2l was injected in the splitless mode and the purge time was 1 min. The MS (Hewlett–Packard 5889B MS Engine) with selected ion monitoring (SIM) was used. The mass spectrometer was operated at 70eV and scan fragments from 50 to 650 m/z. Peak identification of crude extract was performed based on comparing the obtained mass spectra with those available in NIST library.

RESULTS AND DISCUSSION

Sixty-four compounds were identified from the studied extracts **(Table, 1)**. The prevailing compounds in these extracts were Alloaromadendrene (8.8); Hexadecanoic acid, methyl ester (9.4); batilol (12.5); Dimethyl Sulfoxide (2.4). The compounds produced by the studied soft corals possess an antimicrobial activity against a number of Gram-positive and Gram-negative bacteria, fungi and Cancer cell lines **(Table, 2)**.

The results of GC-MS of *Sinularia maxima* with different extracts were as follow:

1. Methanol crude extract contained Ergosta-5, 24-dien-3-ol, (3. beta.)-, Bis(2-ethylhexyl) phthalate, Octadecane and aldehydes derivatives **(Fig. 1a)**.
2. Methylene Chloride solvent contained Bis(2-ethylhexyl) phthalate, Alloaromadendrene, Octadecane, Ergosta-5,24-dien-3-ol, (3. beta.)- and aldehyde derivatives **(Fig. 1b)**.
3. Ethanolic crude extract had main constituents as; Ergosta-5,24-dien-3-ol, (3. beta.)-, Diisooctyl phthalate, Hexadecanoic acid, hexadecyl ester, Alloaromadendrene and aldehydes derivatives **(Fig. 1c)**.

4. Ethyl acetate crude extract had main constituents as; Alloaromadendrene, Pregna-5,16-dien-20-one, 3-hydroxy-, (3. beta.)- Hexadecanoic acid, ethyl ester, octadecyl ester and aldehydes derivatives **(Fig. 1d)**.
5. Acetone crude extract had main constituents as; Pregna-5,16-dien-20-one, 3-hydroxy-, (3. beta.), Phthalic acid, di(2-propylpentyl) ester, Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl] bis-, esters and aldehydes derivatives **(Fig. 1e)**.
6. Chloroform crude extract contained 1,2-is (trimethylsilyl)benzene, Androst-5,15-dien-3ol acetate, Hexadecanoic acid, hexadecyl ester and aldehydes derivatives **(Fig. 1f)**.

The genus *Sinularia* is the most widespread soft corals. They are rich source of bioactive substances with intriguing and unique structural features **(Chen *et al*., 2012).** The present work reviews the latest progress in the chemistry and pharmacological activities of *Sinularia* which the extracts show abundance of Alloaromadendrene compounds in the construction of the species. Also, this study revealed that compound may be considered as an important medicinal organism. In fact, average from one hundred to one hundred and forty chemical compound has been identified from all extracts of coral by GC-MS analysis. A high percentage of these compounds is represented by fatty acids and alkenes compound Such as Alloaromadendrene, Dimethyl Sulfoxide, ethyl ester, methyl, Octadecane and n-Hexadecanoic acid which possess many desirable biological activities. The importance of this study is due to the biological activity of some of these compounds. It could be concluded that Soft Coral *S. Maxima* contains various bioactive compounds. So it is recommended as soft corals of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile**.**

According to the analysis of GC-Ms in **Table (2)**, most of the identified compounds have been reported to possess interesting biological activities. Among the identified chemicals, Alloaromadendrene has the property of antibacterial activity **(Palazzo *et al*., 2009).** Hexadecanoic acid, methyl ester has both antioxidant, anti-inflammatory activities and seems to have the ability to decrease blood cholesterol **(Asghar *et al*., 2011).** Dimethyl Sulfoxide has ability to enhance TGF-b activity (Transforming growth factor) **(Huang *et al*., 2016).** Acetone crude extract of *Sinularia* contain Batilol which act as effective antimicrobial agent **(Hassan *et al*., 2016). Ghaidaa *at al*. (2016)** found that n-Hexadecanoic acid has several activities such as Anti-inflammatory, antispasmodic, anticancer and antiviral. n-Hexadecanoic was extracted from *Sinularia* by all investigated solvent. Heneicosane which extracted by ethyl acetate is reported as microbicide activity **(Vanitha *et al*., 2020).** Also, Octacosanol in Ethanolic extract can be used as nutritional supplement **(Taylor *et al*., 2003).**

Table 1. The most abundant compounds probabilities in the crudes extract of *Sinularia maxima* using GC-mass spectra.

No.	Library/ID	R.T./min	Area Pct.%	M. weight	Molecular formula	Sample No. *
1	Pregna-5,16-dien-20-one, 3-hydroxy-, (3.beta.)-	12.3	11.4	315	$C_{21}H_{30}O_2$	4,5
2	Diisooctyl phthalate	12.2	11.1	390.5	$C_{24}H_{38}O_4$	3
3	Ergosta-5,24-dien-3-ol, (3.beta.)-	12.3	10.8	398.6	$C_{28}H_{46}O$	1,2,3
4	Hexadecanoic acid, octadecyl ester	12.8	9.1	508.9	$C_{34}H_{68}O_2$	4

5	Phthalic acid, di(2-propylpentyl) ester	12.2	8.8	391	$C_{24}H_{38}O_4$	5
6	Hexadecanoic acid, hexadecyl ester	13.7	7.3	482	$C_{32}H_{64}O_3$	3,4,6
7	Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-	12.8	7.2	305	$C_{22}H_{40}$	5
8	Bis(2-ethylhexyl) phthalate	12.2	6.0	390.5	$C_{24}H_{38}O_4$	1,2,4,6
9	Heneicosane	11.6	3.5	296.5	$C_{21}H_{44}$	4
10	Octacosanol	12.5	3.0	411	$C_{28}H_{58}O$	3
11	Batilol	12.5	2.4	345	$C_{21}H_{44}O_3$	5
12	Cholesterol	10.9	1.7	386.6	$C_{27}H_{46}O$	3,5
13	Eicosane	11.6	1.4	282.5	$C_{20}H_{42}$	2,4,6
14	n-Hexadecanoic acid	9.6	1.4	256.4	$C_{16}H_{32}O_2$	1,2,3,4,5,6
15	Octadecanoic acid	10.6	1.3	284.4	$C_{18}H_{36}O_2$	1,2
16	Dimethyl Sulfoxide	2.4	1.3	78	C_2H_6OS	1,2,3,4,6
17	1,8-Nonadiene, 2,7-dimethyl-5-(1-methylethenyl)-	11.4	1.2	192.3	$C_{14}H_{24}$	2
18	9-Octadecenoic acid, methyl ester, (E)-	10.3	1.2	296.4	$C_{19}H_{36}O_2$	1,4,5,6
19	1-Docosanol, methyl ether	10.2	1.0	340.6	$C_{23}H_{48}O$	2,3,5,6
20	Octadecanoic acid, ethyl ester	10.7	1.0	313	$C_{20}H_{40}O_2$	3
21	Hexadecanoic acid, methyl ester	9.4	1.0	270.451	$C_{17}H_{34}O_2$	1,2,3,4,5
22	Alloaromadendrene	6.9	0.9	204.351	$C_{15}H_{24}$	1,2,3,4,5,6
23	cis-13-Octadecenoic acid, methyl ester	10.3	0.9	296.488	$C_{19}H_{36}O_2$	3
24	Cyclotetradecane	10.2	0.9	196.372	$C_{14}H_{28}$	1,3,4,6
25	Bicyclo[6.1.0]nonane, 9-bromo-9-methyl-, (1.alpha.,8.alpha.,9.alpha.)-	11.1	0.9	217.146	$C_{10}H_{17}Br$	1
26	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	11.3	0.8	204.351	$C_{15}H_{24}$	2,4
27	Methyl stearate	10.4	0.7	298.504	$C_{19}H_{38}O_2$	1,2,4,5,6
28	Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)-	10.9	0.7	162.271	$C_{12}H_{18}$	4
29	trans-13-Octadecenoic acid, methyl ester	10.3	0.7	296.488	$C_{19}H_{36}O_2$	2
30	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	7.3	0.7	204.351	$C_{15}H_{24}$	2,3
31	2(1H)-Naphthalenone, octahydro-4a,7,7-trimethyl-, trans-	11.0	0.7	194.313	$C_{13}H_{22}O$	2,4
32	Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-	10.9	0.5	400.6	$C_{28}H_{48}O$	2,4
33	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7	7.6	0.5	222.366	$C_{15}H_{26}O$	1,2,3,4,5,6

	a.beta.,7b.alpha.)]-					
34	Hexadecanoic acid, ethyl ester	9.7	0.5	268.478	$C_{18}H_{36}O$	3
35	1,2-Naphthalenediol, 2-ethyl-1,2,3,4-tetrahydro-1-methyl-, cis-	11.3	0.5	206.281	$C_{13}H_{18}O_2$	4
36	1-Nonadecene	10.8	0.4	267	$C_{19}H_{38}$	4
37	Heptadecanal	9.9	0.4	254	$C_{17}H_{34}O$	3
38	Alloaromadendrene	8.8	0.4	220.3	$C_{15}H_{24}O$	4
39	2-Nonadecanone	9.8	0.4	282.5	$C_{19}H_{38}O$	4
40	1-Octadecene	10.2	0.4	252.4	$C_{18}H_{36}$	4
41	Heptadecanoic acid, 16-methyl-, methyl ester	10.4	0.4	298.5	$C_{19}H_{38}O_3$	3
42	E-10-Methyl-11-tetradecen-1-ol propionate	8.9	0.4	282.461	$C_{18}H_{34}O_2$	4
43	Oxirane, heptadecyl-	9.9	0.4	282.504	$C_{19}H_{38}O$	4
44	Tetradecanal	8.9	0.4	212.372	$C_{14}H_{28}O$	2
45	1-Eicosene	10.4	0.3	280.532	$C_{20}H_{40}$	2
46	10,13-Octadecadienoic acid, methyl ester	10.3	0.3	294	$C_{19}H_{34}O_2$	5
47	Dibutyl phthalate	9.7	0.3	278.344	$C_{16}H_{22}O_4$	5
48	5-Eicosene, (E)-	9.2	0.3	280.532	$C_{20}H_{40}$	1,2,3,5
49	Octadecanal	8.9	0.3	268.478	$C_{18}H_{36}O$	1,2,6
50	Cyclododecanone, 2-methylene-	9.7	0.2	194.313	$C_{13}H_{22}O$	1
51	Tricyclo[4.3.0.0(7,9)]non-3-ene, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7.alpha.,9.alpha.)-	9.0	0.2	204.351	$C_{15}H_{24}$	4
52	Cetene	9.2	0.2	224.425	$C_{16}H_{32}$	4
53	Isolongifolene, 9,10-dehydro-	8.4	0.2	202.335	$C_{15}H_{22}$	4
54	Formamide, N,N-dibutyl-	5.8	0.2	157.253	$C_9H_{19}NO$	2,4
55	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	8.2	0.2	204.351	$C_{15}H_{24}$	4
56	9-Hexadecenoic acid, methyl ester, (Z)-	9.3	0.2	268.435	$C_{17}H_{32}O_2$	4
57	Aromadendrene oxide-(1)	8.1	0.2	220.350	$C_{15}H_{24}O$	2
58	Humulane-1,6-dien-3-ol	8.1	0.2	222.366	$C_{15}H_{26}O$	4
59	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	9.7	0.2	264.343	$C_{13}H_{16}N_2O_2S$	2,3,4,5,6
60	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	8.2	0.1	204.351	$C_{15}H_{24}$	2
61	1-Heptatriacotanol	8.6	0.1	536.999	$C_{37}H_{76}O$	2
62	cis-13-Eicosenoic acid	9.5	0.1	310.514	$C_{20}H_{38}O_2$	2
63	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	7.3	0.1	204.351	$C_{15}H_{24}$	1,2,3
64	Diethyl Phthalate	7.7	0.1	278.344	$C_{16}H_{22}O_5$	3

* *Sinularia maxima* extracts; 1) methanol extract, 2) Methylene Chloride extract, 3) Ethanolic extract, 4) Ethyl acetate extract, 5) Acetone extract and 6) Chloroform extract.

Table 2. The most Reported and identified compounds with biological activities*.

No.	Compound	Biological Activity	References	Sample No.
1	Alloaromadendrene	Antibacterial activity	**Palazzo *et al.* (2009)**	2,4
2	Hexadecanoic acid, methyl ester	Anti-oxidant, decrease blood cholesterol, anti-inflammatory	**Asghar *et al.* (2011)**	4
3	Dimethyl Sulfoxide	Enhances TGF-b activity (Transforming growth factor)	**Huang *et al.* (2016)**	1.4.6
4	Batilol	Antimicrobial activity	**Hassan *et al.* (2016)**	5
5	n-Hexadecanoic acid	Anti-inflammatory, antispasmodic, anticancer and antiviral	**Ghaidaa *et al.* (2016)**	1,2,3,4,5,6
6	Heneicosane	microbicide activities	**Vanitha *et al.* (2020)**	4
7	Octacosanol	Nutritional supplement	**Taylor *et al.* (2003)**	3

* *Sinularia maxima* extracts; 1) methanol extract, 2) Methylene Chloride extract, 3) Ethanolic extract, 4) Ethyl acetate extract, 5) Acetone extract and 6) Chloroform extract.

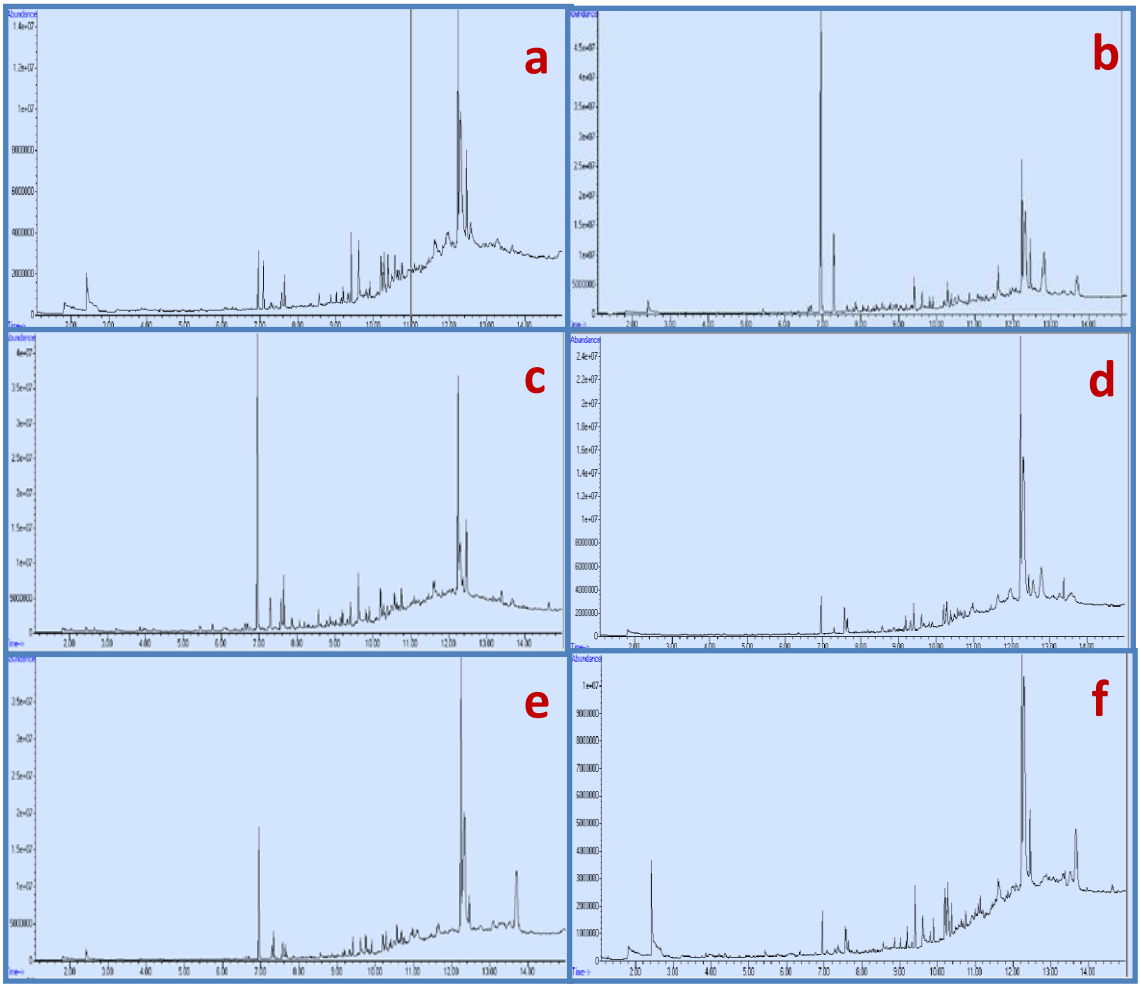

Fig. 1. GC-Mass spectra of the detected extracts of *Sinularia maxima* extracts; a) methanol extract, b) Methylene Chloride extract, c) Ethanolic extract, d) Ethyl acetate extract, e) Acetone extract and f) Chloroform extrac.

GC-Mass is important Methods to identifying chemical compositions of organism which most recently researches applied to get to know important compounds, and there are some studies done by GC-Mass method. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula **(Ghaidaa *et al.,* 2016).** Several other compounds were also detected through GC/MS chromatogram having notable medicinal property. Among the identified chemicals, Ergosta-5,24-dien-3-ol, (3. beta.)- (10.8%), Phthalic acid, di(2-propylpentyl) ester (8.8%) and Bis (2-ethylhexyl) phthalate (6.0%) have the property of antioxidant **(Ghaidaa *et al.,* 2016)**. Also the identified chemicals, E-10-Methyl-11-tetradecen-1-ol propionate, (0.4 %), has the property of Increase zinc bioavailability, oligosaccharide provider, Catechol-O Methyl Transferase-Inhibitor, Methyl Donor, Methyl-guanidine-inhibitor **(Sivakumaran *et al*., 2020)**.

Soft Corals Extracts are well known for their bioactivity. Soft corals especially genus *Sinularia* were proven to be a rich source of Terpenoids. Although this genus has been well studied regarding bioactive constituents **(Jaclyn and Ciufolini, 2011)**, Also **(Thao *et al*., 2013)** resulted in the isolation of seven norditerpenoids from *Sinularia Maxima*, including two new compounds, 12-hydroxy-scabrolide A (2) and 13-epi-scabrolide C (6).

CONCLUSION

Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal organism and this type of study will be helpful for further detailed study. However, isolation of individual chemical substance and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *Sinularia maxima* contains various bioactive compounds. However, further studies are needed to undertake its bioactivity and toxicity profile.

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